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RESEARCH ARTICLE

Effects of Gonadotropin Releasing Hormone (GnRH) Administration on Serum Hormonal and Mineral Profiles and Pregnancy Rate during Breeding and Early Non-Breeding Season in Female Dromedary Camel

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A B S T R A C T

The effects of GnRH on serum concentrations of progesterone, estrogen, luteinizing hormone (LH), follicle stimulating hormone (FSH), mineral profile (phosphorus, magnesium, zinc, calcium and manganese) and pregnancy rate were evaluated in female dromedary camels. Forty female camels were randomly assigned to one of four equal groups: A (GnRH treated) and B (control) during breeding season, and C (GnRH treated) and D (control) during non-breeding season. GnRH analogue Dalmarelin (20µg) was given intramuscularly to the treated animals one day prior to mating, while the controls received 1 ml normal saline intramuscularly. Pregnancy was diagnosis 50 days after mating through trans-rectal palpation. Blood from all the groups was collected at the time of GnRH/normal saline administration (day -1), then daily at days 1, 4, 7 and 10 during breeding and non-breeding seasons and analyzed for serum progesterone, estrogen, FSH and mineral profile. LH was determined by blood sampling from all the groups at -1, 1, 2, 3 and 5 hours with reference to one day after GnRH/normal saline administration (this time corresponded to time of mating in mated females). Results revealed that serum progesterone and LH concentrations were higher ($P<0.05$) in animals of treated than control group during each season. Differences in serum estrogen between treated and control groups in each season were non-significant. However, estrogen levels in both treated and control groups in breeding season were higher ($P<0.05$) than both groups in non-breeding season. Differences in serum FSH, P, Mg, Zn, Ca and Mn levels among all four groups were non-significant. Pregnancy rate was highest in group A and lowest in group D ($P<0.05$); the difference between groups B and C was non-significant. In conclusion, use of GnRH shows positive prospects to manipulate reproductive cycle in camels.

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INTRODUCTION

Reproductive efficiency is the primary factor affecting the productive performance of a female and is hampered by multiple factors in female camels (El-Malky *et al.*, 2018). Pakistan is ranked eighth among camel keeping countries in the world, with estimated population of 1.2 million heads (Anonymous, 2019). Camel is a seasonal breeder, with high breeding activity occurs during winter and spring, while low breeding activity is recorded during summer and autumn (Swelum *et al.*, 2018). It produces few young ones during its rearing life due to 28-36 months long calving interval. The reproductive performance of a female camel is adversely affected due to higher age at first service, longer interval between calving, relatively short breeding season, induced

ovulation and poor conception rates. Moreover, poor herd management, low nutrition availability and disease prevalence can also be involved in poor reproductive performance in this species (El-Malky *et al.,* 2018).

Gonadotropin releasing hormone (GnRH) protocol has been used for the synchronization of ovulation as an alternative to poorly detectable estrus. Regardless of the stage of estrous cycle (follicular development), GnRH is considered to be the most effective hormone to induce ovulation in one-humped and two humped camels (Manjunatha *et al.,* 2015).

Macro and micro minerals like zinc and manganese are involved in almost all physiological and metabolic activities of an animal. Minerals are essentially required for the physiological processes encompassing reproductive biology of any mammal (Molefe and Mwanza, 2020). Changes in serum mineral profile can predispose animals to various gynecological and obstetrical problems. The normal serum mineral profile is especially important during the physiological stress of gestation for fetal growth and placental health (Mostafa *et al.,* 2020).

In the she camels, no document reflects the clear picture for the effect of GnRH on serum calcium, phosphorus, magnesium, zinc and manganese during winter and summer seasons and their interaction with ovarian activity. Moreover, seasonal effects of exogenous GnRH hormone administration on pituitary and ovarian activity and pregnancy rates have not been studied under ecological conditions of Pakistan. Therefore, the present study was designed to investigate the effect of exogenous GnRH administration on ovarian steroidogenesis activity, pituitary FSH and LH production, blood mineral profile and pregnancy rates in dromedary camels maintained under ecological conditions of Pakistan.

