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RESEARCH ARTICLE

Hormonal Profiles in the Serum and Follicular Fluid of Female Camel (*Camelus dromedarius*) During the Peak and the Low Breeding Season

Shujait Ali, Nazir Ahmad*, Nafees Akhtar, Zia-ur-Rahman[1] and Maqbool Ahmad

Department of Theriogenology; [1]Department of Physiology & Pharmacology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: profnazir53@hotmail.com

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ABSTRACT

Serum and follicular fluid concentrations of some hormones during the low (May to October) and the peak (November to April) breeding seasons in female camels with small and large Graafian follicles were investigated. Ovaries of 25 adult female camels slaughtered at a local abattoir were collected. Jugular blood samples and follicular fluid aspirated from small (5-9 mm) and large (10-20 mm) follicles were analyzed for progesterone, estradiol, T3, T4 and cortisol concentrations. Serum progesterone and cortisol concentrations were significantly higher ($P<0.05$) during the low than the peak breeding season. However, reverse was true for the serum estradiol, T3 and T4 levels. Animals with small follicles had higher ($P<0.05$) serum progesterone and cortisol concentrations than those with large follicles, while reverse was true for serum T3 levels. Follicular size had non significant effect on serum estradiol and T4 levels. In the follicular fluid, estradiol concentration was higher ($P<0.05$) during the low breeding season. However, season had non significant effect on follicular fluid levels of other hormones. Size of the follicle affected only progesterone level in the follicular fluid, which was higher ($P<0.05$) in large follicles. In conclusion, serum contents of estradiol, T3 and T4 were higher during the peak than the low breeding season; while reverse was true for serum progesterone and cortisol contents. For the follicle fluid, contents of estradiol were higher during the low than the peak breeding season. Follicle size influenced its progesterone contents only, which were higher in large than in small follicles.

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INTRODUCTION

Follicular fluid contains a number of biochemical metabolites derived from the serum or produced within the follicle and are associated with the metabolic activity of follicular cells (Gerard *et al*., 2002). Previous studies in several mammalian species clearly indicate that follicular fluid contains essential substances which are required for oocyte maturation and its fertilization. Changes in biochemical metabolites in the follicular fluid may influence oocyte maturation and quality (Gode *et al*., 2011). The metabolic activity, as well as the barrier properties of the follicular wall may show remarkable changes during the growth phase of the follicle (Godsen *et al*., 1988). Follicular fluid levels of biochemical metabolites also change due to diseases of the ovary (Khan *et al.*, 2011). Therefore, differences in concentrations of various biochemical metabolites in the fluid from small and large follicles may be anticipated. Since the camel is a seasonal breeder, concentrations of various biochemical metabolites in the serum or follicular fluid can also vary in different seasons.

Earlier, Ali *et al*. (2008) reported on changes in metabolite contents of blood serum and fluid from small and large sized Graafian follicles in one-humped camels during the low and the peak breeding seasons. Rahman *et al.* (2008) recorded significantly higher T3 concentrations in fluid from small than that from large follicles, while there was no difference in follicular fluid concentrations of T4 between small and large follicles. Furthermore, serum concentrations of these two hormones were much lower than those of follicular fluid. The aim of the present study was to monitor changes in the profiles of progesterone, estradiol, T3, T4 and cortisol in the serum

and fluid from small and large follicles during the peak and the low breeding seasons in one-humped female camels (*Camelus dromedarius*) kept under climatic conditions of Pakistan.

MATERIALS AND METHODS

Collection of samples

Ovaries of adult female one-humped camels (*Camelus dromedarius*) slaughtered at a local abattoir over a 24-month period were collected immediately after slaughter. Pre-slaughter information regarding the nutritional or reproductive status of these camels was not available. After cleaning each ovary off the extraneous tissue, diameter of Graafian follicles was measured using Vernier Calipers. On the basis of their size, follicles were classified into two groups viz. small (5-9 mm) and large (10-20 mm). Fluid from each follicle was aspirated aseptically and stored at –20°C. Animals having ovaries with any pathological lesions, or those with cystic follicles (>20 mm in diameter; Tibary and Anouassi, 1997) were not included in the study. Thus, data on 25 camels were available for analysis. Before slaughter, about 15 ml peripheral blood was collected from each animal, serum was separated and stored at –20°C for hormonal analysis. The period from November to April was taken as the peak breeding season, while May-October was considered as the low breeding season.

