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EFFECTS OF BREEDS OF THE MALE DROMEDARY CAMEL ON SEMEN CHARACTERISTICS, SPERM SURVIVABILITY AND HISTOLOGICAL CHANGES OF THE TESTIS

By

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ABSTRACT

Fifteen male dromedary camels at < 6 to 10 years of age (Fellahi, n = 5, Maghrebi, n = 5 and Sudani, n = 5) were used in the present study (Two experiments). Semen samples from each breed were collected using an artificial vagina (AV). Copulation time, semen characteristics, sperm mensuration and histological changes of the camels testes were recorded (The first experiment). In the second experiment, semen was collected and diluted with lactose-yolk-citrate (LYC) extender for each breed and stored at 5ºC for 3 days. *In vitro* response of spermatozoa and its ability to penetrate cervical mucus in different breeds of she-camel, during incubation at 37ºC for 4 hrs, was recorded.

The obtained results showed that copulation time (min), semen-ejaculate volume (ml), sperm motility (%) and sperm-cell concentration ($x10^6$/ml) were significantly ($P<0.05$) higher, however, the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage of spermatozoa and chromatin damage of spermatozoa were significantly ($P<0.05$) lower of Fellahi and Maghrebi than Sudani camels. Semen colour was Creamy, Creamy and Thin creamy, while semen consistency was Viscous, Viscous and Semi-viscous of Fellahi, Maghrebi and Sudani camels, respectively. On the other hand, seminal hydrogen-ion concentration (pH) value and sperm mensuration showed insignificantly differences among the studied breeds. With regard to histological changes, seminiferous tubules of the testis were significantly ($P<0.05$) improved and highly active of Fellahi and Maghrebi breeds than Sudani camels (Experiment 1). The percentages of sperm motility and sperm storagability were significantly ($P<0.05$) higher, while the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa were significantly

(P<0.05) lower in Fellahi and Maghrebi than Sudani camels during storage at 5ºC for 3 days (Experiment 2). The advancement of storage time at 5ºC for 3 days decreased significantly (P<0.05) semen quality in different breeds. The ability of spermatozoa into penetrate cervical mucus showed significantly (P<0.05) better in Fellahi and Maghrebi than Sudani camels spermatozoa, during incubation at 37ºC for 4 hrs. The advancement of incubation times at 37ºC was significantly (P<0.05) decreased the penetrating ability in different breeds into she-camel cervical mucus. In conclusion, copulation time, semen quality, histological changes of the testis and sperm survivability showed better in Fellahi and Maghrebi than Sudani camel spermatozoa.

Keywords:

Dromedary camel, Breeds, Semen characteristics, Testis histology, Penetration.

INTRODUCTION

The extant Camelidae are classed in two genera. The old world genus of Camelus is generally accepted comprise two species. Camelus dromedaries, namely one-humped or Arabian camel and Camelus bactrianus, the Bactrian or two-humped camel **(Wilson, 1984).** In the new world there also exists a single genus of the Camelidae, comprising four species. Two LIma guanacoe and Lama vicugna, the vicuna, are wild and two, LIma glama, the LIma and LIma pacos, the alpaca, are domesticated **(Wilson, 1984).** The vicuna is occasionally considered as a separate genus**.**

In Egypt, increasing camel productivity can help to solve the insufficient amount of animal meat and milk and depends firstly and mostly on reproductive efficiency. A management strategy that promotes maximum reproductive efficiency depends, in turn, on an understanding of reproductive biology of the camel **(Zeidan *et al.*, 2001).**

The camel is an important livestock species that can uniquely adapted to live in hot arid areas. Four camel breeds are found in Egypt (Sudani, Maghrebi, Fellahi and Al-Mowalled). Al-Fellahi camel breed is dominated in the Nile delta region, but not in desert environments, while Al-Mowalled camel breed is much more suitable as a farm and desert animal. Al-Sudani and Al-Maghrebi camel breeds were raised for meat and milk production **(Wilson, 1997).**

Achievement of high reproductive levels partially depends on the success of Artificial Insemination (AI) which in turn is dependent on the quality of semen obtained and its capacity for dilution and storage with minimum loss of fertilizing ability **(Tibary and**

Anouassi, 1997). Generally, the live spermatozoa can be prolonged for several days in chilled state (2-5ºC). However, satisfactory fertility results are not always achieved after, as little as, one day of storage **(Zeidan *et al.*, 2001 and Matter, 2019)** in the dromedary camels.

The objective of the present study was to investigate the effect of camel breeds (Fellahi, Maghrebi and Sudani) on copulation time, semen characteristics, sperm mensuration and histological changes of the testes (Experiment 1). Semen quality and sperm storagability of different camel breeds, during storage at 5ºC were recorded. The penetrating ability of spermatozoa into she-camel cervical mucus with different breeds during incubation at 37ºC for 4 hrs was also assessed (Experiment 2).

MATERIAL AND METHODS

The experimental work was carried out in Private Camel Farm, Marsa Matrouh Governorate during the period from January, 2016 till November, 2016, and Egypt.