MATERIALS AND METHODS

Experimental animals and treatments: The study was conducted during breeding season (December-January) and early non-breeding season (April-May) of camels. Forty clinically healthy, sexually mature, non-pregnant female Dromedary camels, maintained at the Camel Breeding & Research Station, Rakh-Mahni, District Bhakkar, Pakistan, were used in this study. This research station is located over 9171 acres, having animal sheds, offices and jungle area. All the experimental camels were kept under similar feeding and housing management conditions of semi-intensive type under desert climate. The animals were left loose for grazing from 9:00 am to 04:00 pm daily and then housed in cemented shed with open areas till the next morning. In the evening, each animal was also fed three Kg Gram straw. The animals had *ad libitum* access to water in the sheds and water was also offered thrice daily during grazing by attendants through tube wells. Onset of estrus was marked if the female did not resist to male during natural mating.

For the experiment, camels were randomly divided into groups A and B (Breeding season) and groups C and D (non-breeding season), with 10 animals in each group. Groups A and C (treatment group) were injected intramuscularly with 20µg commercially available GnRH analogue Dalmarelin (Lecirelin, Fatro, Italy), as described earlier (Monaco *et al.,* 2017). Animals of Groups B and D served as control and were injected intramusculary with 1 ml normal saline at the same time when the respective treatment group was injected with GnRH. These treatments were given irrespective of the physiological state of estrus cycle i.e. whether animals were cyclic or non-cyclic. Animals showing signs of heat were allowed to mate with fertile males one day after GnRH/normal saline administration. However, 3, 4, 3 and 10 females in group A, B, C and D, respectively were not mated, as they were not in heat). Pregnancy diagnosis in mated animals was done through trans-rectal palpation on day 50 after mating (Koc *et al.,* 2016) and the pregnancy rate (%) for each group was determined following the formula of Skidmore and Billah (2006).

Collection of blood: About 10 ml of blood was collected from each camel of Groups A and B through jugular veni-puncture, using a sterilized syringe before treatment (day -1) and then at days 1, 4, 7 and 10 after treatment with GnRH/normal saline, serum was separated by centrifugation at 1800xg for 15 minutes and stored at -20ºC till use. For LH determination, blood sampling was done from mated, as well as non-mated, animals at -1, 1, 2, 3, and 5 hours with reference to 24 hours after GnRH/normal saline administration (this time corresponded to time of mating in mated females). The similar pattern was followed during the experiment performed in non-breeding season in animals of Groups C and D.

Hormonal analysis: The preserved serum samples were analyzed for progesterone, estrogen, LH and FSH concentrations through enzyme-linked immunosorbent assay (ELISA), using commercially available kits (BioCheck Inc., USA). For progesterone (Catalogue Number BC-1113), the assay sensitivity was 0.05ng/ml, while inter and intra-assay coefficients of variation were 3.85 and 6.4%, respectively. For estradiol (Catalogue Number BC-1111), the value was 10pg /ml for sensitivity, while inter and intra-assay coefficients of variation were 10.85 and 12.5%, respectively. For LH (Catalogue Number BC-1031), assay sensitivity was 0.14ng/ml and inter and intra-assay coefficients of variation were 10 and 12%, respectively. For FSH (Catalogue Number BC-1029), assay sensitivity was 1.0 mIU/ml, while inter and intra-assay coefficients of variation were 10 and 12%, respectively.

Determination of serum mineral profile: Concentrations of zinc, magnesium and manganese in the serum of female camels were measured with the help of atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan), following the method of Fedor *et al.* (2017)**.** Serum calcium and phosphorus concentrations were determined using commercially available kits, by using spectrophotometer, as described earlier (Abdel-Raheem *et al.,* 2019).

Statistical analysis: The data obtained from the trial were analyzed using Statistical Package for the Social Sciences (SPSS) software version 16 for windows and MS Excel 2010. The effects of treatments on serum hormonal and

mineral profiles were analyzed by ANOVA, following completely randomized design. Different means were compared by Tukey's test (Lee and Lee, 2018). Data on pregnancy rates among all four groups were analyzed using Chi-Square test.