Enzyme immunoassay for hormones

Blood serum and follicular fluid samples were analyzed for progesterone, oestradiol, T3, T4 and cortisol, through EIA technique, using a Microstrip Elisa Reader (Stat-Fax-303, Awareness Technology, Inc.). Progesterone and estradiol concentrations were determined by using kits from Bremancos Diagnostic INC–GmbH, Germany (Cat. # BC-1113 & BC-1111, respectively). The lowest detectable level of progesterone during this test was 0.05 ng/ml, while the cross reactivity with other steroid hormones was <0.74%. For estradiol, the lowest detectable level was 5.9 pg/ml and cross reactivity with other steroids was <2.10%.

T3 and T4 concentrations were determined by using kits from DRG Instrument GmbH, Germany (Cat. # EIA-1780 & EIA-1781, respectively). The minimum detectable concentrations of T3 and T4 were 0.2 ng/ml and 0.4 µg/ml, respectively. Cortisol concentrations were determined by using kit from DRG Instrument GmbH (Cat. # EIA-1887). The minimum detectable concentration of cortisol in the assay was 2.5 ng/ml. Data regarding minimum detection levels and cross reactivity for each hormone was available with the respective kits.

Statistical analysis

The data collected during the study were subjected to statistical analysis using ANOVA, following completely randomized design. The GLM of statistics was used for this purpose. Correlation co-efficients between different hormonal concentrations of serum and follicular fluid were also computed.

RESULTS AND DISCUSSION

Progesterone

In the female camels, overall mean serum progesterone level was 0.98 ± 0.26 ng/ml. Camel is known to be an induced ovulator, and ovulation and corpus luteum formation in females of this species occurs only after mating (Hafez and Hafez, 2006). Since all camels included in the present study were non pregnant, serum progesterone levels were expected to be low. According to Cristofori *et al*. (1986), mean progesterone level in 53 non pregnant camels was 0.04 ± 0.4 ng/ml; in only two subjects serum progesterone level exceeded 1 ng/ml. According to Agarwal *et al*. (1989) and Tibary and Anouassi (1997), serum progesterone levels were below 1.0 ng/ml in non pregnant camels in the absence of ovulation.

Serum progesterone level was significantly higher ($P<0.05$) in the low than the peak breeding season (Table 1). However, Chamany and Khazali (1998) were unable to record any difference in serum progesterone levels in camels between the breeding and the non breeding seasons. They used only six animals in their study which was much less than the number of camels included in the present study.

The camel is an induced ovulator and in the absence of mating, the follicles continue to grow and then regress. Luteinization of the follicles without ovulation has been reported in cattle and mare (Collins *et al*., 1997). It is possible that such phenomena also occurs in camel and its incidence might have been higher during the low than the peak breeding season, resulting in higher progesterone levels during the low breeding season. Moreover, animals with small ovarian follicles showed higher ($P<0.05$) serum progesterone levels compared to those with large ovarian follicles (1.62 ± 0.04 ng/ml versus 0.37 ± 0.05 ng/ml; Table 1).

The overall mean follicular fluid progesterone concentration was 1.17 ± 0.27 ng/ml. This value is much less than 35, 37 and 39 ng/ml in small, medium and large follicles, respectively in cattle (Henderson *et al*., 1982), 13 ± 2 and 43 ± 10 ng/ml in dominant and pre-ovulatory follicles, respectively in mares (Collins *et al*., 1997) and 16.92 ± 1.13 ng/ml in buffaloes (Eissa, 1996). Species difference seems to be the main factor responsible for these discrepancies. The reproductive physiology of camels differs from other species in that the camel is an induced ovulator, while other species reported above are spontaneous ovulators. However, the difference in follicular fluid progesterone contents between the peak and the low breeding seasons was non significant (Table 2).