Two experiments were carried out. The first experiment aimed to investigate the effects of breed of the male dromedary camels (*Camelus dromedarius*) on copulation time, semen characteristics, sperm mensuration and histological changes of the testes. The second experiment aimed to define the effects of different breeds on the diluted camel semen quality with LYC extender, during storage at 5ºC for 3 days. The pentrating ability of the diluted spermatozoa into she-camel cervical mucus in different breeds, during incubation at 37ºC for 4 hrs was also assessed.

1. Materials:

1.1. Experimental animals:

Three breeds of the male dromedary camels (*Camelus dromedarius*) aging, < 6-10 years old and 500-600 kg live body weight, were used in the first and second experiments. Fifteen camels were divided into three groups according to their breeds (Fellahi, n=5, Maghrebi, n=5 and Sudani, n=5).

All camels were in healthy condition and clinically free from external and internal parasites with a sound history of fertility in the herd.

1.2. Feeding and management:

The rations offered to different breeds of 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 2kg commercial concentrate mixture, 2 kg Egyptian clover hay and 9 kg rice straw daily. Clean fresh water was offered freely to all camels. Different camel breeds 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. Camels semen collection:

Seven ejaculates were collected from each camel breed (Fellahi, Maghrebi and Sudani) of the dromedary camel between 0.8:00 and 10:00 a.m using an artificial vagina (AV) during the breeding season according to **Abd El-Raouf *et al.* (1975)**. A modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France) as the method described by **Zeidan (2002).** AV was filled with water at 50-55ºC and the temperature inside the inner liner was stabilized at 45-50ºC. The ejaculates were usually comes in fractions. Fresh camel semen that has a jelly-like consistency is left for liquefaction for about 30-60 minutes to make the sperm attained motility.

2.2. Semen extension:

Semen samples were collected, pooled and evaluated for each breed (Fellahi, Maghrebi and Sudani) at <6-10 years and then diluted with lactose-yolk-citrate (LYC) extender (2.9g sodium citrate dehydrate, 0.04g citric acid anhydrous, 1.25g lactose and 10ml egg-yolk per 100ml distilled water, 500 I.U/ml penicillin and 500 µg Streptomycin sulphate) according to **Salisbury *et al.* (1978).** Semen extension was carried out by adding the appropriate volume of the semen slowly to the extender as the method described by **Salisbury *et al.* (1978).** The dilution rate was 1 ml semen: 3 ml extender accoding to **Musa *et al.* (1992).**

Semen samples were immediately diluted with LYC extender and kep at 25-30ºC for liquefaction in waterbath for 45 mins, where semen samples were shaked throughly at this time. Thereafter, the mixture was transported in glass containers to a cooled chamber cabinet at 5ºC for 3 days (**Salisbury *et al.*, 1978)**.

2.3. Chilling of semen at 5ºC:

The test tubes containing extended semen for each camel breed (Fellahi, Maghrebi and Sudani) were placed in a 500 ml beaker containing water at 30ºC with a thermometer in order to facilitate periodic check 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 5ºC during a period of 1.5-2.0 hours according to **Musa *et al.* (1992).** The cooled spermatozoa were kept at 5ºC for up to 3 days. After each storage time (0, 1, 2 and 3 days), the percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa were recorded.

The present study included two experiments as follows:

The first experiment aimed to investigate the effect of breeds (Fellahi, Maghrebi and Sudani) of the male dromedary camels *(Camelus dromedarius)* on:

1. Copulation time (minutes):

Duration of copulation was measured from the time of penile intromission into the artificial vagina until withdrawal as the method described by **Bravo *et al.* (2000).**

2. Semen characteristics:

2.1. Semen Colour:

Semen colour was evaluated by direct visual examination from the collecting tube **(Bravo *et al.*, 2000).**

2.2. Semen consistency:

Semen consistency was qualified as viscous when semen did not dropped from a pasteur pipette, semi-viscous when some semen dropped from the pasteur pipette to glass slide and liquid when semen was fluid and dropped readily from the pasteur pipette according to **Bravo *et al.* (2000).**

2.3. Semen-ejaculate volume (ml):

Semen-ejaculate volume (ml) was determined using a conical graduated tube.

2.4. Hydrogen-ion concentration (pH):

Seminal pH value was measured using universal indictor paper and standard commercial stains according to **Karras (1952).**

2.5. Sperm motility (%):

In general, camel sperm motility (%) was detected as oscillatory motion of the flagellum, but not progressive due to viscous materials **(Campbell *et al.*, 1956).** The percentage of sperm motility was determined using one drop of the diluted semen after each storage period on dry, clean and pre-warmed (37ºC) glass slide. The drop of the diluted semen was covered by a warmed cover slip and immediately examined using high power magnification (40x).