RESULTS

Serum hormonal profile: The overall mean serum progesterone concentration in GnRH treated group during breeding season was significantly higher (P<0.05) than control, as well as than both groups of non-breeding season (Table 1). Moreover, serum progesterone concentration was significantly higher in treated groups than the respective control groups during both the seasons, indicating that GnRH treatment had positive effect on serum progesterone concentration both during breeding and non-breeding season. The mean progesterone concentration in the four groups at different days with reference to GnRH/normal saline administration is shown in Fig. 1. Progesterone level remained low on day before and day of treatment. Then it increased in both GnRH treated and non-treated groups of breeding season, as well as in treated group of non-breeding season, but no increase was seen in the control group of non-breeding season. On each day of blood collection (except day 1), serum progesterone remained significantly higher (P<0.05) in treated group of breeding season compared to other three groups.

The overall mean serum estrogen concentration in both groups of breeding season was significantly higher (P<0.05) than in both groups of non-breeding season. However, serum estrogen did not differ between GnRH treated and control camels during the breeding season. The same was true for the non-breeding season (Table 1). The mean estrogen concentration in the four groups at different days with reference to GnRH/normal saline administration is shown in Figure 2. Estrogen concentration was significantly higher in both groups of breeding season than non-breeding season during the period from Day -1 to Day 4 of GnRH/normal saline administration. However, estrogen concentration in the both groups of breeding season decreased rapidly by day 7. Overall, estrogen concentration in both groups of non-breeding season did not vary during the study, except that it increased unexpectedly at day 10 for the control group.

During the breeding season, overall mean serum LH concentrations of the treated group was significantly higher (P<0.05) compared to the control group. The same was true for the non-breeding season (Table 1). Moreover, mean serum LH levels for treated and control groups of breeding season were significantly higher than the respective groups of non-breeding season. In the dromedary female camel, plasma LH concentrations increased gradually in groups A, B and C to reach a maximum value at 3 h after the start of blood sampling, which was done at -1, 1, 2, 3, and 5 hours with reference to one day after GnRH/normal saline administration (this time corresponded to time of mating in mated females); at this stage, it was highest in group A (P<0.05), followed by Group B and Group C (Fig. 3). In camels of Group D, serum LH concentration was significantly lower (P<0.05) compared to other three

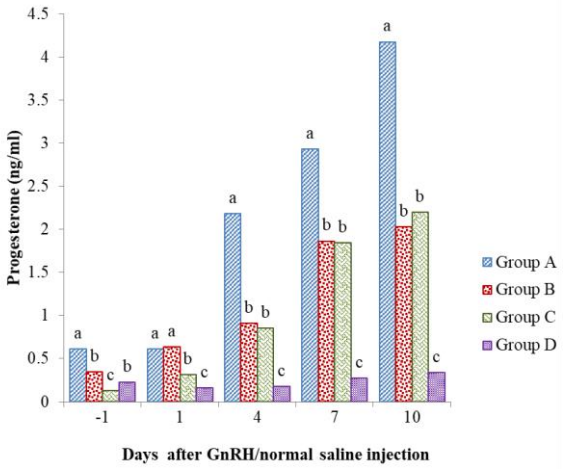

Fig. 1: Serum progesterone concentrations in camels of four groups at various days after respective treatment. Different alphabets at the bars indicate significant difference (P<0.05) among groups for the same day.

