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ORIGINAL ARTICLE

Effect of age, pubertal stage and season on testosterone concentration in male dromedary camel

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KEYWORDS

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Abstract The present study was conducted in the Laboratory of Animal Physiology and Biotechnology, Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt. The present investigation aimed at studying effects of ages, pubertal stages and seasons of the year on testosterone concentrations in blood plasma and tissue homogenate of the testes. The testes used in the current study were collected from a total of 104 one-humped male camels (*Camelus dromedarius*). Samples were taken from pre (1–3.5 years) and post (3.5–13 years) pubertal camels. Testes were studied for a two consecutive seasons. The freshly prepared homogenate of the testicular tissue and blood plasma were used for determining the concentrations of testosterone in plasma and testicular extract. The concentrations of testosterone in blood plasma and testicular tissue were significantly increased during the breeding season compared with that of non-breeding season; the concentration of testosterone was higher in testicular tissue than in blood plasma.

Testosterone concentrations in plasma and testicular tissue were increased in breeding than in non-breeding season. In addition, the testosterone concentrations were closely related with seasonal changes, stage of puberty and advancing age.

1. Introduction

Camel plays vital socio-economic roles and support millions of human beings in the dry and arid zones of Asia and Africa. Camels proved as the most fit domestic animals during sever drought periods, not only surviving such droughts, but also producing and reproducing (Wardeh, 1989).

Testes are responsible for the production of spermatozoa and secretion of androgens. The spermatozoon is the result of a complex process of cellular differentiation. During this process, morphofunctional modifications occur based on biochemical and cytochemical changes (Baccetti, 1972; Fawcett, 1975).

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).

Therefore, the present investigation was carried out to study the effects of different ages, pubertal stages and seasons

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of the year on testosterone concentrations in blood plasma and tissue homogenate of the testes.

2. Materials and methods

The present study was conducted in the Laboratory of Animal Physiology and Biotechnology, Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt. The testes used in the current study were collected from a total of 104 one-humped male camels (*Camelus dromedarius*). Tissue samples were taken from pre (1–3.5 years) and post (3.5–13 years) pubertal camels. The materials were collected from Bilbase slaughterhouse during breeding season (December–April) and non-breeding season (June–August). The age determination was based on the description given by Wahby (1938). Within 3 h after slaughter, testes were transported to the laboratory in cool box.

2.1. Testosterone assay

Jugular blood samples were collected in EDTA-containing vials after slaughtering and centrifuged at 5000 rpm for 20 min. Plasma was separated and stored at −20 °C. Samples of each testis were diced in acetone (1:4, w/v), completely homogenized for about 3–4 min using a stirrer. The homogenized samples of testes were combined and left at 4 °C for 12 h. The precipitate was separated by centrifugation at 5000 rpm for 10 min and washed three times with acetone ether mixture (1:1, v/v). Normal saline was added to the precipitate to bring the solution back to its original volume (2 g of testicular tissue was added to 4 ml saline). Testicular extract was stored at −20 °C for subsequent hormonal assay.

The concentrations of testosterone in plasma and testicular extract were measured without column separation from dihydroxytestosterone (DHT) using the method described by El-Belely et al. (1995). The cross-reactivity of the antibody was 100% testosterone, 58.6% 5a-DHT, 54.3% 5b-DHT, 15.6% androstenedione, 7.8% corticosterone and <1% oestriol and oestradiol-17a. The sensitivity of the assay was 8 pg/ml. The average within and between assay coefficients of variation in eight replicates were 15.8% and 14.2%, respectively. Testosterone concentration in plasma samples was determined by ELISA (testosterone EIA te ELISA st kit cod 1115 Taiwan).

2.2. Statistical analysis

The obtained data were analyzed statistically using SAS (1996) procedures of personal computer. The least significant difference among means was carried out according to Duncan (1955).

3. Results

The present data of testosterone concentration in blood plasma and testicular tissue (Table 1) indicate that the testosterone in plasma and testicular tissue significantly increased in breeding than in non-breeding season. Also, testosterone concentrations in plasma and tissue of testes significantly increased in post-pubertal than in pre-pubertal camels (Table 1).

Generally, the concentration of testosterone is always higher in testicular tissue than that in plasma. There is an interaction between season and pubertal stage (Table 1).