Storageability (%) of different breeds (Fellahi, Maghrebi and Sudani) of the diluted cooled spermatozoa was refered to the percentage of original motile spermatozoa still motile after 3 days of storage time at 5ºC according to **Yassen and El-Kamash (1970).**

2.6. Dead spermatozoa (%):

The eosin/nigrosin staining procedure was carried out bydissolving1.67gm esion and 10.00gm nigrosin in distilled water up to 100ml according to **Hackett and Macpherson (1965).**

2.7. Abnormal spermatozoa (%):

The morphological of abnormal spermatozoa (%) were determined in the same smears prepared for live/dead spermatozoa ratio **(Watson, 1975).**

2.8. Acrosome damage of spermatozoa (%):

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

2.9. Chromatin damage of spermatozoa (%):

Toluidine blue staining was performed as previously described by **Erenpreiss *et al.* (2004).** Smears were fixed in ethanol-acetic acid glactial (3: 1, v/v) for 1 min and 70% ethanol for 3 mins. Smears were hydrolyzed for 20 mins in 1 mM HCL, rinsed in distilled water and air-dried. One droplet of 0.025% Toluidine blue in Mcllvaine buffer (sodium citrate-phosphate) pH 4.0 was placed over each smear and then cover slipped. Smears were evaluated with light microscope magnification (x1000). The percentage of chromatin damage was estimated by evaluating 300 sperm cells in each smear. Spermatozoa stained as green to light blue were considered to have normal chromatin, while those stained dark blue to violet were considered to have damaged chromatin.

2.10. Sperm-cell concentration ($x10^6$/ml):

Spermatozoa were counted using haemocytometer according to **Khan (1971).**

2.11. Sperm mensuration (µm):

Mensuration of spermatozoa was measured using calibrated eye-pice micrometer scale **(Hemeida, 1972)**. Mensuration of spermatozoa was carried out on sperm-abnormality smears stained by Eosin-Nigrosin stain according to **Campbell *et al.* (1956).** Every pixel of the micrometer scale was represented of 0.085µm when an oil immersion lens (x100). The parameters of the mensuration of spermatozoa included length and width of sperm head as well as length and width of sperm tail according to **Kononov (1968) and Banaszewska *et al.* (2011).**

3. Histological changes in the camel testis:

For histological study, three testes from each breed (Fellahi, Maghrebi and Sudani) were taken and put in neutral formaline saline (10%) to be preserved, then it passes in ordinary histological set as the method described by **Carleton and Drurg (1967).**

After staining with Haematoxylin and Eosin (H&E) stains, the slides were examined by binuclear microscope and photographed by magnification (x10 & 40) according to **Culling (1975)**.

The second experiment was carried out to study the effects of breed in the dromedary camels *(Camelus dromedarius)* during storage at 5ºC for 3 days on:

1. Extended semen quality with LYC extender (percentages of sperm motility, sperm storagability, dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa) during storage at 5ºC for 3 days.
2. Sperm penetration into she-camel cervical mucus with different breeds (Fellahi, Maghrebi and Sudani) was recorded.

1. Sperm penetration (score):

Sperm penetration for each breed (Fellahi, Maghrebi and Sudani camels) into she-camel cervical mucus was assessed as the follows: Cervical mucus was obtained from a she-camel. A portion of the mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide a column of 6cm length. From different breeds (Fellahi, Maghrebi and Sudani), semen was collected and diluted with LYC extender according to **Musa *et al.* (1992)** and then placed into 2 ml cuvettes (1ml 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 **Hanson *et al.* (1982).**

3. Statistical analysis:

Data were statistically analyzed by one-way and 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. Penetration score was analyzed by Chi-square test.

The following model used in the first and second experiments was as follows:

The first experiment:

$Y_{ij}= \mu + B_i + e_j$

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

μ= is the overall mean.

B_i= is the fixed effect of breed.

e_j= is the residual error

The second experiment:

$Y_{ijk}= \mu + B_i + S_j + (B_i \times S_j) + e_{ijk}$

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

μ= is the overall mean.

B_i= is the fixed effect of breed.

S_j= is the fixed effect of storage time

B_iXS_j= is the first order interaction between breed and storage time.

e_{ijk}= is the residual error.

RESULTS

The first experiment:

1. Copulation time (min):

Data presented in Table 1 showed that copulation time was significantly ($P<0.05$) longer in the male Fellahi and Maghrebi than Sudani camels.

2. Semen characteristics:

Semen colour was Creamy, Creamy and Thin creamy of Fellahi, Maghrebi and Sudani camels, respectively (Table 1). In addition, semen consistency was Viscous, Viscous and Semi-viscous of Fellahi, Maghrebi and Sudani camels, respectively (Table 1). On the other hand, seminal pH value was not significantly among different breeds (Table 1) in the camels. Semen-ejaculate volume, (ml), the percentage of sperm motility and sperm-cell concentration ($x10^6$/ml)showed significantly($P<0.05$) higher, however, the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa were significantly ($P<0.05$) lower of Fellahi and Maghrebi camels than Sudani camels (Table 1).

Table (1): Copulation time and semen characteristics of breeds of the male dromedary camels (Means ± SE).

