Vol 10 No 2, p 183-190

PHYSIO-BIOCHEMICAL CHANGES IN THE MALE DROMEDARY CAMELS DURING BREEDING (RUTTING) AND NON-BREEDING SEASON

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ABSTRACT

The results showed that seminal pH value, total erythrocyte count (TEC), calcium and potassium concentrations were insignificantly higher during breeding (rutting) than non-breeding season. Semen colour was creamish white during breeding and watery white during non-breeding season. Testes weight (gm), testis tone firmer (score), testicular volume (cm^3), scrotal circumference (cm^3), percentage of sperm motility, sperm-cell concentration (x 10^6/ml), total leucocyte count (TLC), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), inorganic phosphorus and testosterone concentrations were significantly ($p<0.01$) higher during breeding than non-breeding season. However, dead spermatozoa, sperm abnormalities, acrosomal integrity percentages, haemoglobin, packed cell volume, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), acid phosphatase (ACP), sodium and oestradiol-17β concentrations were significantly ($p<0.01$) higher during non-breeding than breeding season. The histopathological status of the testes showed full complement of germinal elements, more developed and consisted of mature seminiferous tubules with mature spermatocytes during breeding than non-breeding season. Penetrating ability of the camels spermatozoa into she-camel cervical mucus was insignificantly better during breeding than non-breeding season.

Keywords : Breeding (rutting) and non-breeding season, male camel, physio-biochemical changes

Although the dromedary camel is a seasonal breeder but, the breeding season varies in the different climatic zones of the World (Wilson, 1984). In Egyptian and Sudanese camels, there are fluctuations in the sexual activity and number of spermatogonia, spermatids and spermatozoa due to seasonal variations and the rate of spermatozoa and sperm production is higher in spring and lower during autumn (Osman and El-Azab, 1974). In Indian camels, the normal breeding season is from November to March (Khanna *et al,* 1990) and from mid January to the end of May in the Turkman dromedary camels (Abdunazarov, 1970).

The breeding season in the male camels has many physiological and behavioural peculiarities. El-Wishy (1988) observed that Leydig cells are less active in the non-breeding season with a resulting reduction in steroidogenic activity by the testes. So, the testicular activity and blood components have been helpful in evaluating gonadal activity in the animals.

The aim of the present study was to investigate the effects of breeding and non-breeding season on the testicular activity, semen characteristics, blood haematology and its components of the male dromedary camels. Histopathological status of the camel testis was studied. Penetrating ability of the camels spermatozoa into she-camel cervical mucus, was also assessed.

Materials and Methods

Fourty eight male one-humped camels (*Camelus dromedarius*) of 8 to 12 years of age and of 600 to 700 kg body weight were chosen randomly before slaughter from Belbies City Abattoirs, Sharkia Province (30°N), during breeding (rutting) (n=26) and non-breeding (n=22) season.

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Before slaughter, testis tone firmer (score) testicular volume (cm^3) and scrotal circumference (cm) were recorded. Testis tone firmer was determined via manual palpation (scored from 1: very soft and 9: very firm) as described by Wildeus and Hammond (1993). Testicular volume (cm^3) was calculated by multiplying length x breadth x depth of the testis by ordinary caliper as described by Ismail (1979). The scrotal circumference was measured with a flexible, cloth measuring tape around the largest diameter of the testis and scrotum placed after pushing the testes firmly into the scrotum (Mickelsen *et al,* 1982).

Blood samples were also collected pre-slaughter from the jugular vein of each camel, during breeding and non-breeding season. Blood haemoglobin (g/dl), packed cell volume (%), total erythrocyte count (TEC) (x $10^6/mm^3$) and total leucocyte count (TLC) (x $10^6/mm^3$) were estimated immediately after blood collection. Blood samples were then centrifuged at 3000 r.p.m. for 15 minutes. Plasma samples were collected and transferred gently into glass vials and stored at -20°C until biochemical analysis. Glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase (ALP) and acid phosphatase (ACP) concentrations were determined colorimetrically using commercial kits purchased from Bio-Merieux (Marcy L'Eltoile, Charbonnieres, Les Bains, France). Lactic dehydrogenase (LDH) concentration was determined according to Anon (1970). Calcium (Ca), inorganic phosphorus (IP), sodium (Na) and Potassium (K) concentrations were determined as described by Tietz (1982). Testosterone and oestradiol -17β concentrations were estimated using Radioimmunoassay technique of coat-Ab-Count kits (Diagnostic Products Corporation, Los Angeles, USA) according to Abraham (1977) and Pratt (1978).

