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INFLUENCE OF AGE ON SEMEN QUALITY AND HISTOLOGICAL CHANGES IN THE DROMEDARY CAMELS TESTES

By

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ABSTRACT

Two experiments were carried out through the period from January to April 2019. Fifteen Fellahi camel bulls were used in the present work. Animals were divided into three groups according to their ages (five each). The age of the camels in the first (G1), second (G2) and third groups (G3) was <5 to 11, < 11 to17 and <17 to 23 years, respectively. Semen was collected using an artificial vagina (AV). Copulation time, semen characteristics, sperm mensuration and histological changes of the testes were recorded (Experiment 1). Semen was extended with Lactose-yolk-citrate (LYC) extender and stored at 5 ^{o}C. Sperm penetration into she-camel cervical mucus was recorded, during incubation at 37ºC in different ages of the camels (Experiment 2).

The results revealed that, copulation time (mins) and semen ejaculate-volume (ml) were significantly (P<0.05) better in the first and second groups than third group. Semen colour was creamy white in the first and second groups and milky white in the third group. Moreover, semen consistency was viscous in the first and second groups, while semi-viscous in the third group. Seminal pH value and sperm mensuration (µm) were insignificantly differences among groups. Percentage of sperm motility and sperm-cell concentration (x 10^6/ ml) were significantly (P<0.05) higher, while the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of the camel spermatozoa in the first and second groups were significantly (P<0.05) lower than the third group. Testes of the camels in the first and second groups showed more numerous seminiferous tubules (ST) than the third group. This ST lined by spermatogenic cells of different maturation stages (spermatogonia, spermatocytes, spermatids and spermatozoa) are present and highly active of the camels in the first and second groups as compared to third group (Experiment 1). The percentages of sperm motility and storagability were significantly (P<0.05) higher in the first and second groups, however the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of sperm were significantly (P<0.05) lower in the

first and second groups than the third group, during storage at 5 °C (Experiment 2). The advancement of storage time at 5°C caused significantly (P<0.05) decreased the percentages of sperm motility and storagability, while significantly (P<0.05) increased the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa in all groups. The penetrating ability of spermatozoa into she-camel cervical mucus was significantly (P<0.05) better in the first and second groups than the third group. 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 in all groups. In conclusion, copulation time, semen characteristics, sperm mensuration, histological changes in the testes and penetration score were superior in the first and second groups than the third group of the dromedary camels.

Keywords:

Dromedary camel, Semen characteristics, Mensuration, Testes histology, Penetration score.

INTRODUCTION

Less information is available about management in the male camel during breeding activity. Full reproductive potential in 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, 2019)**.

In addition, the breeding activity in the 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)**. Moreover, reproductive management of females can influence parameters such as age at first service, conception rate, calving rate and intercalving interval **(Khanna, 1990)**.

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 on copulation time, semen characteristics, sperm mensuration and histological changes in the testes in the dromedary camels (Experiment 1). The camel semen quality during storage at 5 °C for 3 days was evaluated (Experiment 2). The penetrating ability of spermatozoa into she-camel cervical mucus during incubation at 37 ºC for 4 hours was also assessed.

MATERIALS AND METHODS

The present study was carried out in the Laboratory of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig,Egypt and Private Camel Farm,Belbies City, Sharkiya Governorate, Egypt

1.Materials:

1.1.Experimental animals:

Fifteen Fellahi camels (*Camelus dromedarius*) aging 5-23 years old and 400 to 700 kg live body weight were used in the present study. All camels were in healthy condition and clinically free from external and internal parasites with a sound history of fertility in the herd.

1.2. Feeding and management:

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

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

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

Animals were divided into three groups according to their ages (five each). The age of the camels in the first (G1), second (G2) and third groups (G3) was <5 to 11, < 11 to17 and <17 to 23 years old, respectively. The age of animals was determined on the basis on dental formula according to **Wilson (1984)** as follow:

1.Temporary or milk teeth:

These teeth in the camel are 22 in number, the dental formula is:

$$\frac{1\text{-}1}{3\text{-}3} \quad \frac{1\text{-}1}{1\text{-}1} \quad \frac{3\text{-}3}{2\text{-}2} \quad =22$$

Incisors Canines Premolars:

2. Permanent teeth: The permanent teeth number 34, the dental formula is written as:

$$\frac{1\text{-}1}{3\text{-}3}\quad\frac{1\text{-}1}{1\text{-}1}\quad\frac{3\text{-}3}{2\text{-}2}\quad\frac{3\text{-}3}{3\text{-}3}=34$$

Incisors Canines Premolars Molars

1.3. Camel semen collection:

Semen was collected and evaluated in different ages on several occasions from an intact male camel using an artificial vagina (AV) (30 cm long and 5 cm internal diameter, IMV, France) as described by **Zeidan (2002)** containing warm water (50 ^{o}C). Semen was evaluated and diluted within 15 mins after collection. The AV was filled with water at 50-53 ^{o}C for reached to 45-50 ^{o}C inside the inner liner. Fresh camel semen that has a jelly-like consistency is left for liquefaction for about 30-60 mins to make the sperm attain motility.