Items	Breed			P-value
	Fellahi	Maghrebi	Sudani	
Copulation time (min)	**7.03±0.13^{a}**	**7.03±0.11^{a}**	**4.10±0.08^{b}**	**0.002**
Semen cooler	**Creamy**	**Creamy**	**Thin-creamy**	**0.001**
Semen consistency	**Viscous**	**Viscous**	**Semi-visocous**	**0.001**
Semen-ejaculate volume (ml)	**6.18±0.11^{a}**	**6.12±0.014^{a}**	**4.82±0.06^{b}**	**0.002**
Hydrogen-ion-conc. (pH)	**7.53±0.16^{a}**	**7.48±0.15^{a}**	**7.64±0.18^{a}**	**0.237**
Sperm motility (%)	**76.18±1.45^{a}**	**75.15±1.22^{a}**	**62.81±1.14^{b}**	**0.001**
Dead spermatozoa (%)	**11.78±0.14^{c}**	**18.30±0.16^{b}**	**22.73±0.19^{a}**	**0.002**
Abnormal spermatozoa (%)	**5.82±0.12^{c}**	**11.52±0.14^{b}**	**16.32±0.18^{a}**	**0.003**
Acrosome damage (%)	**2.14±0.06^{c}**	**5.65±0.10^{b}**	**8.16±0.13^{a}**	**0.006**
Chromatin damage (%)	**1.12±0.06^{c}**	**2.96±0.08^{b}**	**4.72±0.10^{a}**	**0.002**
Sperm-cell conc. (x10^{6}/ml)	**462.19±13.82^{a}**	**458.12±11.54^{a}**	**411.23±12.67^{b}**	**0.001**

a-c Within rows, within breed, means with different superscripts letters differ significantly (P<0.05).

n = 5

3. Sperm mensuration of the camels (µm):

Data presented in (Table 2) revealed that sperm mensuration**,** head shape index, tail shape index and total length of Fellahi and Maghrebi camels was insignificantly higher than Sudani camels.

Table (2): Effects of different breeds on sperm mensuration (µm) of the male dromedary camels (Means ± SE).

Sperm mensuration (µm)	Breed			P-value
	Fellahi	Maghrebi	Sudani	
Head length	**6.25±0.08**	**6.18±0.08**	**6.14±0.07**	**0.732**
Head width	**2.91±0.06**	**2.67±0.07**	**2.58±0.06**	**0.0651**
Head breadth	**3.42±0.07**	**3.29±0.07**	**3.11±0.05**	**0.430**
Head shape index	**0.46**	**0.43**	**0.42**	**0.624**
Tail of spermatozoa (Mid, Main and End piece) :				
Tail length	**46.01±0.73**	**45.89±0.68**	**45.10±0.57**	**0.327**
Tail width	**1.64±0.04**	**1.53±0.05**	**1.25±0.04**	**0.261**
Tail breadth	**1.85±0.06**	**1.81±0.06**	**1.37±0.05**	**0.472**
Tail shape index	**7.36**	**7.42**	**7.34**	**0.526**
Total length	**52.26±0.82**	**52.07±0.74**	**51.24±0.64**	**0.328**

Head shape index = Width / length ratio.

Tail shape index = Tail length / Head length ratio.

4. Histological changes in the testis:

Plate 1 shows that seminiferous tubules of Fellahi testes camels had a typical stratified germinal epithelium rest on clear basement membrane with different maturation stages (Spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids). In addition, characteristics of moropathological changes with regard to changes in tunica albuginea, Leydig cells proliferation and number of affected seminiferous tubules, characteristics changes represented by thick tunica albuginea (TA,) marked proliferation of the Leydig cells (LYC) associated with atrophy (AST), compression and inactivation of about 35-40% (average 37.8%) of the seminiferous tubules were recorded. Other tubules, about 20-25% (average 22.2%) showed degenerative and apoptotic changes in the spermatogonia and spermatocytes with partial arrest of spermatogenesis.

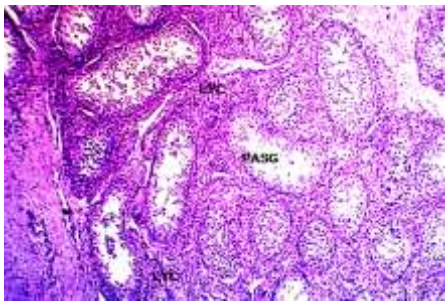

Plate (1): Cross section of Fellahi camels showing normal spermatogenesis and sperm- genesis with a normal morphology of most of spermatogonia, Sertoli cells, spermatocytes, spermatids and spermatozoon. **H & E X 200.**

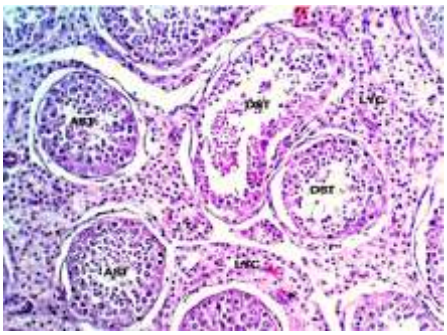

Plate (2): Cross section of Meghrabi camels showing marked proliferation of the Leydig cells (LYC) associated with atrophy (AST),compression and inactivation of some seminiferous tubules .Other tubules, about 20-25% showed degenerative and apoptotic changes in the spermatogonia and spermatocytes with partial arrest of spermatogenesis and sperm genesis. **H & E X 200.**