After slaughter, a total number of 82 clinically normal testes were collected during breeding (n=42) and non-breeding (n=40) season. Each epididymal corpus region was cut to allow the escape of its contents into buffer citrate solution (camel spermatozoa is stored in the body of epididymis) for the determination of the semen characteristics immediately after collection.

The testes were then weighed to the nearest gram by an ordinary balance and fixed in Bouins solution. Representative samples were washed, dehydrated in ascending grades of ethyl alcohol, cleared and embedded in paraffin wax. Thereafter, the samples were sectioned at 5 microns thickness and stained with Haematoxylin and Eosin, then examined using x 20 objective of a phase contrast microscope, to study the histopathological status of the testis. Semen colour was determined directly from the collecting tube. pH of the semen was measured by Universal indicator paper and standard commercial stains (Karras, 1952). Individual spermatozoal motility (%) detected as an oscillatory motion of the flagellum, but not progressive due to the viscous materials, was determined as a percentage of motile sperm in three different fields at 40 x magnification. Dead spermatozoa (%) and sperm abnormalities (%) were determined according to Campbell *et al* (1956). Acrosomal integrity (%) was determined by the dual stain procedure described by Didion *et al* (1989). Sperm cell concentration (x 10^6/ml) was determined by haemocytometer method (Khan, 1994).

Sperm penetration into she camel cervical mucus was tested as follows: cervical mucus was obtained from she camel. A portion of mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide columns of 6 cm length. The extended semen with lactose yolk citrate extender as described by Salisbury *et al* (1978) was placed into 2 ml cuvettes (1 ml in each). The tubes containing the mucus were inserted (open end) into the cuvettes containing the extended semen and incubated at 37°C for up to 4 hours. Sperm penetration was judged as the rank score as described by Eskin *et al* (1973) and Hanson *et al* (1982).

Minimum and maximum values of air temperatures (°C), relative humidity (%), temperature humidity index (THI) and length of day light (hours) during breeding and non-breeding season were as shown in table 1. The temperature humidity index (THI) was estimated according to Livestock and Poultry Heat Stress Indices suggested by Agricultural Engineering Technology Guide, Clemson University, Clemson,

SC 29634, USA (1990), using the following formulae: THI = db°F - (0.55 - 0.55 RH) (db°F-58.00) where db°F = dry bulb temperature in Fahrenheit and RH = relative humidity (RH% ÷ 100). The obtained values of THI were classified as follows: 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.

Data were statistically analysed by analysis of variance according to Snedecor and Cochran (1982). Percentage values were transformed to Arc-Sin values before being statistically analysed. Duncan's Multiple Range Test (Duncan, 1955) was used for the multiple comparisons.

Results

Temperature - humidity index (THI)

The temperature humidity index (THI) estimated in table 1 indicated exposure of the male dromedary camels to very severe heat stress during non-breeding season.

Testicular activity

Table 2 shows that testes weight, testicular volume, testis tone firmer and scrotal circumference of the male dromedary camels were significantly ($p<0.01$) higher during breeding than non-breeding season. Similar trends were reported by Wildeus and Hammond (1993) and El-Sherief (1997). Photoperiod seems to play a major role in regulating the seasonal activity (breeding season) of the camel testes which are regarded as short day breeders, in which change from long to short day seems to stimulate synthesis and release of gonadotropins hormones from the anterior pituitary gland, which in turn stimulate testicular activity and sexual behaviour (Lincoln *et al,* 1977).

Camel semen characteristics

Semen characteristics of the male dromedary camels during breeding and non-breeding season are shown in table 3.

Table 2. Testis weight, testicular volume, testis tone firmer and scrotal circumference of the male dromedary camels, during breeding (rutting) and non-breeding season.

