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Research article

Seasonal variations in the sex-steroid hormones and behavior of one humped male camel *(Camelus dromedarius)* in Punjab, Pakistan

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Abstract
Dromedary camel is a seasonal breeder showing aggressive behavior during winter. Present study was intended to study the season-wise serum steroid hormone pattern and characterized the sexual behavior of camel in Punjab, Pakistan during 2010-11. The blood samples were collected at regular monthly intervals and behavioral observations were made for at least two hours interval for a week during beginning, mid and end of each season. Serum samples were analyzed for the steroid hormones by Radioimmunoassay (RIA) using commercially available kits. Steroid hormones were recorded as significantly higher ($P<0.01$) during the cooler months (rutting season of camel) at all the experimental areas which affect the male behavior. Regional variations were recorded in the hormonal profile and behavior. Serum testosterone started decreasing during spring and reached baseline during summer, maintained almost same during autumn at Faisalabad zone however increased again in autumn at Attock and Bhakkar zones. Serum estradiol 17-β started declining in spring and summer; little increased level was observed in the month of June but again sloped down in July and remained on baseline till end of autumn. Study revealed that seasonal changes have direct correlation with the serum steroid hormones which alter the sexual behavior of camels.
Keywords: Camel, Testosterone, Estrogen, Serum, Sexual behavior, Punjab

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Introduction

Dromedary camels were formerly domesticated in Central and Southern Arabic countries [1]. From these areas they gradually dispersed to North Africa, Middle East, some parts of Europe and Asia. There are about 19.3 million camels world-wide of which the dromedary camel accounts for 95%; the remaining 5% are bactrianus camel. Pakistan with one million head spossesses about 23% camel population of Asian countries and it rates fourth in the world after Somalia, Sudan and Mauritania [2]. The arid climate of Pakistan is characterized by hot summers and cold winters, and wide variations exist between extremes of temperature at different locations. Pakistan has four seasons: a cool, dry winter from December to February; a hot, dry spring from March to April; the summer rainy season, or southwest monsoon period, from May to September; and the diminishing monsoon period of October and November. The commencement and duration of these seasons vary according to ecological locality of different zones.

Dromedary camel is a seasonal breeder, showing sexual bustle during winter and early spring season in Pakistan [3-5]. The variations in both the initiation and duration of the breeding season of camel amongst different regions of the world demonstrate that the local seasonal factors must instigate the sexual activity. Changes in the day length stimulate seasonality [6-7], but clearly some other factors like rainfall [8], management, pasture condition and nutrition [9], are also important in the camels found near the equator. The androgen level is highest during the cooler months [10], a significant correlation found between testosterone secretion and the environmental temperature. However Arthur *et al.* [11] suggested that dromedary camel can reproduce during all the seasons of year, provided food supply abundant along with good management. Agarwal and Khanna [12] reported off-season breeding in the dromedary camel but Zhao [13] reported vice versa and documented the loss of libido in male camel during off-season. The cyclic reproductively in the camel is also obvious in the form of endocrine surge of gonadotropins [14-15]. There is a dramatic increase in the blood testosterone and in the testicular tissue during the rutting season of the one humped camel [15-16]. The average month-wise testosterone concentration of Jaisalmeri camel (4-12 years old) ranged from 616.80±47.49 pg/ml to 8658.2±485.45 pg/ml of plasma. Testosterone level is low during summer season (April-September) while this increase during October and November continue to increase during December and peaked in the January and February winter). It starts decreasing in March and reached to low level in subsequent months. Testosterone concentration becomes low during hot months of the year including May, June, July, August and September because of decline in endocrine surge. Peak testosterone concentrations reported as

Table 1: Behavioural signs of male one-humped camels as recorded during different seasons at Faisalabad zone.

Obs.	Season	D/A	IPA	RFI	M	FD	HC	F	ESP	GRS	ISSG	US	PGS	FS
12	Winter	A	+++	++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
	Spring	A	++	+	++	+	++	++	++	++	++	++	++	++
	Smmer	D	-	-	+	-	+	-	+	-	-	-	+	-
	Autumn	D	-	-	+	+	+	++	+	+	+	+	+	-

Table 2: Behavioural signs of male one-humped camels as recorded during different seasons at Attock zone.