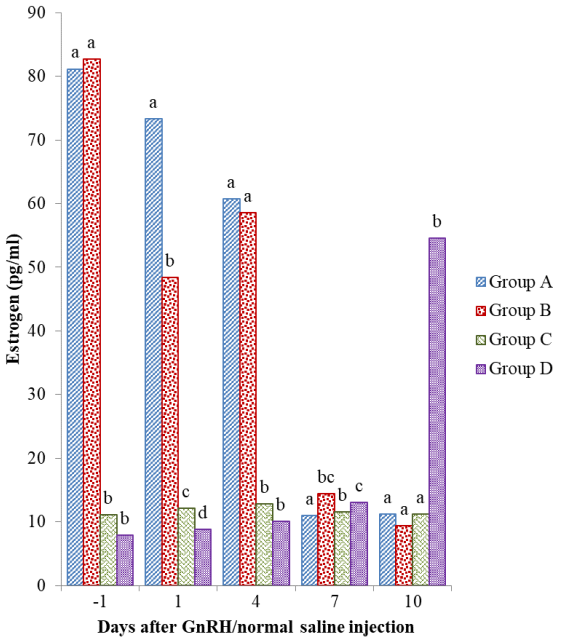

Fig. 2: Serum estrogen concentrations in camels of four groups at various days after GnRH/normal saline treatment. Different alphabets at the bars indicate significant difference (P<0.05) among groups for the same day.

groups and remained almost unchanged throughout the study, suggesting that no pre-ovulatory LH surge was noted in females of this group.

The treatment of camels with GnRH did not affect serum FSH level in any of the four groups. Marginal but non-significant differences were observed among the groups, with the control group of non-breeding season had the lowest mean FSH concentration, while the treated group of breeding season had the highest mean FSH concentration (Table 1). The day wise mean FSH concentration did not follow any specific pattern, except that it remained higher for the treated group of breeding season than both groups of non-breeding season (Fig. 4).

Pregnancy rate: Pregnancy rate in camels of treated group of breeding season was significantly higher (P<0.05) than that of other three groups B, C and D (Fig. 5). Moreover,

Table 1: Mean (± SE) values of progesterone, estrogen, LH and FSH in female dromedary camels of treated and control groups during breeding and non-breeding seasons

Seasons	Groups	Progesterone (ng/ml)	Estrogen (pg/ml)	LH (ng/ml)	FSH (mIU/ml)
Breeding	A	2.10±0.32[a]	47.49±5.57[a]	6.73±1.01[a]	12.82±1.05[a]
	B	1.16±0.33[b]	42.68±9.23[a]	4.15±0.93[b]	12.25±.65[a]
Non- Breeding	C	1.07±0.24[b]	18.87±4.81[b]	5.56±0.81[c]	12.02±0.42[a]
	D	0.24±0.02[c]	11.77±0.72[b]	1.05±0.15[d]	11.82±0.65[a]

Values in the same column with different superscripts differ significantly (P<0.05).

Table 2: Mean (± SE) values of serum Calcium, Phosphorus, Zinc, Manganese and Magnesium in dromedary camels of different groups during breeding and non-breeding seasons

Seasons	Groups	Calcium (mg/dL)	Phosphorus (mg/dL)	Zinc (µg/dl)	Manganese (mg/dl)	Magnesium (mg/dl)
Breeding	A	13.62±1.27[a]	3.75±0.21[a]	90.01±0.62[a]	27.55±1.05[a]	2.69+0.02[a]
	B	14.05±0.59[a]	3.96±0.34[a]	92.29±0.54[a]	31.94±1.19[a]	2.71+0.02[a]
Non- Breeding	C	13.77±1.27[a]	4.06±0.32[a]	90.01±0.62[a]	30.09±0.76[a]	2.69±0.02[a]
	D	13.31±0.63[a]	3.67±0.29[a]	92.29±0.54[a]	30.14±1.30[a]	2.71±0.02[a]

Values in the same column with same superscripts indicate non-significant difference.

Table 3: Mean (± SE) values of serum Calcium, Phosphorus, Zinc, Manganese and Magnesium in dromedary camels of four group at different days with reference to GnRH/normal saline treatment

