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Manipulation of Reproductive Hormones Disorder in Sub-Fertile Male Dromedary Camels Using Exogenous Gonadotropic-Releasing Hormone (GnRH)

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Abstract: Six adult male dromedary camels were divided into 2 groups of 3 animals each. The first group was characterized as "fertile" and served as control, whereas the second group was characterized as "sub-fertile" and received 200 µg GnRH subcutaneously during the breeding season. At the time of GnRH treatment, the mean base-line LH concentration was significantly lower ($P < 0.01$) in the treated sub-fertile group (0.8 ±0.3 ng/ml) compared to the fertile one (7 ±1.0 ng/ml). Mean values of LH remained in these low levels until 4 hours following the treatment, thereafter it increased significantly reaching the highest value at 8 hours after GnRH injection. The average values of blood serum testosterone were significantly lower ($P < 0.01$) in the sub-fertile group (1.7 ±0.2 ng/ml) compared to the fertile one (3.7 ±0.2 ng/ml), while it reached an intermediate value (2.2 ±0.1 ng/ml) in sub-fertile camels following GnRH treatment. Meanwhile, the average values for prolactin concentrations were significantly higher ($P < 0.01$) in the sub-fertile group (276.3 ±5.8 mIU/l) compared to the fertile one (136.3 ±13.9 mIU/l), but it decreased to an intermediate value (243.0 ±6.6 mIU/l) in sub-fertile camels following GnRH treatment. However, neither day of the breeding season nor the reproductive status had significant effects on blood serum estradiol profile. These results indicate that sub-fertile camels responded to GnRH treatment, which may give a futuristic insignia on the use of hormones to improve the reproductive efficiency in bull camels during the breeding season.

Key words: Camel · Sub-fertile · GnRH · LH · Testosterone · Prolactin

INTRODUCTION

Generally, the reproductive performance of dromedary camels is extremely low compared to other farm animal species [1], where males have low mating efficiency throughout their reproductive lives [2]. The limited breeding opportunity, due to the short breeding season and limited libido of males, is considered the major factor contributing to low fertility in camels [3]. Recent experience in breeding management of camel herds has revealed an unexplained sort of sub-fertility and sterility prevailing in a significant number of males [4]. In the literature, there is a lack of publications concerning diagnosing and treating sub-fertility in male dromedary camels. However, determination of the blood circulating levels of different reproductive hormones has allowed good chances in the diagnosis of physiological status and pathological conditions in animals [5]. Anabolic steroids or testosterone therapies, which are sometimes used in attempts to improve male characteristics and libido, are not recommended for dromedary bulls in breeding work [6]. Stimulation of the reproductive function in males using GnRH has been effective in enhancing sexual behavior and semen parameters in different species [7-9]. However, only limited trials were conducted to stimulate libido and sexual activity in camels [10, 11]. But there is still uncertainty about the optimal treatment modality, schedule and duration of the therapy to treat sub-fertility using GnRH [9]. Monitoring the responsiveness of the pituitary gland to a single or repetitive dose of exogenous GnRH with the subsequent release of LH and FSH has been reported in stallions [8]. On the other hand, prolactin concentration has been reported to decrease significantly when transiting from the non-breeding season into the rutting season [12]. Monitoring the changes in blood serum prolactin levels is

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of great importance in determining reproductive disorders in male dromedaries, since the elevated prolactin levels is considered a causative factor of the reduced fertility and libido [13]. The aim of the present study is to improve the reproductive performance in dromedary camels during the breeding season by enhancing the efficiency of sub-fertile males using GnRH treatment.

MATERIALS AND METHODS

Animals and Management: This study was carried out at Maryout Research Station, Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Six adult male dromedary camels(*Camelus dromedaries*) aged 12-15 years and average weight 625 kg were used for two consecutive breeding seasons (December 2008 - March 2009) and (December 2009- March 2010). The animals were fed on a maintenance ration composed of a concentrate mixture (50% corn, 47% barley, 2% minerals, 1% salt) at the rate of 4 kg /head/day, while Egyptian clover (*Trifolium alexandrinum*) hay was offered *ad lib*. Fresh water was presented once daily in mid-day. The animals were divided into 2 groups of 3 animals each. The first group was characterized as "fertile" and served as control, where the animals showed normal sexual and mating behaviors, semen donation and physical characteristics. However, the reproductive status history for the second group for the last five years reveals disorders in sexual behavior, semen donation and accessibility. Therefore, this group of animals was characterized as "sub-fertile".