Obs.	Season	D/A	IPA	RFI	M	FD	HC	F	ESP	GRS	ISSG	US	PGS	FS
6	Winter	A	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++
	Spring	A	++	++	++	++	+++	+++	++	+++	+++	++	+++	++
	ummer	D	-	-	-	-	-	-	-	-	-	-	-	+
	Autumn	D	+	+	++	++	+	+	+	++	+	++	++	++

7457±271.9, 7624.5±666.9, 14644.6±936.4 and 15204.2±954.3 pg/ml of plasma in dromedaries [17]. The testosterone concentration in plasma goes to baseline before rutting (342.93±43.90 ng /ml). Initiation of rut activity is associated with significant rise in testosterone level (4213.94±278 ng /ml), which is maintained for 11-18 weeks followed by turn down to basal levels. Libido in males subsides in March or April. Cessation of libido and inability to copulate appears to be associated with decline in testosterone concentration [18].

Estradiol 17-β (E2) is a steroid hormone predominantly present in the female but also important in regulating male sexual behavior and process of spermatogenesis. In males, estrogen derives from circulating androgens. Sertoli cells convert testosterone to estradiol (E2), which moves to the compartments of the seminiferous tubules and also into blood stream [19]. These cells are the major source of blood and seminal estradiol in dromedary camels [16].

The functional significance of Estradiol 17-β (E2) in males is not yet clearly known [20]. However some workers have reported the significance of the estradiol in males, like Hull *et al.* [21], documented that estrogenic metabolites play a central role in male mating behavior of mammalian species. Estradiol also prevents apoptosis of the sperm cells [22]. It works together with the testosterone to exhibit the sexual behavior and occurrence of successful copulation in adult male green anoles [23]. In the literature cited, only one report is available on the estradiol concentration in dromedary male camels [24], who recognized that hormonal profile (Testosterone and estradiol) is positively correlated with the sexual libido (Copulation time and volume of semen ejaculate) and sexual libido is manifested in male camel due to increased level of testosterone and estradiol. Estradiol concentration is recorded as significantly higher during February (241.13±12.27 pg/ml) followed by March (238.93±29.21 pg/ml) and lowest during December (57.05±1.91 pg/ml) and January (86.69±2.69 pg/ml). Estradiol concentration declined during April (137.45±14.98 pg/ml) and May (114.27±22.04 pg/ml) and increase again during the month of June (190.59±49.21 pg/ml) [24].

The objectives of the this study were to evaluate the seasonal changes and correlation among the serum steroid hormones (Testosterone and estradiol 17-β) and sexual behavior pattern of dromedary camel in the natural ecology of Punjab, Pakistan.

Materials and Methods

Animals and study design

Twenty four adult sexually mature (6-9 years of age) male dromedary camels from three districts located in different ecological zones of Punjab, Pakistan (Fig. 1), namely Faisalabad (Fsd) (n=12), Bhakkar (Bkkr) (n=6) and Attock (Attk) (n=6), kept under traditional management conditions at the Government farms and neighboring localities, were used. The study was conducted during four seasons of the year 2010-11, namely winter (December to February), spring (March to April), summer (May to September) and autumn (October to November).

Collection of samples

The blood samples were collected at regular monthly intervals from the jugular vein of each animalat all the experimental zones. These samples were centrifuged at 15000 rpm for 10 min, thus serum was separated and stored at -20 °C till biochemical analysis.

Serum steroid hormones

Serum samples were analyzed for testosterone& estradiol 17-β as described by Wilson and Foster

[25], by using commercially available kits; RIA Testosterone, direct IM1119IMMUNOTECH (A Beckman Coulter Company, France) and RIA Estradiol A21854 kits IMMUNOTECH (A Beckman Coulter Company, France) respectively. The sensitivity of the assay was 0.025 ng/ml for testosterone &<6 pg/ml for estradiol kit , the intra- and inter-assay coefficient of variation were 14.8 % &15% for testosterone and 12.1% & 11.2% for estradiol 17-β kits respectively.