2. Methods:

2.1.Copulation time (mins):

Copulation was measured from time of penile intromission into the artificial vagina until withdrawal as the method described by **Bravo *et al.* (1997).**

2.2. Semen characteristics:

2.2.1. Semen colour:

Semen colour in the dromedary camels was determined directly from the collecting tube by visual method.

2.2.2. Semen consistency:

Semen consistency in the dromedary camels was qualified as viscous when semen did not drop from a Pasture pipette, semi-viscous when semen dropped from the Pasture pipette to glass slide and liquid when semen was fluid and dropped readily from the Pasture pipette according to **Bravo *et al*. (1997).**

2.2.3. Semen-ejaculate volume (ml):

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

2.2.4. Hydrogen-ion concentration (pH):

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

2.2.5. 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 (40 x).

2.2.6. Storagability (%):

Storagability (%) of the cooled camel spermatozoa refers to the percentage of original motile spermatozoa still motile after 3 days of storage time at 5^{o}C as the method described by **Yassen and El-Kamash (1970).**

2.2.7. Dead camel spermatozoa (%):

The eosin/nigrosin staining procedure was carried out by dissolving 1.67 gm eosin and 10.00 gm nigrosin in distilled water up to 100 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.

2.2.8. Abnormal camel spermatozoa (%):

The morphological abnormalities of spermatozoa (%) were determined in the same smears prepared live/dead spermatozoa ratio according to **Watson (1975)**.

2.2.9. 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 (x l00).

2.2.10. Chromatin damage of spermatozoa (%):

Toluidine blue staining was performed as the method described by **Erenpreiss *et al.* (2004)**. Smears were fixed in ethanol-acetic glactial (3:1.5 v/v) for 1 min and 70% 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.

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

The spermatozoa were counted using haemocytometer according to **Salisbury *et al*. (1978).**

3. Semen extension:

Semen samples were evaluated for each camel. Semen was diluted with lactose-yolk-citrate (LYC) extender containing 11% w: v Lactose and 20% w: v egg yolk according to **Anouassi *et al.* (1992)**. Semen extension was carried out by adding the appropriate volume the semen slowly to the extender using the method described by **Salisbury *et al*. (1978).** Semen samples were immediately diluted with LYC extender and kept at 25-30°C for liquefaction in waterbath for 45 mins. Thereafter, the mixture was transported in glass container to a cooled chamber cabinet at 5°C for 3 days. The final extension rate was 1ml semen: 4ml extender.

4. Sperm mensuration (µm):

Sperm mensuration was carried out with an eye-piece micrometer (Filar balloted; Bausch and Lomb, Lancaster, PA) which was calibrated with a stage of micrometer scale. Every pixel of the micrometer scale was represented of 0.085µm when using an oil immersion lens (x100). The following sperm morphometric measurements were taken. Sperm head area was limited by a curve running along the perimeter of the sperm head and sperm head perimeter length of the boundary limiting the sperm head according to **Banaszewska *et al*. (2011).**

5. Chilling of semen at 5 ^oC:

The test tubes containing extended Fellahi camel semen at <5-11 years old 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 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 according to **Salisbury *et al.* (1978).**

6. Sperm penetration (Score):

Sperm penetration into she-camel cervical mucus (which obtained from she-camel throw estrus period) with different ages in the male camels was assessed. Cervical mucus was obtained from she-camel (at <5-11 years). A portion of the mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide a column of 6 cm length. With different ages, camel semen was collected and extended with LYC extender as described by **Murase *et al*. (1990)** 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 **Hanson *et al*. (1982)**.

7. Histological changes in the camel testes:

For histological changes, three testes from each age (after slaughter the experimental animals) 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)**. The sections were stained by Haematoxylin and Eosin (H&E) stains according to **Culling (1975).** After staining, the slides were examined by binuclear microscope and photographed by magnification (x 10 & 40).

3. Statistical analysis:

Data were statistically analyzed by one-way and two-way design (ANOVA) using General Linear Model (GLA) 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 and storagability were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Experiment 1:

1. Copulation time (mins):

Data presented in (Table 1) showed that the effect of age on copulation time was significantly ($P<0.05$) higher in the first (G1) and second (G2) groups, while significantly ($P<0.05$) shorter in the third group (G3). These results are in agreement with those of **Zeidan (1999)** who reported that, copulation time was significantly ($P<0.05$) longer for the male dromedary camels at 5 to 10 and 10 to 15 years than 15 to 20 years old . Steroid hormones secretion by

testes is increased with age until eleven years consequently increase testosterone level which stimulate copulation time then decreased with age **(Leathem, 1977).**

2. Semen characteristics:

2.1. Semen colour:

As shown in (Table 1) semen colour in the dromedary camels was creamy white in the first and second groups, while milky white in the third group. **Ahmadi (2001)** found that semen colour was yellowish white, creamy white and milky white in the dromedary camels at <5-11, <11-17 and <17-23 years old, respectively. The different colour of semen may be due to different concentrations of spermatozoa and semen consistency **(Zeidan *et al*., 2001). Marai *et al*. (2009)** showed that, the semen colour depends on the ratio of the gelatinous fraction which is grey to the sperm fraction which is white. The colour becomes slightly yellowish the sample is contaminated by urine.