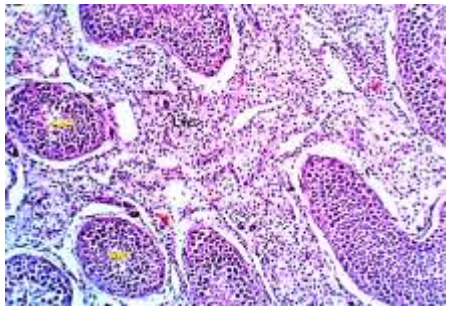

Plate (3): Cross section of Sudani camels showing moderate Leydig cell proliferation (LYC) associated with atrophy and degeneration of seminiferous tubules. **H & E X 200**.

With regard to Maghrebi camels (Plate 2), most of Leydig cells had small size and present singly or scattered here with great reduction in the number of blood and lymphatic vessels. Normal histo-morphological changes with normal spermatogenesis and normal sperm-

genesis, however mild moropathological changes were seen in some seminiferous tubules. About 5-10 % (average 7.2%) of the tubules were affected , such tubules showed partial arrest of spermatogenesis (PASG) as a result of degeneration and apoptosis of both spermatogonia and spermatocytes(ASPG, DSPG, AST, DST) with mild proliferation of the Leydig cells (LYC) .

Few Leydig cells were binucleated and some appeared very large with interstitial granular eosinopilic cytoplasm in Sudani camels (Plate 3). In addition, the amount of interstitial tissues started to diminished. Revealed apparently normal histo-morphological characters with normal spermatogenesis (NSG) and presence of normal intra-luminal sperm cells. A few seminiferous tubules, (About 10-12%, average 10.4 %) showed (focal) histo-morphological abnormalities especially degeneration and apoptosis of spermatocytes and spermatid (AST, DST, DSD, ASD) together with moderate Leydig cell proliferation (LYC) associated with atrophy and degeneration of about 15-20% (average (17.8%) of the seminiferous tubules (AST). The tunica albuginea appeared markedly thickened.

None of the available literature studied on the effects of breed on histological changes in the tests of the dromedary camels.

The second experiment:

1. Camel semen quality during storage at 5^{o}C:

The percentage of motile spermatozoa (Table 3) increased significantly ($P<0.05$), while percentages of dead spermatozoa (Table 4), abnormal spermatozoa (Table 5), acrosome damage (Table 6) and chromatin damage of spermatozoa (Table 7) decreased significantly ($P<0.05$) of the diluted Fellahi and Maghrebi spermatozoa as compared to Sudani camels spermatozoa with LYC extender.

The advancement of storage time at 5ºC decreased significantly ($P<0.05$) the percentage of motile camel spermatozoa (Table 3), however, percentages of dead spermatozoa (Table 4), abnormal spermatozoa (Table 5), acrosome damage (Table 6) and chromatin damage of spermatozoa (Table 7) increased significantly ($P<0.05$) of Fellahi and Maghrebi spermatozoa diluted with LYC extender as compared to Sudani camel spermatozoa with different successive storage times at 5ºC for 3 days..

Table (3): Mean percentage of motile camel spermatozoa in different breeds during storage at 5ºC for 3 days (Means±SE).

Storage time (day)	Breed			Mean
	Fellahi	Maghrebi	Sudani	
0	67.11±0.82	65.389±0.81	60.56±0.78	64.52±0.80^{A}
1	56.87±.72	56.12±0.73	48.61±0.70	53.86±0.71^{B}
2	44.91±0.68	43.56±0.67	37.82±0.42	42.09±0.62^{C}
3	30.56±0.40	29.78±0.40	23.50±0.36	27.94±0.38^{D}
Overall mean	49.86±0.72^{a}	48.83±0.71^{a}	42.62±0.64^{b}	47.10
Storagability	45.53^{a}	45.19^{a}	38.80^{b}	40.30

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

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

Table (4): Mean percentage of dead camel spermatozoa in different breeds during storage at 5ºC for 3 days (Means±SE).

Storage time (day)	Breed			Mean
	Fellahi	Maghrebi	Sudani	
0	18.22±0.14	20.87±0.16	25.43±0.19	21.50±0.18^{D}
1	25.46±0.19	26.18±0.22	33.60±0.28	28.41±0.27^{C}
2	39.17±0.29	41.50±0.32	48.23±0.46	42.96±0.32^{B}
3	56.34±0.48	56.40±0.45	64.85±0.67	59.19±0.53^{A}
Overall mean	34.79±0.26^{b}	36.23±0.27^{b}	43.02±0.35^{a}	38.0

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

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

Table (5): Mean percentage of abnormal camel spermatozoa in different breeds during storage at 5ºC for 3 days (Means±SE).

