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17β - Estradiol and testosterone level in post – racing plasma of mature female and male racing camels.

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Abstract: The true ovarian hormone in females, 17β-estradiol, and the principle androgen testosterone in males, were measured in plasma of 45 mature female and 36 male racing camels respectively. Plasma samples were subjected to liquid extraction, and the levels of 17β- estradiol and testosterone were measured by ELISA (Enzyme Linked Immunosorbent Assay). The data obtained showed normal distribution for both 17β-estradiol and testosterone. The mean ± SD of 17β - estradiol was 77.65 ± 20.02 pg/ml and for testosterone, the values were 118 ± 95 pg/ml.

Key words: 17β - estradiol, testosterone, plasma, racing, camel

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Introduction

Determination of the blood circulating levels of different reproductive hormones has allowed good chances in the characterization of reproductive phenomena and diagnosis of physiological status and pathological conditions in animals. Accuracy of hormonal assay has increased with the development of new sensitive techniques (e.g. ELISA). The main reproductive hormones 17β-estradiol and testosterone have been recently characterized, and very sensitive assay were set up for this measurement that could be very helpful in the study of reproductive process and diagnosis of pathological cases. (Combarnous and Anouassi, 1994; Comin et al., 1994) 17β - estradiol is considered the true ovarian hormone in females. It is the most potent oestrogen, inducing estrone and striol. 17β- estradiol is responsible for the reproductive epithelia, breast growth and maturation of long bones and development of secondary female sex characteristics. The 17β - estradiol level in plasma is used in assaying for fertility, amenorrhoea and premature development of somatic puberty changes in young females. Testosterone is the main natural androgen secreted by the interstitial cells of the testes under influence of the lutenizing hormone (LH). It is responsible for the pubertal growth, development and maintenance of the male secondary characteristics, sex organs and for normal spermatogenesis. It is also exerts growth promoting effect and important protein anabolic response to exercise. These later effects have been utilized as doping agent. Therefore due to the endogenous presence of testosterone in camel's plasma, the detection of the equivalent exogenous administration of the cross-reactive synthetic anabolic hormones with testosterone requires the determination of the threshold value. Testosterone is also secreted by the adrenal cortex of both sexes and from the ovaries in the female. The plasma testosterone levels are useful in the investigating hypogonadism and hormone replacement therapy in males. It is also useful as a marker in hyper androgenism in females.

Materials and Methods

The samples for this study were collected during the final race in March 2001 with daily maximum and minimum temperature of 25 and 8 °C, respectively. The daily relative humidity varied between 20 and 60%. 17β-estradiol and testosterone test kits for the quantitative analysis in biological fluid were commercially available

from Neogen Corporation of Lexington, KY, USA. Each kit contained EIA buffer, wash buffer, extraction buffer and K – blue substrate, in addition to the specific enzyme conjugate, standard and antibody coated plate of 96 wells for each hormone.

EXTUBE, a family of disposable extraction columns designed for rapid sample preparation by a highly efficient liquid liquid extraction resins were obtained from Varian, USA. Brand new glass tubes (10 x 75 min) were used. Diethyl ether was of analytical grade. Water was purified by reverse osmosis and filtered over an Elgasta UHQUII water purification system (Jencons, England) before use. Reference 17β-estradiol and testosterone were provided with the kits. Kits were kept at 4 °C until uses.

Sample preparation

The samples were extracted by liquid liquid extraction in Chem Elute columns (EXTUBE part No. 1219 – 8002 with 1 ml. maximum sample capacity and 4 ml. elution solvent). One millilitre of camel plasma was added to the dry column. The plasma samples were allowed to percolate for 5 min to be adsorbed and distributed into a thin film over the hydrophilic packing material. The steroids were eluted by 2×4ml. diethylether into glass test tube under gravity. The extracts were then combined and were evaporated under a stream of nitrogen under reduce pressure (Zymark) at 40 °C. The residue was reconstituted into 1 ml. of extraction buffer diluted 5 fold with deionised water. The dissolved residue was then vortex and 50 µl. was directly used for ELISA as described by the manufacturer. Each sample was analysed in duplicate.

Results

Accuracy and precision of the ELISA – intra-assay coefficient of variation (CV%) was assayed for pooled plasma of mature female and male racing camel for 17β-estradiol and testosterone respectively. The samples were treated as mentioned in the sample preparation, then pipetted in duplicate (n = 4) onto a single specified plate for each hormone. The mean in concentration was 73±15.5pg/ml (CV = 21%) for 17β-estradiol and 163±41pg/ml (CV = 25%) for testosterone. Inter-assay coefficient of variation and recovery for 17β–estradiol were determined in a young male camel (less than tow years) plasma spiked at 6 different concentrations (0.02, 0.04, 0.1, 0.4, 1and 2 ng/ml). Each concentration was extracted four times and analysed in duplicate. The calibration curve (0.02 – 2 ng/ml) was prepared in EIA buffer to determine the recovery of added 17β–estradiol. The mean values obtained from different assay are summarised in Table 1.