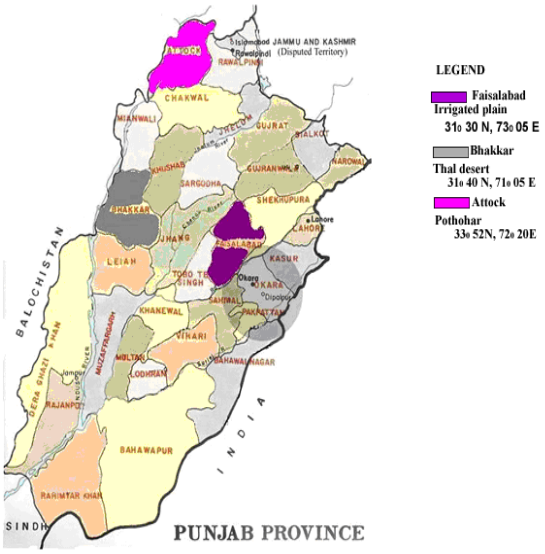

Fig. 1: Locations of different experimental zones under study in Punjab, Pakistan

Observations on sexual behaviour

The behavioural observations were made for at least two hours on each experimental animal for a whole week during the beginning, mid and end of all the seasons of year. The degree of following rutting behaviour signs were recorded: pacing and anxiety, reduction in food intake, frequent diarrhoea, herd chasing, fighting, extrusion of soft palate, gurgling and roaring sounds, increased secretion of saliva, urine spraying, smudging of poll gland secretions and female seeking.

Statistical analysis

The means, standard error means (± SEM) and ranges of steroid hormones were worked out using the computer software Microsoft Excel. Means were compared by two-way ANOVA. Significance among different parameters was calculated at P>0.05 by Duncan's multiple range (DMR) test. Correlation among different calculated parameters was estimated by Pearson Correlation. Descriptive statistics (Frequencies, percentages) and factorial correspondence analysis of rutting behaviour signs were derived using STATISICA 6.0 for windows with its sub program.

Results

Serum testosterone

The means (±SEM) of serum testosterone concentration (ng/ml) were recorded as (8.29±0.54), (13.49±1.37) and (15.51±1.15) in winter, (2.10±0.28), (6.19±0.65) and (6.58±0.74) in spring, (0.66±0.15), (1.58±0.30) and (1.48±0.29) in summer, (0.66±0.05), (8.03±1.57) and (4.14±1.03) in autumn at Faisalabad (Fsd), Attock (Attk) and Bhakkar (Bkkr) zones respectively. Statistical analysis exposed that the overall means of serum testosterone concentration were highly significantly (P<0.01) more at Attk (6.40±0.72) and Bkkr (6.28±0.76) ecological zones as compared to Fsd (2.81±0.31) but showed same trend of seasonal changes which was highly significantly (P<0.01) greater in winter season (11.40±0.64) and lowest during summer (1.10±0.13) (Fig. 2).

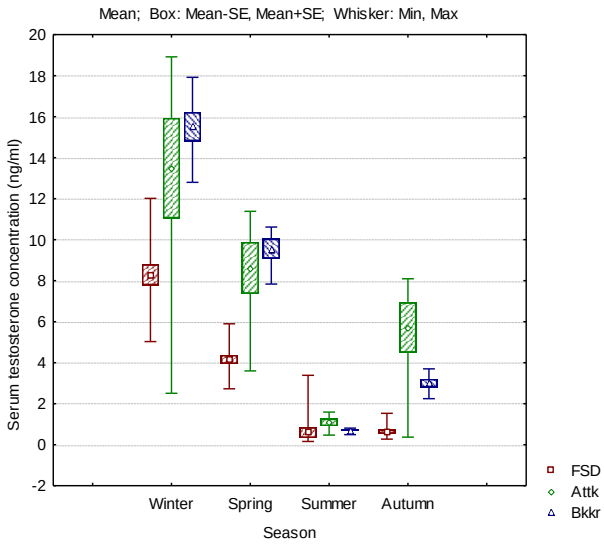

Fig. 2: Season-wise means (±SEM) and ranges of serum testosterone concentration (ng/ml) of dromedary camel at all experimental areas

Serum estradiol 17- β

The means (±SEM) of serum estradiol concentration (pg/ml) were (156.47±10.24), (137.70±11.75) and (117.65±16.61) during winter, (126.63± 5.63), (129.56±15.88) and (127.12±14.42) during spring, (97.47±4.72), (92.23± 5.20), and (94.03±4.94) in summer and (61.17± 5.06), (70.29±7.38) and (76.26±11.95) during autumn at Fsd, Attk and Bkkr zones respectively. Statistically there was no

Table 3: Behavioural signs of male one-humped camels as recorded during different seasons at Bhakkar zone.

