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Effect of age and season on the testicular sperm reserve and testosterone profile in camel (*camelus dromedarius*)

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Abstract

The present investigation aimed to determine the effects of age and seasons of the year on the testicular sperm reserve and serum testosterone concentration in dromedary camels. Testes were collected from a local slaughterhouse during a period of one year (breeding and non-breeding seasons) from two age groups (GI: 4-6 yr and GII: 8-10 yr). Testicular sperm reserve was determined during the breeding (December-May) and non-breeding (June-November) seasons. Blood samples were collected for determination of serum testosterone concentration (ng/ml). There was highly significant difference ($P < 0.01$) in the testicular sperm reserve between breeding (13.58×10^9) and non-breeding (9.90×10^9) seasons. The serum testosterone concentration (ng/ml) was significantly increased during the breeding season (10.94 ng/ml) compared with that of the non-breeding season (4.43 ng/ml). Testicular sperm reserve (14.34 in GII *vs*. 9.13 in GI) and serum testosterone concentration (10.43 *vs*. 4.94 ng/ml) were significantly ($P < 0.01$) increased by age. It could be concluded that the age of the animal and season of the year have significant effects on the testicular sperm reserve and serum testosterone concentration in dromedary camels.

Keywords: age, camel, season, testicular sperm reserve, testosterone.

Introduction

Camels play a vital socio-economic role and support millions of human beings in the dry and arid zones of Asia and Africa (El-Harairy and Attia, 2010). They are proven to be fit domestic animals during severe drought periods, not only surviving such droughts, but also producing and reproducing (Wardeh, 1989). Camels are considered seasonal breeders, however, information about the breeding season in the dromedary is rather conflicting. Thus, the breeding season has been reported from December to May in Egypt (Shalash, 1965). Among farm animals, reproduction in camels has received the least attention. Moreover, the effect of season on the reproductive performance of the camel seems intricate and the information given in the available literature is rather controversial. Osman and Polen (1986) found that spermatogenesis was continuous throughout the year in camel. The seasonality of reproduction is also evident in the form of an endocrine surge of reproductive steroids and gonadotropins (Azouz *et al*., 1992; Tibary and Annouassi, 1997). The higher testosterone level has been recorded during the rutting season (Yagil and Etzion, 1980; Agarwal and Khanna, 1990). There is a general agreement that semen characteristics are not the only criteria to evaluate the reproductive capacity of the male, and the study of testicular sperm reserve seems to be essential for a careful assessment of male fertility (Osman and El-Azab, 1974). The age is an important aspect in considering the potential fertility of a camel. Young and old dromedary bulls may have problems with tacking on a full breeding labor with consistent success rates (Al-Qarawi, 2005). Based on the testicular morphometry and rutting behavior, a young bull may be sexually active and used for service as early as 3 yr of age as reported in Saudi Arabia (Arthur *et al*., 1985), Egypt (El-Wishy and Omar, 1975), India (Matharu, 1966), and Kenya (Gombe and Odour-Okele, 1977). By 4.5-5 yr of age the males are capable of producing adequate number of spermatozoa to mate as many females as an adult bull but full fertilizing capacity is not attained until 6 yr of age on average (Al-Qarawi *et al*., 2000). Conversely, old age may be a problem due to an age related decline in fertility over 18 yr of age (Yagil, 1985).

The influence of age and season on the testicular sperm reserve and serum testosterone concentration is important to understand the pattern of reproduction in the camel. Therefore, the present investigation was carried out to: (i) study the effect of age on the testicular sperm reserve and serum testosterone concentration and (ii) clarify the effect of seasons of the year on serum testosterone and testicular sperm reserve in dromedary camel.

Materials and Methods

Sample collection and their assignment to groups

A total of 120 testes and epididymides of clinically healthy, one-humped camels were collected

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from El-Basateen (Cairo) abattoir during a period of 1 yr (breeding and non-breeding seasons). All samples used were grossly normal and free from pathological lesions. The testes were transferred to the laboratory of Theriogenology Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt in a thermos flask containing sterile physiological saline (0.9%) supplemented with 100 µg/ml streptomycin at 25°C according to Goto *et al*. (1989) within 3-4 h post-slaughter. The camels were allotted to two groups according to their age (GI: 4-6 yr, GII: 8-10 yr) according to the dentations formula given by Rabagliati (1924).