Parameters	Seasons	
	Rutting	Non-breeding
Testis weight (gm)	123.82 ± 6.75[a]	105.26 ± 5.14[b]
Testicular volume (cm^3)	116.53 ± 4.64[a]	92.48 ± 4.72[b]
Testis tone firmer (score)	7.96 ± 0.38[a]	6.18 ± 0.46[b]
Scrotal circumference (cm^3)	19.76 ± 1.78[a]	16.85 ± 1.34[b]

Camels with different superscripts in the same row, differ significantly ($p<0.01$).

Table 3. Semen characteristics of the male dromedary camels, during breeding (rutting) and non-breeding season.

Semen characteristics	Seasons	
	Rutting	Non-breeding
Semen colour	Creamish white	Watery white
pH	8.04 ± 0.32	7.78 ± 0.32
Sperm motility (%)	68.12 ± 1.43[a]	54.25 ± 1.84[b]
Dead spermatozoa (%)	19.26 ± 1.85[b]	28.54 ± 1.26[a]
Sperm abnormalities (%)	18.42 ± 1.75[b]	25.38 ± 1.73[a]
Acrosomal integrity (%)	13.67 ± 1.68[b]	21.16 ± 1.45[a]
Sperm-cell concentration ($x 10^6$/ml)	418.52 ± 25.40[a]	305.25 ± 21.84[b]

Camels with different superscripts in the same row, differ significantly ($p<0.01$).

Semen colour was creamish white and watery white during rutting and non-breeding season, respectively. Similar trend was reported by Rai *et al* (1997). Hydrogen - ion concentration (pH) was insignificantly higher during rutting than non-breeding season, similar to that recorded by Abd El-Azim (1996). In addition, the

Table 1. Meteorological data, temperature - humidity index (THI) and length of day light, during breeding (rutting) and non-breeding seasons.

Seasons	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)		Length of day light (hrs.)
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Rutting	12.26 ± 0.16	21.28 ± 0.42	45.16 ± 0.65	60.48 ± 1.25	52.88 ± 1.37	67.63 ± 1.28	11.16 ± 0.25
Non-breeding	18.72 ± 0.34	32.65 ± 0.54	40.83 ± 0.38	58.12 ± 1.42	63.19 ± 1.84	83.22 ± 1.45	13.84 ± 0.28

alkalinity reaction of the camel semen increased during the sexual activity (rutting season) period than during the sexually rest period (Musa *et al*, 1992). The percentages of sperm motility of the dromedary camels was significantly ($p<0.01$) higher during rutting than non-breeding season, similar to that reported by Abdel-Raouf and Owaida (1974) and Abd El-Azim (1996). These results may be attributed to the increase of the mature Leydig cells and spermatogenesis process during the rutting season. Semen quality is expected to be improved during the rutting season, since the Leydig cells are mainly responsible for testosterone production (Charnot, 1994). However, the percentages of each of dead spermatozoa, sperm abnormalities and acrosomal integrity of the male dromedary camels were significantly ($p<0.01$) lower during non-breeding season which occurs throughout the summer which may cause disturbance in the spermatogenesis process due to degenerative changes with diminished number of mature spermatozoa or destruction or even death of spermatozoa (Abdel Raouf and Owaida, 1974 and Musa *et al*, 1992). Sperm cell concentration (x 10^6/ml) was significantly ($p<0.01$) higher during rutting than non-breeding season, similar to that reported by Rai *et al* (1997). Sinha and Prasad (1993) attributed the low sperm cell concentration of the camel semen during non- breeding season to the long day length, as well as heat stress which lead to reduction in the interstitial cells stimulating hormones and consequently, reduction in androgen production.

Blood haematology and biochemistry

Blood haematology and biochemistry of the male dromedary camels during breeding (rutting) and non-breeding season are shown in table 4.

Table 4. Blood haematology and its components of the male dromedary camels, during breeding (rutting) and non-breeding season.