Seasons	Groups	Minerals	Days of GnRH/normal saline injection				
			-1	1	4	7	10
Breeding	A	Calcium	12.50±0.58[a]	13.06±0.59[a]	13.69±1.25[a]	14.99±6.45[a]	13.87±1.00[a]
		Phosphorus	3.40±0.55[a]	4.20±0.68[a]	3.75±0.52[a]	3.77±0.22[a]	3.63±0.29[a]
		Zinc	90.06±1.3[a]	91.14±1.16[a]	90.29±1.81[a]	88.71±1.84[a]	89.86±0.67[a]
		Magnesium	2.70±0.02[a]	2.70±0.06[a]	2.70±0.03[a]	2.63±0.04[a]	2.69±0.05[a]
		Manganese	24.60±1.64[a]	28.07±2.38[a]	28.50±2.99[a]	27.55±3.15[a]	29.03±1.42[a]
	B	Calcium	12.43±0.24[a]	16.40±0.58[a]	11.70±0.09[a]	12.67±0.09[a]	17.07±0.23[a]
		Phosphorus	4.43±0.34[a]	2.85±0.16[a]	4.16±0.24[a]	3.90±0.31[a]	5.64±0.31[a]
		Zinc	91.77±1.53[a]	92.48±1.88[a]	93.29±0.89[a]	91.12±0.47[a]	92.80±1.38[a]
		Magnesium	2.71±0.03[a]	2.73±0.07[a]	2.72±0.04[a]	2.67±0.07[a]	2.75±0.01[a]
		Manganese	32.51±2.59[a]	35.00±0.84[a]	28.11±2.60[a]	29.51±4.00[a]	34.59±0.92[a]
Non-breeding	C	Calcium	12.17±1.01[a]	13.29±1.04[a]	13.41±1.11[a]	15.50±6.31[a]	14.51±1.16[a]
		Phosphorus	5.07±0.33[a]	3.67±0.22[a]	4.20±0.21[a]	2.20±0.12[a]	3.70±0.38[a]
		Zinc	90.40±1.30[a]	91.57±1.08[a]	91.24±1.58[a]	89.39±1.64[a]	90.55±0.90[a]
		Magnesium	2.70±0.02[a]	2.70±0.06[a]	2.72±0.03[a]	2.63±0.04[a]	2.69±0.05[a]
		Manganese	28.76±2.16[a]	31.55±1.70[a]	29.78±1.60[a]	30.12±1.65[a]	30.21±1.65[a]
	D	Calcium	11.80±1.15[a]	14.80±1.55[a]	11.23±0.24[a]	12.40±0.21[a]	16.30±1.05[a]
		Phosphorus	3.97±0.58[a]	3.73±0.47[a]	4.617±0.37[a]	3.11±0.65[a]	4.49±0.35[a]
		Zinc	92.28±1.66[a]	93.22±1.23[a]	93.88±0.95[a]	91.38±1.03[a]	93.02±1.32[a]
		Magnesium	2.71±0.03[a]	2.73±0.07[a]	2.72±0.04[a]	2.67±0.07[a]	2.75±0.01[a]
		Manganese	27.45±3.93[a]	32.25±4.23[a]	29.34±2.84[a]	28.85±1.3[a]	32.79±2.24[a]

Values in the same row with same superscripts indicate non-significant difference.

pregnancy rate in the treatment group was higher than the respective control group during non-breeding season, indicting the efficacy of GnRH during non-breeding season. However, none of the 10 animals of the control group in non-breeding season (Group D) showed estrus and were not mated. Therefore, no pregnancy was recorded in females of this group. Moreover, difference in the pregnancy rate for the control group of breeding season and treatment group of non-breeding season was non-significant.

Serum mineral profile: The overall mean serum concentrations of Calcium, Phosphorus, Magnesium, Zinc and Manganese did not differ among four groups (Table 2). Similarly, mean serum concentrations of these minerals for four groups at different days with reference to GnRH/normal saline administration did not differ (Table 3).

DISCUSSION

Gonadotropin releasing hormone has long been considered as the main regulator of pituitary to release FSH and LH for subsequent ovarian estrogen and progesterone production to regulate female reproductive biology (Aritonang *et al.*, 2017). Manipulation of this hypothalamic-pituitary-ovarian axis is the main strategy to manipulate estrus cycle for enhanced livestock production. In the present study, exogenous GnRH administration resulted in significantly increased pregnancy rate both during breeding and non-breeding seasons. More impactful finding of the study is increased pregnancy rate by GnRH treatment during non-breeding season as compared to the respective control (28.6% vs 0%). However, it is note-worthy that none of the 10 animals of the control group in non-breeding season (Group D) showed estrus and were not mated. Therefore, no pregnancy was recorded in females of this group.