2.2. Semen consistency:

Semen consistency in the dromedary camels was viscous in the first and second groups and semi-viscous in the third group (Table 1). **Zeidan *et al*. (2007)** showed that semen consistency was viscous in the camels at 5 to 10 and 10 to 15 years and semi-viscous in the camels at 15 to 20 years old. Viscosity of the camel semen is usually attributed to the presence of mucopolysaccharides **(Garnica *et al*., 1993)** which only secreted from bulbourethral or the prostate glands. Immediately after semen collection, the ejaculate becomes aqueous in consistency.The physiological role of mucopolysaccharides is not clear.

2.3. Semen-ejaculate volume (ml):

As shown in (Table1), the effect of age on Semen-ejaculate volume was significantly ($P<0.05$) higher in the first and second groups, while significantly ($P<0.05$) lower in the third group. Similar trends were recorded by **Ahmadi (2001)** in the dromedary camels.

2.4. Hydrogen-ion concentration (pH):

Seminal hydrogen-ion concentration (pH) value of the male dromedary camels (Table 1) was insignificantly higher in the first group than the second and third groups. Similar trend was reported by **Agarwal *et al*. (2004)** who found that seminal pH value was fairly constant between various seminal ejaculates within a narrow range of 7.2 to 7.4 in camels. Similarly **Matter (2019)** found also that seminal pH value of the male dromedary camels showed insignificantly higher in the male dromedary camels at 10 to 15 years or 15 to 20 years than 5 to 10 of age.

2.5. Percentage of sperm motility:

The percentage of sperm motility was significantly (P<0.05) higher in the dromedary camels in the first and second groups, while lower in the third group (Table 1). The highest (P<0.05) value of the percentage of sperm motility was recorded in the first group and the lowest (P<0.05) value was recorded in the third group. Similar trends were reported by **Zeidan *et al.* (2001) and Matter (2019)** in the dromedary camels**.** The advancement of age revealed hypoactive Leydig cells which are considered to be testosterone hormone producing factor, so this reflected on a bad semen characteristics produced by the aged animals **(Tingari *et al.*, 1993).**

2.6. Percentage of dead spermatozoa:

The percentage of dead spermatozoa was significantly (P<0.05) higher in the dromedary camels in the third group than the first and second groups (Table 1). The highest (P<0.05) value of the percentage of dead spermatozoa was recorded in the third group and the lowest (P<0.05) value was recorded in the first group. Similar trends were reported by **Zeidan *et al.* (2001) and Matter (2019)** in the dromedary camels. Similarly, **Ahmadi (2001)** showed that, the highest value of the percentage of dead spermatozoa was recorded in the dromedary camels at 12 to 20 years and the lowest value was recorded at 6 to 11 years of age. These results may be attributed to the advancement of age which may cause disturbance in spermatogenesis or destruction or even death of spermatozoa **(Musa *et al.* 1992).**

2.7. Percentage of abnormal spermatozoa:

The effect of ages on the percentage of abnormal spermatozoa was insignificantly effects between the first and second groups, while significantly (P<0.05) higher in the third group (Tble1). Similar trends were recorded by **Ahmadi (2001).** Testosterone hormone producing cells is reflected in bad semen characteristics produced by aged male camels **(Tingari *et al.*, 1993)**.

Table (1): Effects of different ages on copulation time and semen characteristics in the dromedary camels.

Items	Age (year)		
	G1 (< 5- 11)	G2 (< 11 – 17)	G3 (< 17 – 23)
Copulation time (mins)	6.11 ± 0.06[a]	6.08 ± 0.07[a]	4.52 ± 0.04[b]
Semen colour	Creamy white	Creamy white	Milky white
Semen consistency	Viscous	Viscous	Semi-viscous
Semen–ejaculate volume (ml)	6.18 ± 0.09[a]	6.10 ± 0.04[a]	4.72 ± 0.06[b]
Hydro.ion concen. (pH)	8.00 ± 0.06[a]	7.82 ± 0.08[a]	7.50 ± 0.05[a]
Sperm motility (%)	72.19 ± 0.81[a]	71.35 ± 0.68[a]	67.91 ± 0.73[b]
Dead spermatozoa (%)	24.33 ± 0.18[b]	24.92 ± 0.16[b]	28.65 ± 0.20[a]
Abnormal spermatozoa (%)	10.61 ± 0.12[b]	12.74 ± 0.05[b]	16.43 ± 0.11[a]
Acrosome damage (%)	4.15 ± 0.04[b]	5.13 ± 0.08[b]	7.92 ± 0.06[a]
Chromatin damage (%)	2.75 ± 0.01[b]	2.96 ± 0.02[b]	4.16 ± 0.02[a]
Sperm-cell concentration ($x10^6$ /ml)	381.63 ± 12.80[a]	376.19 ± 11.76[a]	319.72 ± 14.26[b]

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

G1: Group1 G2: Group 2 G3: Group3

2.8. Percentage of acrosome damage:

The percentage of acrosome damage was significantly (P<0.05) higher in the dromedary camels in the third group than the first and second groups (Table 1). The highest (P<0.05) value of the percentage of acrosome damage was recorded in the third group and the lowest (P<0.05) value was recorded in the first group. Similar trends were reported by **Zeidan *et al.* (2001)** in the dromedary camel.