Obs.	Season	D/A	IPA	RFI	M	FD	HC	F	ESP	GRS	ISSG	US	PGS	FS
6	Winter	A	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Spring	A	+++	+++	+++	++	++	++	++	+++	+++	++	++	+++
	Summer	D	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	D	+	-	+	+	+	+	+	-	+	-	+	+

Notes: D/A=Docile or aggressive (D = docile, A =aggressive), IPA= Increased passing and anxiety, RFI= Reduction in food intake, M= Micturition, FD = Frequent diarrhoea, HC= Herd chasing, F= Fighting, ESP= Extrusion of soft palate, GRS= Gurgling and roaring sounds, ISSG= Increased secretion of salivary glands, US= Urine spraying, PGS= Poll gland secretion, FS= Female seeking, +++ = high, ++ =medium, + = low, - = absent

Table 4: Chi-square values of different behavioural signs from the correspondence analysis.

	RFI	M	FD	HC	F	ESP	GRS	ISSG	US	PGS	FS
IPA	51.12**	63.48**	50.24**	48.81**	60.64**	61.94**	62.70**	60.27**	66.42**	54.59**	57.28**
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RFI		49.36**	80.96**	52.00**	57.65**	50.33**	52.46**	65.51**	59.48**	55.82**	48.26**
		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
M			49.38**	54.30**	47.48**	43.71**	47.68**	57.50**	53.11**	69.09**	71.31**
			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FD				51.50**	56.77**	54.97**	59.05**	67.00**	66.07**	68.95**	51.29**
				0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
HC					69.42**	50.99**	50.35**	69.46**	49.12**	46.30**	58.31**
					0.000	0.000	0.000	0.000	0.000	0.000	0.000
F						53.61**	55.23**	74.24**	62.02**	56.29**	49.24**
						0.000	0.000	0.000	0.000	0.000	0.000
ESP							81.10**	67.88**	74.74**	59.78**	60.00**
							0.000	0.000	0.000	0.000	0.000
GRS								77.04**	67.20**	56.36**	60.35**
								0.000	0.000	0.000	0.000
ISSG									77.00**	65.44**	63.86**
									0.000	0.000	0.000
US										54.88**	63.86**
										0.000	0.000
PGS											60.69**
											0.000

Notes: ** = Significant (P<0.01)
IPA=Increased passing and Anxiety, RFI=Reduction in food intake, M=Micturation, FD=Frequent diarrhoea, HC=Herd Chasing, F=Fighting, ESP=Extrusion of soft palate, GRS=Gurgling and roaring sounds
ISSG=Increased secretion of salivary glands, US=Urine spraying, PGS=Poll gland secretion, FS= Female seeking

difference of serum estradiol concentration among three different zones including Fsd (111.03± 4.37), Attk (106.16±5.45) and Bkkr (102.49± 5.83), however overall means showed highly significantly (P<0.01) more estradiol concentration during winter (142.07±7.37) and spring (127.48±5.89) seasons as compared to summer (95.30±2.95) and autumn (67.22±4.32) at all the zones (Fig. 3).

Correlation analysis of steroid hormones

Correlation analysis revealed that overall there was a highly significant (P<0.01) positive correlation (r = 0.454**) between the serum testosterone and estradiol concentration, same positive correlation (r = 0.796**) was recorded at Faisalabad zone, however this correlation was positive but non-significant at Bhakkar (r = 0.417) and Attock (r = 0.485) zones.