The strategy of exogenous GnRH administration seems to be useful to get pregnancy and avoid increased calving interval in camels if the breeding season has passed without conception. However, to achieve pregnancy through the use of GnRH or any other hormone in the female, either artificial insemination should be performed or prospective male should also be induced to seasonality for mating, as the reproductive ability of the male camel is more adversely affected by the season than that of she-camel (El-Allali *et al.*, 2018).

In the present study, GnRH treatment of she-camels resulted in increased serum LH and progesterone concentrations both during breeding and non-breeding seasons compared to the respective controls. These results regarding increased levels of LH after GnRH treatment agree with a previous study in 140 female camels (Quzy *et al.*, 2013). Similarly, our results regarding serum

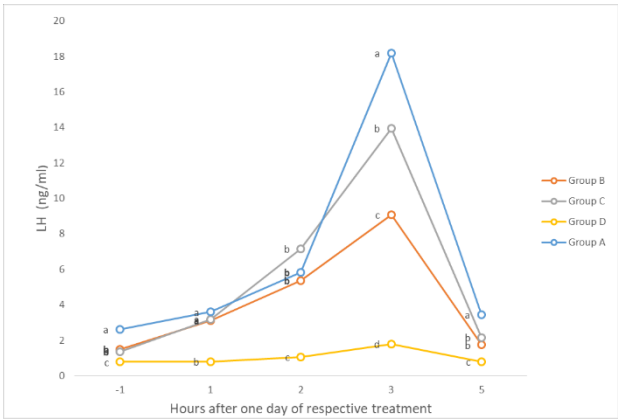

Fig. 3: Mean serum LH concentrations for treated and non-treated dromedary camels at different hours with reference to one day after GnRH/normal saline injection. Different alphabets indicate significant difference (P<0.05) among groups for the same day.

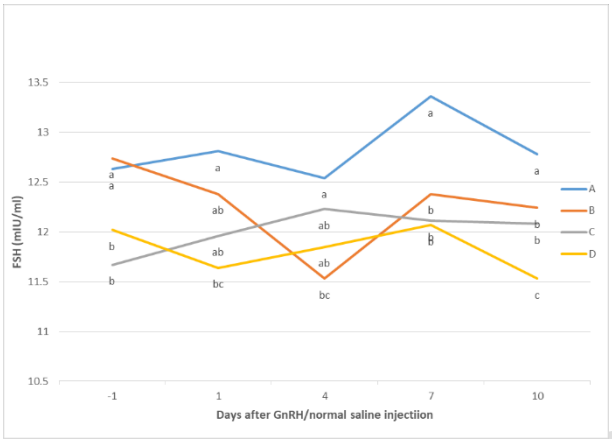

Fig. 4: Serum FSH concentrations in female dromedaries of four groups at different days after GnRH/normal saline treatment. Different alphabets indicate significant difference (P<0.05) among groups for the same day.

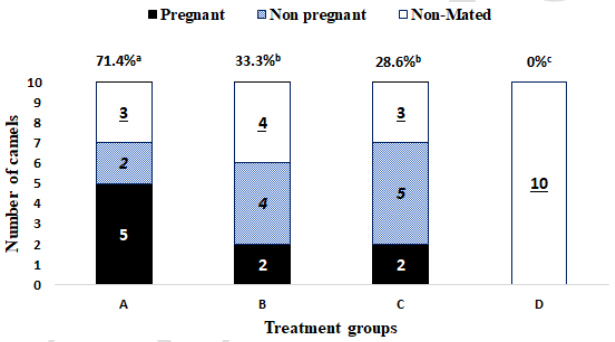

Fig. 5: Pregnancy rate in dromedary camels of the four experimental groups. Different alphabets with the values indicate significant difference (P<0.05) among groups.

progesterone are supported by an earlier study during breeding and non-breeding season in dromedary camels (Rawy *et al.*, 2014). Another study in 20 dromedary camels by Skidmore *et al.* (1996) revealed that serum concentrations of progesterone reached peak values in non-breeding season after GnRH treatment. Higher serum progesterone in treated and control groups of breeding season as compared to treated and control groups of non-breeding season indicates relatively higher number of treated animals ovulated and luteinized in response to GnRH administration. The reason is obvious as the GnRH, either exogenous or endogenous, stimulates the pituitary gland to release pre-ovulatory LH surge, which in turn stimulates a complicated cascade of events to induce ovulation and the resultant corpus luteum releases progesterone (Christou *et al.*, 2017).