Storage time (day)	Breed			Mean
	Fellahi	Maghrebi	Sudani	
0	9.64±0.13	10.36±0.14	10.12±0.14	10.04±0.13^{C}
1	10.25±0.14	12.84±0.16	12.67±0.17	11.92±0.18^{C}
2	12.73±0.19	14.35±0.23	16.89±0.25	14.62±0.23^{B}
3	17.82±0.24	17.41±0.26	28.89±0.29	21.39±0.27^{A}
Overall mean	12.61±0.19^{b}	13.74±0.21^{b}	17.16±0.26^{a}	14.50

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

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

Table (6): Mean percentage of acrosome damage of spermatozoa in different breeds during storage at 5ºC for 3 days (Means±SE).

Storage time (day)	Breed			Mean
	Fellahi	Maghrebi	Sudani	
0	**3.16±0.08**	**3.42±0.09**	**4.53±0.12**	**0.70±0.09^{C}**
1	**3.87±0.11**	**4.78±0.13**	**5.64±0.16**	**0.76±0.12^{C}**
2	**4.53±0.13**	**5.16±0.16**	**7.13±0.19**	**5.60±0.17^{B}**
3	**6.74±0.22**	**7.19±0.20**	**11.86±0.26**	**8.59±0.23^{A}**
Overall mean	**4.57±0.13^{b}**	**5.13±0.17^{b}**	**7.29±0.21^{a}**	**5.66**

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

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

Table (7): Mean percentage of chromatin damage of spermatozoa in different breeds during storage at 5ºC for 3 days (Means±SE).

Storage time (day)	Breed			Mean
	Fellahi	Maghrebi	Sudani	
0	**1.45±0.08**	**1.53±0.09**	**1.87±0.11**	**1.61±0.12^{C}**
1	**1.83±0.09**	**2.17±0.14**	**2.76±0.16**	**2.25±0.16^{B}**
2	**2.14±0.15**	**2.82±0.16**	**3.19±0.19**	**2.71±0.17^{B}**
3	**3.71±0.19**	**4.06±0.23**	**7.65±0.26**	**5.14±0.24^{A}**
Overall mean	**2.28±0.16^{b}**	**2.64±0.15^{b}**	**3.86±0.19^{a}**	**2.92**

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

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

2. Assessment of the camel fertility using a mucus penetration test:

Fig. (1) showed that, the penetrating abitity of spermatozoa into she-camel cervical mucus was significantly (P<0.05) better of the diluted Fellahi and Maghrebi camels spermatozoa with LYC extender than Sudani camels spermatozoa during incubation at 37ºC for 4 hrs. Moreover, the advancement of incubation time at 37ºC for 4 hrs was significantly (P<0.05) lower the penetrating ability of the diluted spermatozoa with LYC extender into she-camel mucus in different breeds (Fellahi, Maghrebi and Sudani camels).

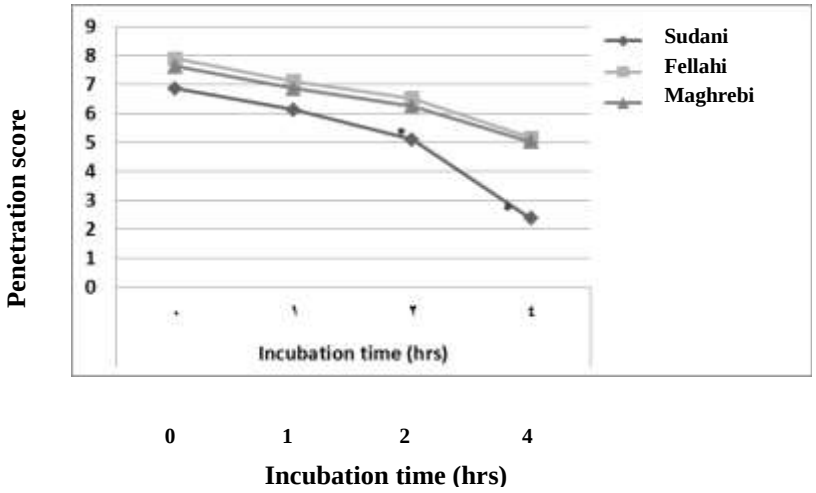

Fig. (1): Penetrating ability of different breeds in the dromedary camel spermatozoa into she-camel cervical mucus, during incubation at 37ºC for 4 hours.

* Significantly different from control in the same time point (P<0.05).

DISCUSSION

In the present study, copulation time (mins) was significantly (P<0.05) improved in the male Fellahi and Maghrebi than Sudani camels (Table 1). The longest time of copulation was recorded in Fellahi camels, while the shortest time was recorded in Sudani camels. These findings may be due to the increase of steroid hormone secretion in Fellahi and Maghrebi camels, consequently, increase of testosterone hormone level **(Abd El-Azim, 1996)** which stimulate copulation time as compared to Sudani camels. In addition, Fellahi and Maghrebi camels were more adapted to Egyptian environmental conditions than Sudani camels. Similar trends were recorded by **El-Mahdy (2019)** of the dromedary camels.