Mean follicular fluid progesterone concentration was higher ($P<0.05$) in large than in small follicles (Table 2). These results confirm some earlier findings in camels (Rahman *et al*., 2008) and gilts (Meurer *et al*., 1991). Higher concentrations of progesterone in large follicles observed in the present study suggest that luteinization of granulose cells occurs in camels, as has been reported in mares and cattle (Collins *et al*., 1997).

The overall mean progesterone concentration in the follicular fluid (1.17 ± 0.27 ng/ml) was higher than that in

Table 1: Mean values (± SE) for concentrations of different hormones in serum of camels with small and large sized follicles during two breeding seasons

Hormones	Breeding seasons	Follicular size		Overall mean
		Small (5-9 mm)	Large (10-20 mm)	
Progesterone (ng/ml)	Peak	0.23 ± 0.09	0.49 ± 0.10	0.36 ± 0.19b
	Low	3.01 ± 0.10	0.25 ± 0.10	1.64 ± 0.43a
	Mean	1.62 ± 0.04a	0.37 ± 0.05b	0.98 ± 0.26
Estradiol (pg/ml)	Peak	68.11 ± 1.14	67.05 ± 1.26	67.58 ± 1.36a
	Low	14.96 ± 1.26	15.54 ± 1.26	15.25 ± 1.54b
	Mean	41.53 ± 0.29a	41.29 ± 0.31a	41.42 ± 5.44
Tri-iodothyronine (T3;	Peak	9.05 ± 0.82	11.88 ± 0.86	10.47 ± 0.19a
ng/ml)	Low	5.22 ± 0.86	7.44 ± 0.86	6.33 ± 0.21b
	Mean	7.14 ± 0.19b	9.66 ± 0.21a	8. 46 ± 0.66
Thyroxine (T4; µg/ml)	Peak	10.34 ± 0.94	10.17 ± 1.40	10.25 ± 0.32a
	Low	5.88 ± 0.70	6. 26 ± 0.80	6.07 ± 0.35b
	Mean	8.11 ± 0.32a	8.22 ± 0.34a	8.08 ± 1.02
Cortisol (ng/ml)	Peak	33.23 ± 5.46	29.01 ± 6.34	31.12 ± 2.94b
	Low	104.81 ± 6.34	13. 01 ± 6.34	58.91 ± 3.61a
	Mean	69.02 ± 2.94a	21.01 ± 3.16b	43.97 ± 9.89

Mean values with different letters within a row or a column for each hormone differ significantly from each other (P<0.05).

Table 2: Mean values (± SE) for concentrations of different hormones in follicular fluid of camels with small and large sized follicles during two breeding seasons

Hormones	Breeding seasons	Follicular size		Overall mean
		Small (5-9 mm)	Large (10-20 mm)	
Progesterone (ng/ml)	Peak	0.39 ± 0.24	1.51 ± 0.30	0.95 ± 0.06a
	Low	1.15 ± 0.30	1.72 ± 0.30	1.44 ± 0.07a
	Mean	0.77 ± 0.06b	1.61 ± 0.07a	1.17 ± 0.27
Estradiol (pg/ml)	Peak	16.50 ± 4.13	20.83 ± 3.61	18.67 ± 1.10b
	Low	44.33 ± 2.81	57.33 ± 2.75	50.83 ± 5.47a
	Mean	30.42 ± 0.94a	39.08 ± 1.03a	34.75 ± 4.21
Tri-iodothyronine (T3; ng/ml)	Peak	11.51 ± 0.39	9.08 ± 0.32	10.29 ± 0.17a
	Low	10.52 ± 0.45	12.72 ± 0.33	11.62 ± 0.21a
	Mean	11.01 ± 0.21a	10.90 ± 0.22a	10.91 ± 0.61
Thyroxine (T4; µg/ml)	Peak	7.33 ± 1.10	6.92 ± 0.95	7.12 ± 0.21a
	Low	7.25 ± 0.71	6. 52 ± 0.72	6.88 ± 0.26a
	Mean	7.29 ± 0.13a	6.72 ± 0.14a	7.02 ± 0.35
Cortisol (ng/ml)	Peak	35.29 ± 3.72	28.04 ± 3.31	31.67 ± 1.46a
	Low	35.92 ± 4.10	57.78 ± 3.15	46.85 ± 1.57a
	Mean	35.60 ± 1.46a	42.91 ± 1.57a	39.26 ± 7.27