2.9. Percentage of chromatin damage:

The percentage of chromatin damage of spermatozoa in the dromedary camels was significantly (P<0.05) higher in the third group than the first and second groups (Table 1). The highest (P<0.05) value of the percentage of chromatin damage was recorded in the third group and the lowest (P<0.05) value was recorded in the first group (Table 1). Similar trends were reported by **El-Mahdy (2019)** in the dromedary camel spermatozoa. However, **Pradana *et al.* (2016)** found that, the sperm chromatin integrity was not significantly different during

storage at 5°C. This phenomenon may be due to the decrease of adenosine triphosphate which activated apparently ability of resynthesizing. This was accompanied with precipitation fall in the rate of fructolysis, consequently, increased chromatin damage **(Mann and Lutwak-Mann, 1981).**

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

Sperm-cell concentrations was significantly (P<0.05) decreased in the third group, while increased significantly (P<0.05) in the first and second groups (Table1). Similar trend was reported by **Zeidan *et al*. (2001)** in the dromedary camels. The cause of lower reproductive efficiency with the progress of age may be due to hormonal imbalance or deficiency of spermatogenesis processes. **Ali and Najlaa (2014)** noticed that the concentration of sperm obtained from epididymis tail of bulls camel significantly increase with age progress. **Matter (2019)** showed also that sperm-cell concentration (x10^6 /ml) was significantly (P<0.05) higher in the male dromedary camels at 5 to 10 and 10 to 15 years than 15 to 20 years of age.

3. Sperm mensuration measurements (µm) in the dromedary camels.

Sperm mensuration measurements (µm) in the dromedary camels was insignificantly effects with the different ages in the dromedary camels (Table2). Similarly, **Zeidan (1999)** in the dromedary camels reported that the length, the breadth and head area of sperm head was insignificantly higher at 5-10 years and 10-20 than 15-20 years.

Table (2): Effects of different ages on sperm mensuration measurements (µm) of the dromedary camels.

Sperm mensuration (µm)	Age (year)		
	G1 (< 5- 11)	G2 (< 11-17)	G3 (< 17-23)
Head length	6.08 ± 0.09	6.10 ± 0.11	6.18 ± 0.12
Head width	2.87 ± 0.04	2.89 ± 0.05	2.81 ± 0.05
Head breadth	3.24 ± 0.05	3.41 ± 0.06	3.26 ± 0.80
Head shape index:	0.47	0.47	0.45
Tail length	45.91 ± 0.52	45.94 ± 0.64	45.87 ± 0.62
Tail width	1.47 ± 0.03	1.53 ± 0.02	1.42 ± 0.02
Tail breadth	1.86 ± 0.04	1.92 ± 0.04	1.84 ± 0.03
Tail shape index:	7.55	7.53	7.42
Total length	51.99 ± 0.73	52.04 ± 0.92	52.05 ± 0.84

G1: Group1 G2: Group 2 G3: Group3

Head shape index = Width / Length ratio

Tail shape index = Tail length / Head length ratio

Fertilizing ability of spermatozoa depends on the status of their shapes and sizes, which affect the course of the acrosomal reaction and sperm penetration of the ovum **(Kondracki *et al.*, 2011)**. Generally male age is one of the significantly causes of variation in spermatozoa morphology and morphometric dimensions **(Banaszewska *et al.*, 2011)**.

4. Histological changes in the testes of the dromedary camels:

Moste of the examined testicular tissues in the dromedary camel testes at < 5- 11 years (Plate 1) revealed apparently normal histo-morphological characters with normal spermatogenesis. A very few seminiferous tubules (average 4%) showed mild (focal) histo-morphological abnormal especially degenerated spermatocytes (DSTC) and apoptotic spermatids together with focal interstitial oedema (FIE), dilated capillaries (DC) and Leydig cells proliferation (LCP). Similar trends were recorded by **Zeidan and Abbas (2004)** and **Matter (2019).**

In respect of dromedary camel testes at < 11-17 years (Plate 2) showing apparently normal seminiferous tubules (ST) with normal spermatogenesis (NSG). Moreover, few Leydig cells were appeared very large with intensely granular eosinophilic cytoplasm.

There was numerous lymphatic and blood vessels. Leydig cells were loosely associated with disappearance of eosinophilic granular cells. These results are in agreement with those of **Zeidan and Abbas (2004) and Matter (2019)** in the dromedary camels.