In breeding season, the plasma testosterone concentration significantly increased to reach its maximum at 10 years of age (6.3 ng/ml), then decreased sharply at 13 years of age (0.80 ng/ml) (Table 2). Testicular tissue testosterone concentration significantly increased with progressing age. The maximum concentration was observed at 4.5 and 9 years of age (404 and 410 ng/g, respectively) and the minimum value was obtained at 13 years of age (366 ng/g) (Table 2). The data reveal that testosterone concentrations in plasma and testicular tissue are higher in all ages in breeding than in non-breeding

Table 1 Effect of season, puberty stage and their interaction on testosterone concentration of plasma and testicular tissues in male camel (mean ± SE).

Item	Season		Stage		Interaction			
	Non-breeding season (*A*)	Breeding season (*B*)	Pre-pubertal (*C*)	Post-pubertal (*D*)	*A* × *C*	*A* × *D*	*B* × *C*	*B* × *D*
Plasma (ng/ml)	1.11 ± 0.25^{b}	2.74 ± 0.48^{a}	0.31 ± 0.05^{b}	2.92 ± 0.37^{a}	0.20 ± 0.04	1.71 ± 0.33	0.42 ± 0.10	4.13 ± 0.49
Testicular tissue (ng/g)	273.09 ± 17.74^{b}	385.33 ± 4.26^{a}	302.0 ± 25.49^{b}	344.04 ± 11.31^{a}	238.20 ± 38.29	296.35 ± 13.21	374.67 ± 5.28	391.73 ± 5.51

^{a,b} Means in the same row having different letters, differ significantly ($P \leqslant 0.01$).

Table 2 Effect of age on testosterone concentrations of plasma and testicular tissue in male camel during breeding season (mean ± SE).

Item	Age (year)							
	2	3	3.5	4.5	8	9	10	13
Plasma (ng/ml)	0.12 ± 0.01^{e}	0.73 ± 0.12^{d}	0.42 ± 0.01^{de}	3.90 ± 0.06^{c}	5.0 ± 0.17^{b}	4.67 ± 0.20^{b}	6.30 ± 0.12^{a}	0.80 ± 0.12^{d}
Testicular tissue (ng/g)	382.33 ± 1.45^{bc}	386.0 ± 7.21^{b}	355.67 ± 2.60^{d}	404.67 ± 2.91^{a}	370.0 ± 8.66^{cd}	410.0 ± 1.15^{a}	408.0 ± 1.15^{a}	366.0 ± 5.20^{d}

$^{a–e}$ Means in the same row having different letters, differ significantly ($P \leqslant 0.01$).

Table 3 Effect of age on testosterone concentrations of plasma and testicular tissue in male camel during non-breeding season (mean ± SE).

Item	Age (year)							
	1.5	2	3	4.5	5	5.5	6	9
Plasma (ng/ml)	0.15 ± 0.09	0.23 ± 0.02	0.23 ± 0.02	1.71 ± 1.30	1.57 ± 1.16	1.73 ± 0.27	2.13 ± 0.74	1.4 ± 0.12
Testicular tissue (ng/g)	224.5 ± 60.27^{b}	127.33 ± 1.76^{c}	367.33 ± 9.33^{a}	290.1 ± 4.95^{ab}	244.67 ± 1.45^{b}	273.67 ± 13.91^{ab}	302.76 ± 38.67^{ab}	370.67 ± 1.76^{a}

$^{a–c}$ Means in the same row having different letters, differ significantly ($P \leqslant 0.01$).

season (Tables 2 and 3) and almost higher in testicular tissue than in plasma regardless of breeding season.

In non-breeding season, testosterone concentration in plasma insignificantly increased with advanced age reaching its maximum at 6 years of age (2.13 ng/ml), thereafter decreased to 1.4 ng/ml at 9 years of age. In testicular tissue, testosterone concentration significantly increased to reach its maximum at 9 years of age (370 ng/g) (Table 3).

Testosterone concentration in testicular tissue significantly ($P < 0.05$) increased with advanced age, reaching its maximum concentration 410.0 ± 1.15 and 370.67 ± 1.76 ng/g at 9 years during breeding and non-breeding season, respectively, reflecting non-significant seasonal effect on testosterone concentration in testicular tissue (Tables 2 and 3).