Determination of testicular sperm reserve

Testicular sperm reserve was estimated using the homogenization haemocytometric technique (Amann, 1970). Briefly, the left and right testes were trimmed and weighed. After careful removal of the tunica albuginea, each testis was homogenized at 6000 rpm for 2 min. After recording the homogenate volume, a sample of the homogenate was diluted 1:40 v/v using physiological saline containing antibiotics. The suspensions were then stored for 24 h at 5°C before counting in the haemocytometer. Mixing of the suspension was carried out at 6-12 h intervals during the period of storage. For counting the spermatozoa in the suspension, modified Neubauer heamocytometer was used, after shaking the homogenate for 1 min. Two counts were made for each suspension at a magnification of 400X using a binocular microscope. The number counted by the haemocytometer was then multiplied by the volume of the homogenate and the dilution rate to obtain the sperm reserve in right and left testes.

Blood sampling schedules

Jugular blood samples were collected from all animals after slaughter (GI and GII) during the breeding and non-breeding seasons. These samples were drawn into clean vials, left to clot at room temperature for 1 h and centrifuged at 3000 rpm for about 15 min. The serum was then harvested and stored at -20°C until used for hormonal analysis.

Radioimmunoassay

Serum samples were analyzed for testosterone by radioimmunoassay (RIA) as described by Wilson and Foster (1992). Six labeled testosterone antibody-coated tubes were used for the standard curve. Testosterone calibration solutions (50 µl of 0, 0.2, 1, 4, 8 and 16 ng/ml) were transferred to the six tubes from A to F, respectively. Testosterone (1 ml of I^{125}) was added to each standard or sample tube. All tubes were shacked well and incubated in water bath at 37°C for 3 h. All tubes were decanted, dried and subjected to gamma-counter (Berthold MAG 312, Germany) for 1 min. The concentration of testosterone (ng/ml) was determined according to the standard calibration curve. The sensitivity of the assay was 0.01ng/ml, the intra- and inter-assay coefficients of variation were 12.8 and 15.4%, respectively.

Statistical analysis

Data were analyzed using a computerized statistical analysis system (SAS, 2000) and expressed as mean ± SEM. Seasonal and age variations were determined by analysis of variance using a model that included the effect of season, age as well as the interaction between them. Student's t-test was used to differentiate between the right and left testes within the same age or the same season. All statistical analyses were carried out according to Snedecor and Cochran (1980).

Results

Testicular sperm reserve in dromedary camel increased ($P < 0.01$) in the breeding *versus* the non-breeding season for group II and group I (Table 1). There was difference ($P < 0.01$) in the testicular sperm reserve due to age, with increasing testicular sperm reserve with advanced age (Table 1).

Table 1. Effect of age and season on the testicular sperm reserve ($x10^9$) of dromedary camel (Means ± SEM).

Season	Group I (4-6 yr)	Group II (8-10 yr)	Overall means
Breeding season	10.89 ± 1.83^{Ba}	16.24 ± 3.12^{Aa}	13.58 ± 2.84^{a}
Non-breeding season	7.36 ± 1.42^{Bb}	12.44 ± 2.86^{Ab}	9.90 ± 1.96^{b}
Overall means	9.13 ± 1.46^{B}	14.34 ± 2.18^{A}	

^{A,B}Different superscripts within a row differ ($P < 0.01$).
^{a,b}Different superscripts within a column differ ($P < 0.01$).
N = 30 for each season and 15 for each age group.

The testicular sperm reserve of dromedary camel did not differ between the right and left testes within the same age and season (Table 2). Serum testosterone concentration (ng/ml) differed ($P < 0.01$) between the breeding and non-breeding season within the same age group for GI and GII, (Table 3). Moreover, the age had an effect ($P < 0.01$) on the serum testosterone concentration during the breeding season and non-breeding season for GI and GII (Table 3).

Table 2. Testicular sperm reserve ($x10^9$) of camel in the right and left testes in the breeding and non-breeding season in the two age groups (Means ± SEM).

Season	Testis	Group I (4-6 yr)	Group II (8-10 yr)
Breeding season	Right	5.65 ± 0.83^{a}	8.60 ± 1.02^{a}
	Left	5.08 ± 0.70^{a}	8.41 ± 1.05^{a}
Non-breeding season	Right	4.52 ± 0.66^{b}	6.32 ± 0.86^{b}
	Left	4.15 ± 0.82^{b}	6.40 ± 1.04^{b}

^{a,b}Different superscripts within a column differ ($P < 0.01$).
N = 30 for each season and age group, 15 for left and right testes.

Table 3. Effect of age and season on the serum testosterone concentration (ng/ml) of dromedary camel (Means ± SEM).

Season	Group I (4-6 yr)	Group II (8-10 yr)	Overall means
Breeding season	7.88 ± 1.72^{Ba}	14.52 ± 2.12^{Aa}	11.22 ± 2.44^{a}
Non-breeding season	2.62 ± 0.94^{Bb}	6.46 ± 1.54^{Ab}	4.54 ± 1.22^{b}
Overall means	5.25 ± 1.22^{B}	10.48 ± 2.20^{A}	

^{A,B}Different superscripts within a row differ ($P < 0.01$).
^{a,b}Different superscripts within a column differ ($P < 0.01$).
N = 30 for each season and 15 for each age group.