Meanwhile, all the tissue sections obtained from the testes from the camel at <17 t0 23 years old (Plate 3) had slight to moderate degenerative changes and necrosis in spermatocytes I, II and spermatids (DS). At the level of the interstitial connective tissue, hyperplasia of Leydig cells (L), slightly vacuolations of variable sizes and slightly BVS congestion were observed

Sections of the camel testes at <5 to 11 (Plate 1) and <11 to 17 years old (Plate 2) showed normal architecture of the seminiferous tubules and the interstitial tissues including Leydig cells. The seminiferous tubules appeared rounded or oval in shape and were surrounded by a thin basal Lamina**.** The stratified germinal epithelium that lined the seminiferous tubules, were appeared consisted of two distinct populations of cells; the proliferating, highly divided numerous spermatogenic cells and non-dividing fewer Sertoli cells. In between the tubules, the interstitial tissue had blood vessels with clusters of cells of ovoid or polygonal shape and spherical nuclei representing the Leydig cells were observed as well as flat, squamous cells with flat nuclei representing the myoid cells were also noticed**.**

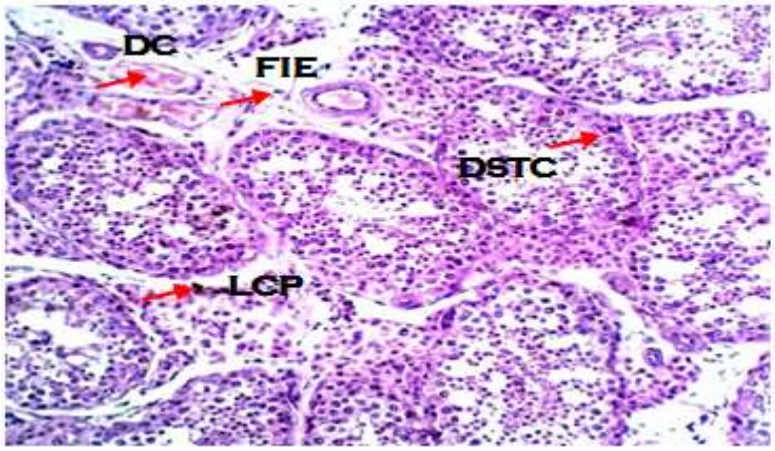

Fig. (1): Cross section of dromedary Camel testes at < 5-11 years, showing a few degenerated spermatocytes (DSTC) together with focal interstitial oedema (FIE) , dilated capillaries (DC) and Leydig cell proliferation (LCP) are seen. (H&E X 200).

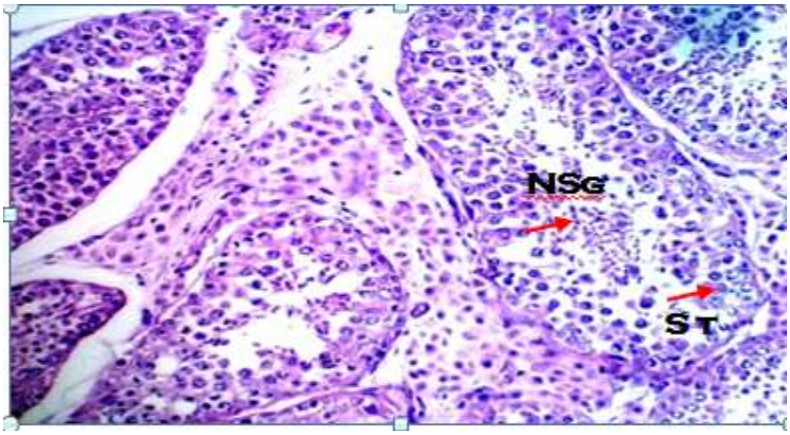

Fig (2): Cross section of dromedary Camel testes at < 11-17 years, showing a apparently normal seminiferous tubules (ST) with normal spermatogenesis (NSG). **(H&E X 200).**

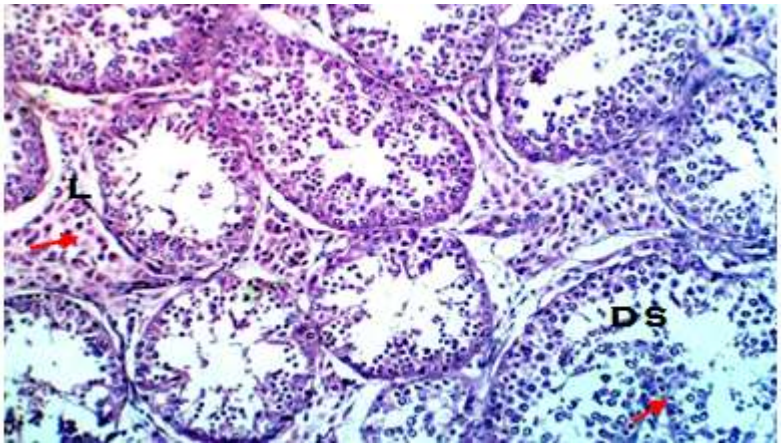

Fig. (3): Cross section of dromedary Camel testes at < 17-23 years, showing few binucleated Leydig cells (L) and different spermatozoa cell types (DS). **(H&E X 200).**

Experiment 2:

Camel semen quality during storage at 5°C:

1. Percentage of sperm motility:

The percentage of sperm motility and storagability were significantly (P<0.05) higher in the first and second than the third groups (Table 3). The highest (P<0.05) values of sperm motility were recorded with the camels at <5-11 years (first group) and at <11-17years (second group), while the lowest value was recorded with the camels at <17- 23 years (third group).