Items	Seasons	
	Rutting	Non-breeding
Blood haematology		
Haemoglobin (g/dl)	12.38 ± 0.27[b]	14.75 ± 0.25[a]
Packed cell volume (%)	26.57 ± 0.98[b]	31.92 ± 0.82[a]
Total erythrocyte count ($x10^6/mm^3$)	8.12 ± 0.38	8.92 ± 0.63
Total leucocyte count ($x10^3/mm^3$)	14.86 ± 0.58[a]	9.48 ± 1.05[b]
Blood biochemistry		
Glutamic-oxaloacetic transaminase (U/L)	40.81 ± 3.48[b]	62.18 ± 3.16[a]
Glutamic-pyruvic transaminase (U/L)	46.25 ± 2.18[b]	50.86 ± 2.21[a]
Lactic dehydrogenase (IU/L)	360.42 ± 13.84[a]	212.62 ± 12.94[b]
Alkaline phosphatase (U/L)	53.28 ± 2.26[a]	48.92 ± 2.34[b]
Acid phosphatase (U/L)	16.22 ± 1.20[b]	20.28 ± 1.82[a]
Calcium (mg/dl)	10.37 ± 0.42	9.18 ± 0.24
Inorganic phsophorus (mg/dl)	5.26 ± 0.25[a]	3.15 ± 0.32[b]
Sodium (mg/dl)	192.28 ± 5.84[b]	207.25 ± 6.45[a]
Potassium (mg/dl)	12.42 ± 1.28	11.85 ± 1.21
Testosterone (ng/ml)	3.82 + 0.38[a]	1.64 ± 0.15[b]
Oestradiol - 17 b (pg/ml)	6.92 ± 1.13[b]	12.85 ± 1.28[a]

Camels with different superscripts in the same row, differ significantly ($p<0.01$).

Blood haemoglobin (g/dl) was significantly ($p<0.01$) higher during non-breeding (hot season) than breeding season. The increase of haemoglobin during non-breeding season may be due to that iron and copper essential for haemoglobin synthesis, since male camels during breeding season lost their appetite and body condition with diarrhoea (Schalm *et al*, 1975 and El-Mougy *et al*, 1984) and consequently, protein intake was low and haemoglobin decreased. However, packed cell volume (%) was significantly ($p<0.01$) lower during breeding than non-breeding season. The decrease of the packed cell volume during breeding season may be due to haemodilution (Mohamed, 1977). Total erythrocyte count (TEC) were

insignificantly lower during breeding (rutting) than non-breeding season which may be due to haemoconcentration during the hot weather (Kaneko, 1989). However, total leucocyte count (TLC) were significantly ($p<0.01$) higher during breeding (rutting) than non-breeding season, similar to that reported by Amin (1993). The increase of TLC during breeding season may be a response to the stress of the breeding season to help the body resistance against exhaustion (El-Mougy *et al*, 1984). Moreover, Ismail *et al* (1979) concluded that the highest TLC counts during winter (breeding season) may playa major role in raising the body resistance against infection during cold weather exposure as WBC's combating inflammatory and infection conditions.

Glutamic - oxaloacetic transaminase and glutamic - pyruvic transaminase concentrations were significantly ($p<0.01$) lower during breeding (rutting) than non-breeding season. Similarly, Abd El-Samee and Marai (1997) suggested that liver functions may be partially affected by heat stress during non-breeding season. Lactic dehydrogenase concentration was significantly ($p<0.01$) higher during rutting than non-breeding season, similar to that reported by Kataria *et al* (1994). This result may be due to zinc deficiency in the non-breeding season which acts as a prosthetic metal in certain enzymes such as lactic dehydrogenase as reported by Azouz and El-Tohamy (1989).

Alkaline phosphatase concentration was significantly ($p<0.01$) higher during breeding than non-breeding season. However, acid phosphatase concentration was significantly ($p<0.01$) lower during breeding than non-breeding season.

Calcium concentration was insignificantly higher, while inorganic phosphorus concentration was significantly ($p<0.01$) higher during breeding than non-breeding season. These results are in agreement with those findings reported by Dessouky (1994) and Abd El-Azim (1996). The high concentration of calcium and phosphorus during breeding season (winter and spring) may be due to the high calcium and phosphorus values of the green fodder (Ayoub *et al*, 1972).