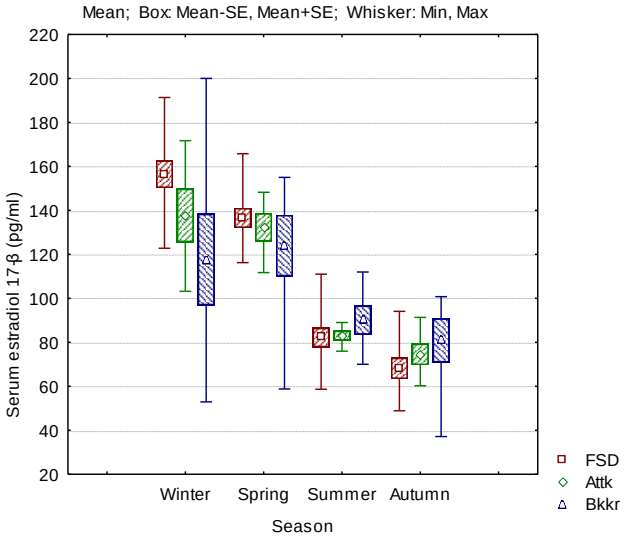

Fig. 3: Season-wise means (± SEM) and ranges of serum estradiol 17-β concentration of dromedary camel at all experimental areas.

Sexual behavioral signs

The results of present study revealed that all experimental animals showed aggressiveness during winter and spring season and docility in summer season at all the ecological zones, however during autumn half of the animals were aggressive at Faisalabad and Attock while 33.3% camels were aggressive at Bhakkar zone. Most of the camels (66.7% at Faisalabad, 100% at Attock and 83.3% at Bhakkar), (33.3% at Faisalabad and Attock, 50% at Bhakkar) were in the condition of restlessness and anxiety during winter and spring seasons. This sign was totally absent (83.3% at Faisalabad, 16.7% at Bhakkar) or very low (66.7% at Bhakkar, 66.7% at Attock, 16.7% at Faisalabad) during autumn season. The majority of camels remained off feed (with moderate or high extent) during the winter and spring season while this was totally absent or very low during summer and autumn seasons of the year. The percentage of degree of micturition was more at Bhakkar zones (66.7% camels), frequent diarrhoea and fighting behaviour was observed in more camels (66.7% and 100%) at Attock, extrusion of soft palate was more at Attock and Faisalabad (83.3%), the percentage of extent of gurgling and roaring sounds was high (100%) at Faisalabad, salivary glands secretions were more copious (83.3%) at Attock and Bhakkar, urine spraying character was high at Bhakkar, high percentage (83.3%) of camels showed poll gland secretion at Bhakkar followed by Attock and Faisalabad, during winter and spring seasons (Fig. 4).

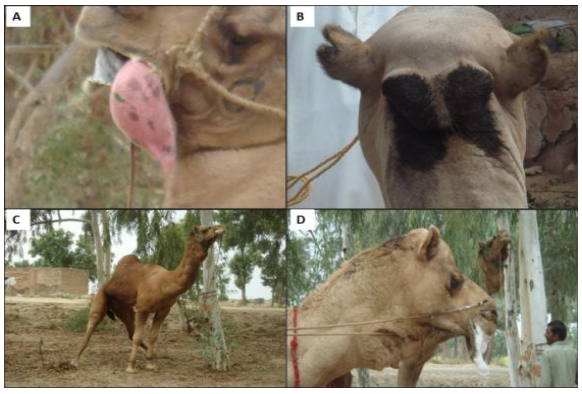

Fig. 4: Photographs of male camels showing different behavioral signs
A= Extrusion of soft palate B= Poll glands secretion
C= Urine spraying D= Salivary glands secretion

These behavioural differences correlated with the steroid hormonal fluctuations which might be attributed to the climatic condition or nutritional status of the camels at different locations. Some behavioural signs like micturition, frequent diarrhoea, fighting, extrusion of soft palate, secretion of salivary glands and poll glands secretion were observed as more extensive at Attock and then Bhakkar zone as compared to the Faisalabad, which is subjective to the recorded higher steroid hormonal concentrations at Attock and Bhakkar zones (Fig. 2 and Fig. 3). Higher hormonal concentrations and degree of behavioural signs observed earlier in the autumn (October, November) and extended up -to spring season of the year, which revealed that the rutting season started earlier at these locations (Attock and Bhakkar) and extended even up to the start of the summer at Attock zone. The behavioural signs and their Chi-square values are documented in table 1, 2, 3 and 4.