Table (3): Mean percentages of motile camel spermatozoa with different ages during storage at5 ^{o}C for 3 days.

Storage time (day)	Age (year)			Mean
	G1 (< 5- 11)	G2 (< 11-17)	G3 (< 17-23)	
0	69.11± 0.90	69.72 ± 0.89	68.47 ± 0.68	69.10 ± 0.92^{A}
1	65.17 ± 0.83	64.15 ± 0.81	62.53± 0.80	63.95 ± 0.86^{B}
2	57.67 ± 0.68	57.19 ±0.65	51.80 ± 0.60	55.53 ± 0.16^{C}
3	44.90 ± 0.36	45.62 ± 0.39	34.75 ± 0.28	41.75 ± 0.47^{D}
Overall mean	59.21 ± 0.83^{a}	59.17 ± 0.80^{a}	54.38 ± 0.73^{b}	57.58
Storagability (%)	64.96^{a}	65.43^{a}	50.75^{b}	60.41

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

A- D Within a column, within ages, means with different superscripts letters differ significantly (P <0.05

G1: Group1 G2: Group 2 G3: Group3

It is of interest to note that, the prolongation of storage time at 5°C for up to 3 days decreased significantly (P<0.05) the percentage of the camel sperm motility in all ages (Table3). These results are in agreement with those of **Ahmadi (2001)**. Similar trend was reported by **Zeidan *et al.* (2008) and Matter (2019)** 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).**

2. Percentage of dead spermatozoa:

The effects of ages on the percentage of dead camel spermatozoa were significantly (P<0.05). The highest (P<0.05) value of percentage of dead spermatozoa was recorded with camels at <17-23 years (third group), while the lowest (P<0.05) value was recorded with camels at <5-11 years (first group) and <11-17years (second group) (Table 4).

The prolongation of storage time at 5°C for up to 3 days was significantly (P<0.05) increased the percentage of dead spermatozoa of the dromedary camels in the all groups (Table 4). The percentage of dead camel spermatozoa increased significantly (P<0.05) as time of storage increased. These results are in agreement with those of **Zeidan *et al.* (2008)** and **Matter (2019)** in camel spermatozoa.

Table(4): Mean percentages of dead camel spermatozoa with different ages, during storage at5 ^{o}C for 3 days.

Storage time (day)	Age (year)			Mean
	G1 (< 5- 11)	G2 (<11-17)	G3 (< 17-23)	
0	28.16± 0.19	28.74 ± 0.17	30.17 ± 0.23	29.02 ± 0.22^{D}
1	32.47 ± 0.28	34.63 ± 0.32	35.41± 0.41	34.17 ± 0.41^{C}
2	39.22 ± 0.47	40.15 ±0.51	44.65 ± 0.63	41.34 ± 0.52^{B}
3	50.11 ± 0.61	52.06 ± 0.63	57.13 ± 0.68	53.10 ± 0.65^{A}
Overall mean	37.49 ± 0.42^{b}	38.89 ± 0.46^{b}	41.84 ± 0.53^{a}	39.40

a-b within a rows, within ages, means with different superscripts letters differ significantly (P <0.05).

A-D within a column, within ages, means with different superscripts letters differ significantly (P <0.05).

G1: Group1 G2: Group 2 G3: Group3

3. Percentage of abnormal spermatozoa:

Data presented in (Table 5) showed that, the effect of ages on the percentage of abnormal camel spermatozoa was significantly (P<0.05). The highest (P<0.05) values of the percentage of abnormal spermatozoa was recorded with camels at <17-23 years (third group), while the lowest (P<0.05) value was recorded with camels at <5-11 years (first group) and <11-17years (second group).

The advancement of storage time at 5°C for up to 3 days showed significantly (P<0.05) increased the percentage of abnormal camel spermatozoa (Table5). 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 **Matter (2019)** in the camel spermatozoa.

Table (5): Mean percentages of abnormal camel spermatozoa with different ages, during storage **at 5 ^{o}C for 3 days.**

Storage time (day)	Age (year)			Mean
	G1 (< 5- 11)	G2 (< 11-17)	G3 (< 17-23)	
0	10.17± 0.08	11.76 ± 0.10	14.81 ± 0.16	12.34 ± 0.16^{D}
1	14.25 ± 0.15	14.80 ± 0.17	19.16± 0.18	16.07 ± 0.18^{C}
2	20.34 ± 0.18	19.14 ±0.19	25.38 ± 0.23	21.62 ± 0.23^{B}
3	31.53 ± 0.27	30.82 ± 0.26	36.45 ± 0.30	32.93 ± 0.29^{A}
Overall mean	19.07 ± 0.16^{b}	19.13 ± 0.17^{b}	23.95 ± 0.22^{a}	20.71

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

A-D Within a column, within ages, means with different superscripts letters differ significantly (P <0.05).