Mean values with different letters within a row or a column for each hormone differ significantly from each other (P<0.05).

the blood serum (0.98 ± 0.26 ng/ml), which is similar to an earlier study in camels (Rahman *et al*., 2008). The main source of serum progesterone is the corpus luteum. However, during steroidogenesis in the Graafian follicle, the theca cells secrete testosterone under the stimulus of LH, which is converted into estrogen by granulose cells under the influence of FSH (Hafez and Hafez, 2006). Some of testosterone may be aromatized into progesterone within the follicles. Perhaps this accounts for higher progesterone concentrations in the follicular fluid than in peripheral blood. However, the correlation co-efficient between serum and follicular fluid progesterone concentrations (r = -0.011) was non-significant.

Estradiol

The overall mean serum estradiol concentration in female camels was 41.42 ± 5.44 pg/ml. Skidmore *et al*. (1996) observed serum oestradiol-17β concentration of 39.0 ± 1.8 pg/ml, when the dominant follicles measured 1.7 ± 0.1 cm. According to Cristofori *et al*. (1986), mean serum total free estrogens were 83.2 ± 11.89 pg/ml. This shows a wide variation in the serum estradiol contents, which could be due to differences in the growth phase of the follicles on the ovary.

In camels, mean serum estradiol levels were significantly higher (P<0.05) during the peak than the low breeding season (Table 1). Similarly, Chamany and Khazali (1998) observed significantly higher plasma oestradiol-17β concentrations in camels during the breeding (64.00 ± 0.15 pg/ml) than non-breeding season (13.4 ± 0.15 pg/ml). The main source of serum estradiol are the Graafian follicles, and in camels used in the present study, higher frequency of animals with active ovaries (having follicles >5 mm in diameter) was recorded during the peak (86.76%) than the low (45.83%) breeding season (Ali *et al.*, 2007). Thus, ovaries had more active follicles during the peak breeding season, resulting in higher serum estradiol levels than the low breeding season. However, in the present study, there was non significant difference in serum estradiol levels between camels with small and large follicles on their ovaries. This suggests that instead of size, number of active Graafian follicles was responsible for increased serum estradiol levels during the peak breeding season.

Contrary to serum estradiol levels, follicular fluid estradiol contents were significantly higher (P<0.05) in the low than the peak breeding season (Table 2). This is surprising as the ovarian activity was observed to be higher during the peak breeding season.

The follicular size has been shown to influence its estrogen contents. In bovines, follicular fluid estrogen contents increased as its size increased (Henderson *et al*., 1982). In mares, the concentration of oestradiol-17β was significantly higher in late dominant than in early dominant follicles and decreased between late dominant and healthy pre-ovulatory follicles (Gerard *et al*., 2002). Similarly, in the present study, higher estradiol contents were observed in fluid collected from large than that from small follicles, however, the difference was statistically non significant (Table 2).

The mean serum estradiol concentration was slightly higher than that of follicular fluid (41.42 ± 5.44 versus 34.75 ± 4.21 pg/ml). Since Graafian follicles are the main source of serum estrogen, its concentration should have been higher in the follicular fluid than in serum. The correlation co-efficient between serum and follicular fluid estradiol concentrations (r = 0.179) was statistically non-significant.

Thyroid hormones

In female camels, the overall mean serum T3 and T4 concentrations were 8.46 ± 0.66 ng/ml and 8.08 ± 1.02 µg/ml, respectively. Pande *et al*. (1997) reported serum T3 and T4 concentrations from 12.0 ± 0.26 to 16.6 ± 0.41 ng/ml and 64.38 ± 6.58 to 80.86 ± 8.10 µg/ml, respectively during post partum period in camels. Variations in the physiological state of the animal can be attributed to these differences in mean serum T3 and T4 values observed in different studies.