Discussion

This study was conducted to demonstrate the month wise, seasonal pattern of the serum steroid hormones (Testosterone and estradiol), their correlation amongst each other and extent of sexual behaviour in mature dromedary camels at three ecological zonesof Punjab, Pakistan, including Faisalabad (Irrigated plain), Attock (Pothohar) and Bhakkar (Thal desert). Serum testosterone concentration was higher ($P<0.01$) during the winter season at all experimental zones under study, it started decreasing during spring and reached baseline during summer season, maintained during autumn at Fsd zone however increased again in autumn at Attk and Bkkr zones. This augmented level in autumn season was higher at Attk as compared to Bkkr zone, which may be due to the climatic variations among the different ecological zones. The degree of rutting or sexual behavioural signs was also more at Attk zones which explore the relation between ecological changes in the testosterone level and sexual behaviour of the camels. The testosterone concentration was recorded as significantly more in rutting season (winter and early spring) of the camel comparatively to the non-rutting season; these results are aligned with the previous findings [10,15,17-18, 24,26]. However, earlier reported [24] serum testosterone level of adult male camels during rutting season in Pakistan was very low (1.92±0.61 ng/ml) as compared to these findings and previously documented level of the hormone in the rut season [10,24]. Month wise testosterone profiles showed that testosterone levels were at peak in the month of February at all the experimental areas, it remained higher during the cooler months of

the year (December, January), became relatively lower in the month of March and reached its baseline in the month of April at Fsd zone, while in the month of June at Attk and Bkkr zones. The testosterone concentration maintained to the baseline till the month of November at Fsd, October at Bkkr and August at Attk zone. It started increasing in the month of December at Fsd, November at Bkkr and September at Attk zone which was attributed to the climatic changes in the zones. Overall the testosterone level and magnitude of sexual behavioural signs remained higher during the cooler months of the year or during the rutting season of the camel and it declined and maintained at the basal level during the hot months of year which constitute the non-rutting period of the camel however zonal variations were observed in the duration of the endocrine surge which attribute to the period of the rutting period in different zones. This period was recorded as more lengthy at Attk zone followed by Bkkr and short in the Fsd zone.

Serum estradiol 17-β was higher ($P<0.01$) during winter including January, February at all the experimental areas which positively affect the sexual behaviour of the camel, it started waning in the months of March, April and May, petite augmented level was observed in the month of June but again decreased in July and baseline level was recorded during the months of August, September, October and November, it sloped up in December at Fsd and Attk while estradiol level shoot up one month earlier in November at Bkkr zone. Present study revealed that both the steroid hormone concentrations and the extent of sexual behavioural signs got the peak level during winter or rutting season of camel. The findings of present study have same trend of estradiol level like previous study in India [17], except few variations during the month of January and March, who compare the hormonal status with the sexual libido in camels. And concluded that sexual libido is manifested in camel due to increased concentration of both testosterone and estradiol. Latham and Wade [23] reported that estradiol works together with the testosterone to exhibit the sexual behaviour and occurrence of successful copulation in adult male green anoles. Hull *et al.* [21] recognized that estrogenic metabolites play a key role in adult male mating behaviour. However, one study reported non-significant difference of serum estradiol concentration between rutting and non-rutting periods of camel [24]. Current study exposed positive correlation between serum testosterone and estradiol concentration which is in-line with previous studies in other animals like male mink whales and in *Neophocaenaphocaenoides asiaeorientalis* [25], who demonstrated that testosterone level is closely correlated with the estradiol concentration, as approximately 70% estradiol in circulation in adult males is due to aromatization of the circulating androgens.

Conclusions

In conclusion, our results provide a deep insight into the seasonal and ecological changes in the serum steroid hormones and sexual behavioural signs of the male camels. It is obvious from these results that season have direct correlation with the serum steroid hormones which alter the sexual behaviour of camels. This studyratifies the earlier inference that steroid hormones are important in regulation of male sexual behaviour [27-29].The sexual behavioural signs of camel vary among different ecological zones in same country which could be used as a guide to determine the best time for mating in dromedary camel in this region.

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