G1: Group1 G2: Group 2 G3: Group3

4. Percentage of acrosome damage of spermatozoa:

Data presented in (Table 6) showed that, the percentage of acrosome damage of spermatozoa as affected by ages was significantly (P<0.05). The highest (P<0.05) value of percentage of acrosome damage of spermatozoa was recorded with camels at <17-23 years (third group), while the lowest (P<0.05) value was recorded with camels at <5-11(first group) years and <11-17years (second group).

It is of interest to note that the advancement of storage time at 5°C for up to 3 days showed significantly (P<0.05) increased the percentage of acrosome damage of the camel spermatozoa in all ages (Table 6). Similar trend was reported by **Zeidan *et al.* (2008)** in the camel spermatozoa.

Table (6): Mean percentages of acrosome damage of the camel spermatozoa with different ages, during storage at 5 ^{o}C for up to 3 days.

Storage time (day)	Age (year)			Mean
	G1 (< 5- 11)	G2 (< 11-17)	G3 (< 17-23)	
0	6.14± 0.11	6.25 ± 0.13	7.26 ± 0.18	6.55 ± 0.14^{D}
1	7.18 ± 0.14	8.91 ± 0.19	8.63± 0.16	8.24 ± 0.15^{C}
2	8.23 ± 0.17	9.87 ±0.20	10.94 ± 0.24	9.68 ± 0.21^{B}
3	10.65 ± 0.21	10.82 ± 0.23	14.70 ± 0.28	12.05 ± 0.26^{A}
Overall mean	8.05 ± 0.16^{b}	8.96 ± 0.19^{b}	10.38 ± 0.26^{a}	9.13

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

A-D Within a column, within ages, means with different superscripts letters differ significantly (P <0.05).

G1: Group1 G2: Group 2 G3: Group3

From another point of view, **Lenz *et al.* (1977)** 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 recovered during winter and autumn seasons with different times of incubation at 37°C for up to 2 hours. 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 the 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)**.

5. Percentage of chromatin damage of spermatozoa:

The percentage of chromatin damage of spermatozoa in the dromedary camels was significantly (P<0.05) decreased with the progress of age (Table 7). The highest (P<0.05) value of the percentage of chromatin damage was recorded with the camels at<17-23 years old, while the lowest (P<0.05) value was recorded with the camel at <5-11 and 11-17 years of age. Similar trends were reported by **Matter (2019)** 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). Lioyd *et al*. (2012)** confirmed that sperm DNA integrity was better in commercial diluent could be significantly increased DNA fragmentation during storage at 5^{o}C for up to 48 hours.

The prolongation of storage time at 5^{o}C for three days was significantly (P<0.05) increased the percentage of chromatin damage of camel spermatozoa in all ages (Table7). Similar trends were recorded by **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).**

Table (7): Mean percentages of chromatin damage of the camel spermatozoa with differentages, during storage at 5 ^{o}C for up to 3 days.

Storage time (day)	Age (year)			Mean
	G1 (< 5- 11)	G2 (< 11-17)	G3 (< 17-23)	
0	3.16± 0.08	3.21 ± 0.09	4.18 ± 0.13	3.48 ± 0.12^{D}
1	4.12 ± 0.12	4.37 ± 0.14	5.63± 0.17	4.70 ± 0.14^{C}
2	5.24 ± 0.19	6.52 ±0.23	6.91 ± 0.27	6.22 ± 0.21^{B}
3	7.93 ± 0.30	8.49 ± 0.36	10.16 ± 0.41	8.86 ± 0.37^{A}
Overall mean	5.11 ± 0.18^{b}	5.64 ± 0.21^{b}	6.72 ± 0.26^{a}	5.82

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

A-D Within a column, within ages, means with different superscripts letters differ significantly (P <0.05).

G1: Group1 G2: Group 2 G3: Group3

6. Sperm penetration score:

Fig. (1) shows that, the penetrating ability of spermatozoa into she-camel cervical mucus showed significantly (P<0.05) better with the camel at <5-11 (first group) and <11-17(second group) years than <17-23 years (third group). The advancement of incubation time at 37 ^{o}C decreased significantly (P<0.05) the penetration score in all ages (first, second, and third groups). **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)** and **Matter (2019)** in the dromedary camels.

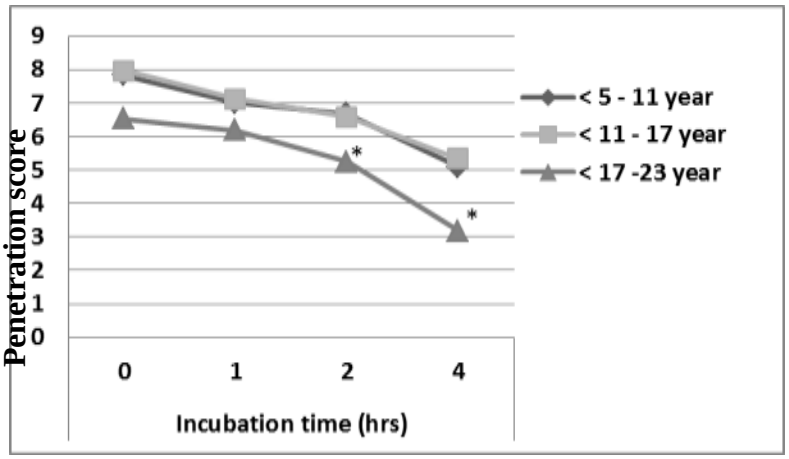

Fig. (1): Penetrating ability of the dromedary camel spermatozoa into she-camel cervical mucus with different age, during incubation at 37ºC for 4 hours.