Semen colour was Creamy in Fellahi and Maghrebi camels and thin creamy in Sudani camels (Table 1). Similar trends were recorded by **Garnica *et al.* (1993).** The different colour of

semen in different breeds may be due to different concentrations of spermatozoa and semen consistency **(Zeidan *et al.*, 2001).** Moreover, semen consistency was Viscous in Fellahi and Maghrebi camels and Semi-viscous with Sudani camels (Table 1). Viscosity of semen is usually attributed to presence of mucopolysaccaharides **(Garnica *et al.*, 1993)** which can only from secretion of the bulbourethral glands or the prostate gland. The physiological role of mucopolysaccaharides is not clear. Similar trends were recorded by **Zeidan *et al.* (2001) and El-Mahdy (2019)** of the dromedary camels.

Our results revealed that, semen-ejaculate volume (ml), percentage of sperm motility and sperm-cell concentration ($x10^6$/ml) increased significantly, while the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa decreased significantly in Fellahi and Magherbi camels as compared to Sudani camels (Table 1). Sudani camels revealed a hypoactive Leydig cell which are considered to be testosterone hormone producing factor, so this reflected on semen characteristics. Similar trends were recorded by **Abdel-Raouf *et al.* (1975), Tingari *et al.* (1993) and Zeidan *et al.* (2001)** of the dromedary camels. **El-Mahdy (2019)** found also that the percentage of chromatin damage of spermatozoa was significantly lower of Fellahi and Maghrebi camels than Sudani camels. 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).**

Moreover, seminal pH value and sperm mensuration (µm) were not significant (Table 1) among different camel breeds (Fellahi, Maghrebi and Sudani).**Foote and Bratton (1960)** indicated the importance of seminal pH value. Similar findings were recorded by **Salisbury *et al.*(1978)**in bulls and **Zeidan *et al.* (2001)** in the dromedary camel spermatozoa. In addition, sperm mensuration (µm) in different breeds (Table 2) was insignificantly which may reflect to similar stimulate gonadal activity and spermatogenesis processes with different breeds (Fellahi, Maghrebi and Sudani). These results are in partially agreement with those of **Zeidan *et al.* (2001)** of the dromedary camels. The present study revealed also that, highly active seminiferous tubules of Fellahi and Maghrebi camels and more stratified germinal epithelium rested on clear basement membrane than Sudani camels (Plates 1, 2 and 3). These findings may be due to the better of spermatogenesis processes of Fellahi and Maghrebi camels than Sudani camels. Similar trends were recorded by **Matter (2019)** in Fellahi, **Abd El-Hag *et al.* (2005)** in Sudani, **El-Khasmi *et al.* (2011)** in Maghrebi and **Matter (2019)** in Fellahi, camels.

In respect to the diluted semen with LYC extender stored at 5ºC, percentages of sperm motility and sperm storagability of Fellahi and Maghrebi camel spermatozoa showed significantly higher, while percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage of spermatozoa and chromatin damage of spermatozoa showed significantly lower than Sudani camels (Tables 3, 4, 5, 6 and 7). These findings may be attributed to low semen quality and sperm storagability in Sudani than Fellahi and Magherbi camels. Moreover, storage of semen at low temperature caused structural damage of acrosome as a result of cold shock. These changes are followed by a decrease in the proportion of spermatozoa with intact acrosome and an increase in the release of enzymes into the extracellular medium **(Zeidan *et al.*, 2001).**

On the other hand, the increase of chromatin damage in Sudani camels spermatozoa may be due to the decrease of adenosine triphosphat which activated, apparently, ability of resynthesizing. This was accompanied with a precipitious fall in the rate of fructolysis, consequently increased chromatin damage of spermatozoa. Similar trends were reported by **Zeidan *et al.* (2001) and El-Mahdy (2019)** of the dromedary camels and **Khalifa *et al.* (2013)** in ram spermatozoa. However, **Pradana *et al.* (2016)** found that sperm chromatin integrity of dog spermatozoa was not significantly different between extenders type during storage at 5ºC.

Our results revealed also that, the advancement of storage time at 5ºC decreased significantly the percentages of sperm motility and storagability, however, the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa increased significantly of the diluted camel spermatozoa with LYC extender (Tables 3, 4, 5, 6 and 7) for different camel breeds (Fellahi, Magherbi and Sudani).These findings may be due increase of sperm motility that caused an increase in sperm metabolic activity, consequently increase of lactic acid production which in turn exerts a toxic effect on the sperm cells (**Mann and Lutwak-Mann, 1981)** in bull.