Testosterone concentration in blood plasma also showed marked increase with advancing age to be the highest (2.13 ± 0.74 ng/g) at 6 years of age during non-breeding season. However, the maximal concentration of testosterone is at older ages during breeding season (Table 2 and 3). It is of interest to note that plasma testosterone concentrations at all ages are almost higher during breeding than during non-breeding season (Tables 2 and 3).

4. Discussion

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). 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). Several studies have shown that testosterone also influence the size and function of epididymis (Goeritz et al., 2003) with a consequence on maturation and survival of spermatozoa during epididymal transit (Robaire and Viger, 1995; Hinton et al., 1996). In addition, testosterone hormone influences the synthesis of a number of caput and cauda epididymal proteins. Some of these proteins could be important for improving spermatozoa maturation, storage and their acquisition of fertilizing ability (De Pauw et al., 2003). Testosterone also plays an essential role in preventing apoptotic cell death in androgen-depended tissues (Thompson, 1994; Sinha Hikim et al., 1989). The level of apoptosis was inversely related to both the proliferation and the testosterone concentration in 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 testosterone in plasma and testicular tissue is highly significantly increased in breeding than in non-breeding season. This is in an agreement with that of Yagil and Etzion (1979) and Nasr and El-Azab (1990) in camel. In addition, Berndston et al. (1983) and Johnson and Thompson (1987) reported that the concentration of testosterone in testicular tissue was increased during breeding season than in non-breeding season. This could be a result of increasing volume of the interstitial tissue during mating season. During the non-mating season, testosterone synthesis is probably impaired only at the final stage of differentiation of the Leydig cell in camel (Friedländer et al., 1984). Johnson and Thompson (1987) reported that leydig cell number/testis was significantly greater in the breeding than in non-breeding season which led to higher concentration of testosterone in breeding season than in non-breeding season. Moreover, increase in the activity of enzymes that synthesize testosterone such as (4-ene-17 alpha-hydroxylase, 4-ene-lyase, and 17 betahydroxysteroid oxidoreductase) was higher in breeding than in non-breeding season (Bedrak et al., 1983; Shan et al., 1993).

The present results show that the testosterone concentrations in plasma and tissue of testes are highly significantly increased in post-pubertal than pre-pubertal camels, and this is in an agreement with Nasr and El-Azab (1990) in camel, who found highly significantly increase in testosterone in the mature ages when compared with those of either young or advanced ages and Matsuzaki et al. (2000) in bull, where serum level of testosterone was low in young animal and then increased with advancing age. From 12 to 48 months of age, serum testosterone concentration increased from 2.0 to 5.8 ng/ml. Also, the data reveal that the concentration of testosterone is higher in testicular tissue than in plasma. Similar findings were reported in camel by Al-Qarawi et al. (2001) who showed that the level of intratesticular testosterone in camels was about 25–30 times greater than the level in peripheral blood and exceeded the intratesticular level reported in the bovine bull (Amann and Ganjam, 1976). The present study shows the same pattern of profile in testosterone concentrations with age in non-breeding and breeding seasons.

The present results agree with that obtained by Al-Qarawi et al. (2000) in camel. Also, Al-Qarawi et al. (2004) reported that testicular testosterone concentration was 168.8 ng/g testis in camel aged 7 years. Such increase was mainly associated with marked increase in size and numbers of Sertoli cells in camels aged between 3 and 4.5 years of age. Furthermore, the onset of puberty coincides with a dramatic increase in the average leydig cell size, which accompanied by a peak in the steroid-producing capacity per leydig cell (Lunstra et al., 1986). In this respect, the profile of testosterone concentration was positively and significantly correlated with the total volume, total number of leydig cells and leydig cell SER content (Fouquet et al., 1984).

5. Conclusion

The testosterone concentrations in plasma and testicular tissue were highly significantly increased in breeding than in non-breeding season. This may be due to the increasing volume of the interstitial tissue which has increasing leydig cells in size, number and activity during breeding season.

From the present results, it is worthy to conclude that the testosterone concentrations in plasma and testicular tissue were increased in breeding than non-breeding season. In addition, the testosterone concentrations were closely related with seasonal changes, stage of puberty and with advancing age.

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