Mean serum T3 and T4 levels were significantly (P<0.05) higher during the peak than the low breeding season (Table 1). This was anticipated as during the peak breeding season, the reproductive and general activity of the animal is increased. Thus, an increase in the concentrations of thyroid hormones is required to enhance the general metabolic rate. According to Spicer *et al*. (2001), T3 and T4 may have a major positive impact on LH-induced androstenedione production by bovine ovarian thecal cells, both of which would result in a net increase in estrogen production by follicles. Perhaps a similar situation exists in the camel, where along with serum T3 and T4, higher levels of serum estradiol were recorded during the peak than the low breeding season.

Camels having large follicles on their ovaries had significantly higher serum T3 contents than those with small ovarian follicles (P<0.05). However, follicular size had non significant effect on serum T4 contents (Table 1).

The overall mean T3 and T4 concentrations in the follicular fluid were 10.91 ± 0.61 ng/ml and 7.02 ± 0.35 µg/ml, respectively. The effects of follicular size or season on follicle fluid concentrations of these hormones were non-significant (Table 2). Rahman *et al*. (2008) also observed non significant difference in follicular fluid T4 contents between small and large follicles in camels; however, follicular fluid T3 levels were significantly higher in small than in large follicles in that study.

The overall mean follicular fluid T3 contents were higher than those of serum (10.91 ± 0.61 ng/ml Vs 8.46 ± 0.66 ng/ml). The correlation co-efficient between serum and follicular fluid T3 concentrations (r = -0.070) was statistically non-significant. The overall mean serum T4 concentration (8.08 ± 1.02 µg/ml) was relatively higher than 7.02 ± 0.35 µg/ml found in the follicular fluid. The correlation co-efficient between serum and follicular fluid T4 concentrations was positive (r = 0.097), but statistically non-significant.

Cortisol

The overall mean serum cortisol concentration was 43.97 ± 9.89 ng/ml. Elias and Weil (1989) recorded serum cortisol concentrations in sexually mature female camels as 8.0 ± 1.3 ng/ml before mating and 45.0 ± 11.9 ng/ml immediately after mating. They also reported an increasing trend (P<0.01) in the serum cortisol concentration from 11.4 ± 1.3 ng/ml 3 days pre partum to 45.3 ± 5.0 ng/ml at the start of parturition.

Mean serum cortisol concentration during the low breeding season was significantly (P<0.05) higher than that found during the peak breeding season (Table 1). In Pakistan, the low breeding season of camel comprises summer and autumn, the former in this area is very hot with ambient temperatures as high as 50°C. The camels might have been under stress due to extreme hot climate which resulted in increased serum cortisol levels. Rahman *et al*. (2007) recorded significantly higher (P<0.05) serum corticosterone in the non-rutting period than in the rutting period of male camels.

Female camels with small follicles had significantly higher (P<0.05) serum cortisol concentrations than those having large follicles (Table 1). However, the physiological significance of this difference remains to be investigated.

The overall mean cortisol concentration in follicular fluid was 39.26 ± 7.27 ng/ml. The follicular fluid cortisol levels were neither influenced by breeding seasons nor by the follicular size (Table 2). Unfortunately, literature regarding follicular fluid cortisol concentrations could not be traced for comparison.

The overall mean serum cortisol concentration (43.97 ± 9.89 ng/ml) was higher than that of follicular fluid (39.26 ± 7.27 ng/ml). A positive (r = 0.036) but non significant correlation co-efficient was recorded between serum and follicular fluid cortisol concentrations.

Conclusions

In conclusion, serum contents of estradiol, T3 and T4 were higher during the peak than the low breeding season; while reverse was true for serum progesterone and cortisol contents. For the follicle fluid, contents of estradiol were higher during the low than the peak breeding season. Follicle size influenced its progesterone contents, which were higher in large than in small follicles.

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