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

In conclusion, copulation time,semen characteristics, sperm mensuration, histological changes in the testes and penetration score were superior in the first and second groups than the third group of the dromedary camels. Hence, it can be recommended to collect and storage in the dromedary camel spermatozoa at 5°C for artificial insemination (AI) programs, especially of the camel at 5 to 17 years old under Egyptian environmental conditions.

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تاثير العمر على نوعية السائل المنوى والتغيرات الهستولوجية فى خصية الجمال العربية

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الملخص العربى

أجريت هذه الدراسة على عدد 15 ذكر جمل فلاحى حيث قسمت الجمال طبقا لاعمارها الى ثلاث مجموعات المجموعة الاولى (G1) كانت اعمارها اكبر من 5 سنوات حتى 11 سنه والثانية(G2) اكبر من 11 وحتى 17 سنة والثالثة (G3) اكبر من 17 حتى 23 سنة على التوالى. تم جمع السائل المنوى بالمهبل الاصطناعى. وتم تقدير فترة الجماع وصفات السائل المنوى وابعاد الحيوانات المنوية والتغيرات الهستولوجية فى خصية الجمال (التجربة الاولى). ثم تم تخفيف السائل المنوى بمخفف اللاكتوز (LYC) وحفظت على درجة حرارة 5 م^0. كما تم اجراء اختبار مدى نفاذية الحيوانات المنوية داخل مخاط عنق الرحم اثناء التحضين على درجة حرارة 37 م^0 وذلك للاعمار المختلفة (التجربة الثانية).

وقد اظهرت النتائج ان هناك تحسن معنوى (على مستوى 0.05) فى فترة الجماع (بالدقائق) وحجم قذفة السائل المنوى (مل) فى المجموعة الاولى والثانية عن المجموعة الثالثة. كما كان لون السائل المنوى كريمى ابيض فى المجموعة الاولى والثانية و ابيض بلون اللبن فى المجموعة الثالثة و كان السائل المنوى لزجا فى المجموعة الاولى والثانية بينما كان شبه لزج فى المجموعة الثالثة. كانت قيمة الـpH فى السائل المنوى وابعاد الحيوانات المنوية (µm) غير معنوية فى كل المجموعات. زيادة النسبة المئوية لحركة الحيوانات المنوية (%) وتركيز الحيوانات المنوية (x 10^6/ مل) معنويا (على مستوى 0.05) بينما انخفضت النسبة المئوية للحيوانات المنوية الميتة والشاذة وشذوذ الاكروسوم والكروماتين فى الحيوانات المنوية للجمال فى المجموعة الاولى والثانية بدرجة معنوية (على مستوى 0.05) عن المجموعة الثالثة. وكان هناك زيادة فى عدد القنيات المنوية (ST) فى خصية ذكور الجمال فى المجموعة الاولى والثانية عن المجموعة الثالثة وكان بها مراحل مختلفة من مراحل النضج (امهات المنى والحيوانات المنوية الاولية والثانوية والحيوانات المنوية الناضجة) وكانت فى صورة نشطة (التجربة الاولى). زيادة النسبة المئوية لحركة الحيوانات المنوية ومقدرتها على الحفظ بدرجة معنوية (على مستوى 0.05) فى حين كان هناك انخفاضا معنويا(على مستوى 0.05) فى النسبة المئوية للحيوانات المنوية الميتة والشاذة وشذوذ الاكروسوم والكروماتين فى المجموعة الاولى والثانية عن المجموعة الثالثة وذلك اثناء الحفظ على درجة 5 م^0 (التجربة الثانية). انخفاض النسبة المئوية لحركة الحيوانات المنوية وكذا قدرتها على الحفظ بدرجة معنوية (على مستوى 0.05) مع زيادة النسبة المئوية للحيوانات الميتة والشاذة وشذوذ الاكروسوم والكروماتين مع تقدم فترة الحفظ فى كل الاعمار. زيادة معدل نفاذية الحيوانالت المنوية داخل مخاط عنق الرحم فى المجموعة الاولى والثانية عن الثالثة بدرجة معنوية (على مستوى 0.05). انخفاض معدل نفاذية الحيوانات المنوية داخل مخاط عنق الرحم بدرجة معنوية (على مستوى 0.05) مع تقدم فترة الحفظ على درجة حرارة التحضين (37 م^0) لمدة 4 ساعات وذلك فى كل الاعمار. الموجز: كانت فترة الجماع وصفات السائل المنوى وابعاد الحيوانات المنوية وكذا التغيرات الهستولوجية فى خصية الجمال ومعدل النفاذية داخل مخاط عنق الرحم افضل فى المجموعة الاولى والثانية عن الثالثة فى ذكور الجمال العربية.