Discussion

The testicular sperm reserve is an indicative value for the spermatogenic activity of the testis. There is general agreement that semen characteristics are not the only criteria to evaluate the reproductive capacity of the male. Therefore, the testicular sperm reserve is essential for a careful assessment of male fertility.

In the present study, the testicular sperm reserve (x 10^9) significantly differed between the breeding and non-breeding seasons with the highest value occurring during the breeding season within the same age group. This is in agreement with Osman and El-Azab (1974), Ismail (1979) and El-Wishy and Omar (1975) who found that the testicular sperm reserve of dromedary camel differed significantly by seasons. Average values of 9.89 and 15.06 x 10^9 were recorded in the present study for testicular sperm reserve in GI and GII, respectively during the breeding season; however, lower values were recorded (3.1 x 10^9) by El-Wishy and Omar (1975) and Ismail (1979) in camel (7.7 x 10^9), which may be attributed to the age of the animals.

The present study recorded that the age exerts significant effect on the testicular sperm reserve with higher values for the adult camel (8-10 yr) within both seasons. These results are in agreement with Osman and El-Azab (1974) and Ismail (1979) who found that the testicular sperm reserve in dromedary camel was significantly increased by the age.

The significant increase in the testicular sperm reserve during the breeding season could be due to the increase in serum testosterone concentration which is responsible for spermatogenesis (Goeritz *et al*., 2003). Furthermore, androgen deprivation led to an immediate arrest in the meiotic transformation of primary spermatocytes to spermatids resulting in an effective block in sperm production (Suresh *et al*., 1995). In addition, testosterone influences the synthesis of a number of caput and cauda epididymal proteins, and some of these proteins could be important to improve spermatozoa maturation and storage (De Pauw *et al*., 2003).

In the present investigation there was not a significant difference in the testicular sperm reserve between right and left testes of dromedary camel. El-Wishy and Omar (1975) have reported similar results.

Testosterone in males is a prerequisite for normal spermatogenesis (McLachlau *et al*., 1996; Goeritz *et al.*, 2003) and normal function of the reproductive tract (Luke and Coffey, 1994). In addition, testosterone influences the synthesis of a number of caput and cauda epididymal proteins. Some of these proteins are important for improvement of spermatozoa maturation, storage and their acquisition of fertilizing ability (De Pauw *et al*., 2003). Moreover, the level of cell apoptosis was inversely related to both the proliferation and the testosterone concentration in the testis. Thus, testosterone is important as a product of the testis as well as a regulator of activities in the testis (Goeritz *et al*., 2003).

The present data indicate that serum testosterone concentration is significantly increased in the breeding compared to the non-breeding season. Similar results were reported by Yagil and Etzion (1979), Nasr and El-Azab (1990), Abdel-Raouf (1993), El-Sherif (1997), and El-Harairy and Attia (2010) in camel. The significant increase in the serum testosterone concentration of dromedary camel during the breeding season may be attributed to the increase in the volume and number of Leydig cells during the rutting period (Hussein, 1980; Johnson and Thompson,

1987). Moreover, increase in the activity of enzymes that synthesize testosterone was higher in the breeding than in the non-breeding season (Bedrak *et al*., 1983; Shan *et al*., 1993). However, Secchairi *et al*. (1976) found that there was no evidence of seasonal effect on serum testosterone concentration in dromedary camel. The present study revealed that the serum testosterone concentration was significantly increased by age in camel. El-Harairy and Attia (2010) and Nasr and El-Azab (1990) recorded similar results in camel; they found a significant increase in testosterone concentration in mature ages compared with those in young ages. Also, in bulls Matsuzaki *et al*. (2000) reported that serum testosterone concentration was low in young animals and then increased by age. The same pattern of profile in testosterone concentration with age in breeding and non-breeding seasons was recorded in the present study. Allali *et al.* (2005) stated that seasonality in camel is controlled by fluctuation in the melatonin hormone which is produced by the pineal gland during the night. Hence, depending on the photoperiod, the concentration of melatonin in the body increases or decreases and regulates the production of GnRH, which controls the testosterone level through LH pulses which increase before the breeding season, reach a peak during that period and drop afterwards. Testosterone is needed for testicular recrudescence and spermatogenesis, thus the hormonal cycle including GnRH and testosterone synchronizes the sexual activity of males.

In the view of the current study, it could be concluded that the testicular sperm reserve and serum testosterone concentration were increased in the breeding compared to the non-breeding season. In addition, age has been found to have a significant effect on the testicular sperm reserve as well as serum testosterone concentration in dromedary camel.

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