Sodium concentration was significantly ($p<0.01$) higher during non-breeding than breeding season, similar to that recorded by Amin (1993). The increase of sodium concentration during non-breeding season (hot season) may indicate effectiveness of mobilisation of the intracellular fluids into extracellular space (Rathore, 1986). Moreover, the combined activity of aldosterone and antidiuretic hormones in maintaining water and salt homeostasis is the cause of water and salt retention during hot environment when compared to winter (breeding season) in the man or animals (Ingram and Mount, 1975). However, potassium concentration was insignificantly higher during breeding than non-breeding season. The lowest values of potassium concentration during non-breeding season may be attributed to increase of aldosterone secretion in the hot and dry climate which is enhanced by renin angiotensin system in response to changes in effective circulating fluid volume where aldosterone balances largely serum potassium (Kaneko, 1989).

Testosterone concentration was significantly ($p<0.01$) higher during breeding than non-breeding season. However, oestradiol - 17b concentration was significantly ($p<0.01$) lower during breeding (rutting) than non-breeding season, similar to that reported by Abd El-Azim (1996). These results may be due to the increase of androgen level, is parallel to the increase of sexual activity during breeding season. This reflects fact that the high androgen in the male camel's blood in the breeding season is the direct cause of the characteristics of its sexual behaviour. In addition, Bedrak *et al* (1983) observed that the relative activity of several enzymes associated with testosterone and its conversion to oestrogen in the blood plasma of the male dromedary camels was lower during non-breeding than breeding season.

Penetration of cervical mucus

Figure 1 shows that penetrating ability of the dromedary camels spermatozoa into she-camel cervical mucus was insignificantly better during breeding than non-breeding season. However, incubation times at 37°C for up to 4 hours significantly ($p<0.01$) decreased the penetration score, similar to that reported by Zeidan *et al* (1998) in Friesian bulls. In addition, Alexander

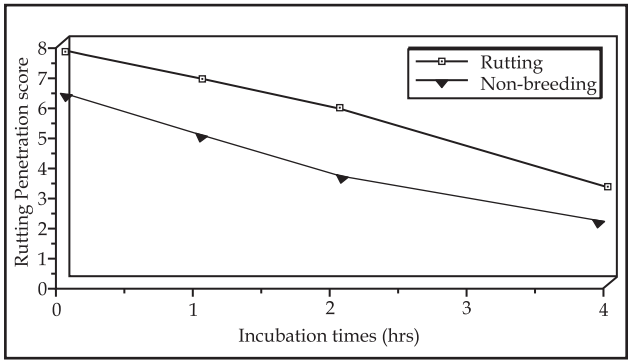

Fig 1. Penetration score value of the male dromedary camels spermatozoa into she-camel cervical mucus, during breeding (rutting) and non-breeding season.

(1981) and Murase *et al* (1990) reported that the duration of sperm motility and penetration distance in the mucus closely correlated to the pregnancy and conception rates.

Histopathological status of the testis

Histopathological status of the camel testis during breeding season showed seminiferous tubules were active in spermatogenesis and the Sertoli cells increased in size and their nuclei became large, elongated with distinct nucleoli and light eosinophilic cytoplasm. The Leydig cells increased also in size more than camel testis during non-breeding season. In addition, minimal incidence of the testicular degeneration occurred during breeding season and maximal degenerative changes were noted during non-breeding season. The finding of minimal incidence of testicular degeneration during breeding season of the camel testis, as well as, the increased frequency and activity of pituitary gonadotropin active spermatogenesis and higher testis weight and sperm production rate were observed (Osman and El-Azab, 1974).

In conclusion, the male dromedary camels showed better testicular activity, blood haematology and its components, semen quality and penetrating ability of spermatozoa into she-camel cervical mucus during breeding (short day light) than non-breeding season (long day light). So it is proposed that the environmental conditions plays a prominent clue in influencing the seasonal physiological and biochemical changes which, in turn, affect the sexual behaviour and reproductive functions of the camels. Therefore, it can be recommended to collection and freezing camel semen during breeding season for artificial insemination to enhance the fertilising ability of she-camel during non-breeding season, under Egyptian environmental conditions.

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