With regard to breeds, *in vitro* response of the spermatozoa-cervical mucus system of Fellahi and Maghrebi camels showed significantly better than Sudani camels with the successive incubation at 37ºC for 4 hrs (Figure 1). **Aitken *et al.* (1983)** found a close correlation between human movement of spermatozoa and their penetrating ability into cervical mucus. **Alexander (1981) and Murase *et al.* (1990)** reported that the duration of sperm motility and penetration distance in the mucus were closely correlated to the pregnancy and conception

rate. Similar trends were recorded by **Zeidan (2002), El-Mahdy (2019) and Matter (2019)** of the dromedary camel spermatozoa.

In addition, the prolongation of incubation time at 37ºC for 4 hrs showed significantly lower the penetrating ability of Fellahi, Maghrebi and Sudani camels with different successive incubation times at 37ºC Fig. (1). these results may related with a decrease in the proportion of motile spermatozoa with different successive incubation times in different camel breeds (Fellahi, Maghrebi and Sudani), similar to that recorded by **Zeidan *et al.* (2001) and El-Mahdy (2019)** in the dromedary camels.

In conclusion, it can be recommended to collect and storage of Fellahi and Maghrebi camel spermatozoa at 5ºC for artificial insemination (AI) programs to enhance of fertilizing ability of she-camels. Particularly in the desert regions where liquid nitrogen may not be available for freezing semen. Further detailed studies are required to establish the reproductive efficiency of the male dromedary camels, under Egyptian environmental conditions.

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الملخص العربي

تأثير سلالات ذكور الجمال العربية المختلفة على صفات السائل المنوي وقدرتها على الحفظ والتغيرات الهستولوجية في الخصية

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أجريت هذه الدراسة على عدد 15 ذكر جمل اعمارهم أكبر من 6-10 سنوات (5 سودانى و 5 فلاحى و5 مغربى) وذلك على تجربتين. تم جمع السائل المنوي لكل سلالة بإستخدام المهبل الاصطناعي. تم تقدير فترة الجماع، صفات السائل المنوي، أبعاد الحيوان المنوي والتغيرات الهستولوجية في الخصية (التجربة الأولى). أما في التجربة الثانية فقد تم جمع وتخفيف السائل المنوي بمخفف اللاكتوز - سترات (LYC) لكل سلالة وحفظت على درجة حرارة 5°م لمدة 3 أيام. كذلك تم تقدير مدى إستجابة الحيوانات المنوية معمليا وكذا قدرتها على النفاذ داخل مخاط عنق الرحم في النوق للسلالات المختلفة اثناء التحضين على درجة حرارة 37°م لمدة 4 ساعات.

أوضحت النتائج أن هناك زيادة معنوية (على مستوى 0.05) في فترة الجماع (دقائق) ، حجم قذفة السائل المنوي (مل)، حركة الحيوانات المنوية (%) وتركيز الحيوانات المنوية ($X10^6$/ml). في حين كان هناك إنخفاض معنوي (على مستوى 0.05) في النسبة المئوية للحيوانات المنوية الميتة والشاذة وشواذ الاكروسوم وشذوذ الكروماتين، في الجمال الفلاحي والمغربي عن الجمال السوداني. كان لون السائل المنوي كريمي، كريمي ، كريمي خفيف ، بينما كانت لزوجة السائل المنوي لزجه، لزجه وشبه لزجه في الجمال الفلاحي ، المغربي والسوداني على الترتيب. من ناحية أخرى لم تختلف قيمة pH في السائل المنوي وكذلك ابعاد الحيوانات المنوية معنويا بإختلاف السلالات المدروسة. كان هناك تحسن معنوي (على مستوى 0.05) في القنيات المنوية وكذلك زيادة نشاطها في الجمال الفلاحي والمغربي عن الجمال السوداني (التجربة الأولى). كان هناك زيادة معنوية (على مستوى 0.05) في النسبة المئوية لحركة الحيوانات المنوية وكذلك قدرتها على الحفظ، بينما كان هناك انخفاض معنوي (على مستوى 0.05) في النسبة المئوية لكل من الحيوانات المنوية الميتة والشاذة وشواذ الاكروسوم وشذوذ الكروماتين في الجمال الفلاحي والمغربي عن الجمال السوداني عند الحفظ على درجة حرارة 5°م لمدة 3 أيام، في حين انخفضت نوعية السائل المنوي في السلالات المختلفة في فترات الحفظ المتتالية على درجة حرارة 5°م لمدة 3 أيام (التجربة الثانية) بدرجة معنوية (على مستوى 0.05). كان هناك إنخفاض معنوي (على مستوى 0.05) في نوعية السائل المنوي في السلالات المختلفة (فلاحي ، مغربي ، سوداني) وذلك مع التقدم في فترة الحفظ. كان معدل نفاذية الحيوانات المنوية داخل مخاط عنق الرحم أفضل بدرجة معنوية (على مستوى 0.05). في الجمال الفلاحي والمغربي عن الجمال السوداني أثناء التحضين على درجة حرارة 37°م لمدة 4 ساعات، ولكن انخفض معدل نفاذية الحيوانات المنوية داخل مخاط عنق الرحم للنوق في السلالات المختلفة مع التقدم في فترة التحضين على درجة حرارة 37°م.