In the present study, serum LH concentrations were higher in treated and control groups of breeding season as compared to treated and control groups of non-breeding season, indicating that GnRH has more pronounced effect during the breeding than the non-breeding season. It seems to be due to the fact that in seasonal breeders including camels, pituitary gland is more sensitive to GnRH and releases higher amount of LH during the breeding than the non-breeding season (Tibary and El-Allali, 2020). Furthermore, another reason for increased LH after GnRH treatment or mating lies in the mechanism of ovulation in camel, where ovulation occurs after mating due to the presence of ovulation inducing factor (OIF) in seminal plasma (Skidmore, 2018). This factor stimulates the release of GnRH, which in turn results in increased LH level, leading to ovulation, CL formation and increased serum progesterone level (Ratto *et al.*, 2019). The findings regarding increased serum LH level after GnRH administration in camels of Group C during non-breeding season are in agreement with the results of Adams and Ratto (2013), who recorded rise of LH level after mating in camels.

Estrus, and the follicular wave pattern in part, is under the effect of estrogen hormone. The estrogen concentration of GnRH treated group in breeding season was significantly higher as compared to treated group of non-breeding season. The most probable reason seems to be the increased sensitivity of pituitary to GnRH during breeding season as compared to non-breeding season (Tibary and El-Allali, 2020) which ultimately stimulated ovarian activity. These results are corroborated by the study on seven female camels injected with GnRH and showed increased estrogen concentration (Elias *et al.*, 1985).

In the present study, GnRH treatment had no effect on serum FSH concentration during the breeding, as well as non-breeding season. Available literature also suggests that FSH increases slowly with the increase in the size of Graafian follicles in camel, therefore present results may not be compared with the functional changes in cattle, buffaloes and other spontaneous ovulators. The FSH related findings of the present study are in close agreement with those of Zhao *et al.* (2001) and Hussein *et al.* (2008), who reported almost similar FSH values in female camel with active ovaries having mature follicle. However, in Bactrian camels, blood LH and FSH increase rapidly when a stimulus caused a mature follicle to ovulate (Li *et al.*, 2002). According to Marie and Anouassi (1986), FSH concentration increased 2.5 times of the basal level within 3-4 hours of ovulation inducing stimulus. It is possible that this rise in FSH secretion may be needed for the next wave of follicles if the previous mating did not result in conception. However, no such changes were recorded during the present study.

Deficiencies of minerals can cause metabolic diseases and infertility in livestock species including camel (Faye *et al.*, 1995). The serum mineral profile in the present

study was not affected by GnRH treatment and was within the normal range reported for camels. Thus, in female Dromedary camel, serum mineral profile does not seem to be affected by GnRH administration for estrus induction. One might expect variation in some minerals between breeding and non-breeding season. Camels are seasonal breeders and this seasonality is due to factors other than variation in serum mineral profile between breeding and non-breeding season (El-Allali *et al*., 2018).

However, in the present study, no consideration could be given to the physiological state of estrus cycle (cyclic or non-cyclic) of camels subjected to GnRH treatment. It would have been better if the animals of the same physiological state had been used for better understanding of possible effects of exogenous GnRH treatment on the parameters investigated in the study.

Conclusions: Based on the results of the present study, it can be concluded that treatment of camels with exogenous GnRH increases pregnancy rate, serum LH and progesterone levels during breeding and non-breeding season. However, it has no effect on serum estrogen, FSH and mineral profile in both the seasons.

Contribution of authors: HJ and ZIQ conceived the presented idea and supervised the work. MA, KAH and GY carried out the experimental work. All authors discussed the results and contributed to the preparation of final version of the manuscript.

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