Table 1. Inter- assay coefficient of variation, recovery and linearity for 17β- estradiol

Concentrations ng/ml	Inter-assay coefficient of variation (%)	Recovery (%)	Linearity R-SQR
0.02	8 (n =4)	125	0.984
0.04	2.38 (n =4)	105	
0.1	2.94 (n =4)	102	
0.4	16.05 (n =4)	88.75	
1	6.35 (n =4)	121	
2	5.368 (n = 4)	93.95	

Table 2. Inter- assay Coefficient of Variation, recovery and linearity for testosterone

Concentrations ng/ml	Inter-assay coefficient of variation (%)	Recovery (%)	Linearity R-SQR
0.004	29 (n =4)	97	0.980
0.008	18 (n =4)	106.6	
0.02	13 (n =4)	98.6	
0.04	10 (n =4)	110	
0.08	5 (n =4)	99	
0.2	21 (n = 4)	81	

The validated analytical method was used to determine the 17β-estradiol levels in the plasma of post racing of 45 mature female camels. The data showed that the level of 17β-estradiol in the post racing plasma of mature female camels was normally distributed with a mean ± SD of 77.65±20.02pg/ml. For testosterone, the inter-assay coefficient of variation and recovery were assayed in a young female camels plasma (one year old) spiked with testosterone at 6 different levels of concentration (0.004, 0.008, 0.02 0.04, 0.08 and 0.2 ng/ml). Each concentration was extracted four time's (n=4) and analysed in duplicate on to a single plate. The calibration curves (0.004 – 0.2 ng/ml) were prepared in EIA buffer to determine the recovery of added testosterone. The mean values obtained from different assays are summarised in Table 2. The validated analytical method was used to determine the natural values of testosterone from 36 post-racing mature male camel plasma. The data were normally distributed. Therefore 118 ± 95 pg/ml were the proposed which safely cover the normal levels of testosterone in post racing plasma of mature male camels.

Discussion

The present study shows that the determination of 17β-estradiol and testosterone in the plasma of mature female and male racing camels respectively is rapid, accurate and with acceptable precision. The proposed values which safely cover the normal levels of 17β-estradiol (77.65±20.02pg/ml.) in post racing plasma of mature female camels in this study, were similar to those observed during oestrus 74.7 ± 6.6 pg/ml (Elias and Yagil, 1984). In United Arab Emirate, Tibary and Anouassi, (1997) observed that well – fed and watered dromedary females show ovarian activity through out the year and the determinant factors for observed seasonality in conception date (November to April) are due to decrease in male lipodo in summer months. During the period of increase activity, the oestrogen plasma level is closely followed the ovarian follicular growth and activity. (Agarwal and Khann, 1990; Cristofori and Quarantal, 1990; Cristofori et al., 1979; 1986 Elisa and Yagil, 1984; Homeida et al., 1988; Zaghloul and Shahta 1991) The oestrogen level increase from 20 pg/ml when no follicle is palpable to more than 80 pg/ml during oestrus. (Homeida et al., 1988).

The high levels of 17 β-estradiol (77.65 ± 20.02 pg/ml) reported in this study could be explained by the fact that, in the absence of mating, the concentration of 17β- estradiol remains high during the entire breeding season (Elias and Yagil, 1984). This phenomena is based on the fact that, the dromedary does not have a luteal phase and its ovaries are consistently producing active follicles (Joshi et al., 1978). In non-breeding season the levels of 17β -estradiol remain low or show irregular slight increase due to incomplete follicular waves. (Elias and Yagil 1984). The post racing plasma testosterone concentration in mature male camels. (Older than 3 years) reported in this study was 118 ± 95 pg/ml. Tibary and Anouassi (1997) reported that, testosterone level in non-rutting animals were similar to those observed in prepubertal males (adults 810 ± 780 pg/ml; prepubertal 990 ± 170 pg/ml) while that of mature studs during breeding season were 1960 ± 180 pg/ml. Age at puberty is generally difficult to determine with precision because of the wide variety of definitions given to the term and the progressive nature of this event. Although display of general behaviour in male dromedary has been reported as early as 2 years of age (Lees, 1927; Sharma and Vays, 1981) field observation suggest that puberty and fertility ability are reach until 3 to 5 years (Khan, 1971; Khan and Kohli 1973; Khanna et al., 1987 Sharma and Vays, 1981). Other author reported that the male dromedary is not fully function as stud until the age of 6 years (Gombe and Oduor-Okelo, 1977; Matharu, 1966). The variability of onset of puberty could be due to different type of camels and their management especially the nutrition.

Seasonal changes in the plasma testosterone concentration have also been reported, higher testosterone in the breeding season may be due to increase syntheses and release of testosterone either by an increase sensitivity of leydig cells to LH and or an enhance secretion of LH from the pituitary gland. (Agarwal et al., 1990; 1991b; Azouz et al., 1992). Difference in endocrionological response to the breeding seasons amongst breed of dromedaries was reported from different geographical locations. (Dixit et al., 1970).

The samples for this study were collected during the racing season (November to April). Although the said period is coincides with low temperature, low humidity and increase rain fall which are favourable condition for rutting (Bono et al., 1989; 1990; Gombe and Oduor-Okelo, 1977; Yagil and Etzion 1980), the testosterone level reported in this study was low compared to what was reported else where. Dixit et al., (1970) reported that testosterone pattern in rutting animal is at inverse relationship to that of cortisol. It was proposed that physical exhaustion due to excitation and mating stress is a causative factor of increase cortisol level after mating (Agarwal et al., 1991a). The low levels of testosterone observed in this study might have resulted from the physical exhaustion of racing secondary to high cortisol levels due to racing stress.

Based on the values obtained in this study and due to the high variation of this data we recommend that much more sample to be analysed so as to narrow down the scatter of the data and thus be able to suggest realistic threshold values for testosterone.

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