Data Collection

Meteorological Parameters: Climatically, location of Maryout Research Station is considered a semi-arid region. Average values for meteorological data including ambient temperature (°C), relative humidity (%) and wind speed (km/h) in the area of the study were obtained from the internet for two consecutive breeding seasons; (December 2008 - March 2009) and (December 2009 - March 2010).

Samples for Hormonal Profiles Analysis: Throughout the period of the study, blood samples were taken bi-weekly from the jugular vein of all animals at 8 a.m. before access to feed and water. The samples were withdrawn into non-heparinized tubes and then were transferred to a refrigerator at 5°C. Blood serum was separated by letting the collection tubes stand oblique in their holder for 24 hours. Blood serum then was collected and stored at -20°C until analysis. Testosterone and estradiol 17-β profiles were assayed using microplate EIA kits obtained from Monobind Inc. Lake Forest, CA, USA. The lower detection limits were 0.38 pg/ml and 0.29 ng/ml plasma, while the intra and inter assay CV's were (4.5, 8.8 %) and (6.3, 9.2 %) for both testosterone and estradiol kits, respectively. These values are based on the means of low, medium and high quality control samples measured in 10 assays. Prolactin profile was analyzed using an ELISA sandwich immunoenzymatic quantitative test. The kit was obtained from Diagnostic System Italy (DSI), Gallarate, Varese, Italy. The lowest detection limit for the kit was 10 mIU/l with a dynamic range between 0-2000 mIU/l based on 10 replicate analyses. The intra and inter assay CV's were 2.2 and 2.7 %, respectively.

GnRH Treatment: At the peak of the breeding season (1st of February), the sub-fertile camels were injected subcutaneously with 200 μg GnRH (Gonabreed®, Gonadorelin acetate, Parnell Laboratories, Australia, PTY. LTD.). The injection was done by using a clinical needle and targeted the back of the neck in the area right under the poll gland location.

Samples for LH Determination: Blood samples were taken from the jugular vein of both control and treated camels immediately before GnRH injection and then every two hours for the subsequent 24 hours following injection. Blood serum then was obtained and stored in the same way that was previously described. Luteinizing hormone (LH) profile in both the control and treated animals was analyzed using an ELISA sandwich immunoenzymatic quantitative test for camel LH determination obtained from ReproPharm SAS, Nouzilly, France.

Statistical Analysis: All blood serum hormonal profiles were statistically analyzed using a general linear model (GLM) procedure for repeated measurements (SPSS, 2006) [14]. The differences between means were detected using Duncan's Multiple Range Test [15].

RESULTS

The results presented in Table 1 showed that LH exhibited an oscillatory releasing pattern in both fertile (control) and sub-fertile (treated) animals throughout the 24 hours. The highest LH concentration in the fertile group was 8 ±0.9 ng/ml while

Table 1: Mean values (± SE) of luteinizing hormone (LH, ng/ml) concentration in fertile and GnRH-treated sub-fertile camels throughout 24 hours following injection

	A.M.				Time (Hours)					P.M.			
Group	0*	2	4	6	8	10	12	14	16	18	20	22	24
Fertile	$7.0^{ab,A}$±1.0	$6.5^{ab,A}$±1.0	5.3^{abc}±0.9	$4.5^{abc,B}$±1.3	$6.0^{abc,B}$±1.3	$4.8^{abc,B}$±2.1	5.5^{abc}±0.8	6.3^{abc}±1.0	3.0^{bc}±1.2	$8.0^{a,A}$±0.9	1.9^{e}±0.7	$2.5^{bc,B}$±1.2	5.6^{abc}±1.7
GnRH-treated	$0.8^{c,B}$±0.3	$0.5^{c,B}$±0.1	$1.3^{de,B}$±0.6	$6.8^{ab,A}$±0.8	$8.7^{a,A}$±0.7	$6.0^{abc,A}$±0.8	6.0^{abc}±1.0	6.3^{abc}±1.7	3.8^{bcde}±1.9	$3.5^{bcde,B}$±1.2	2.3^{cde}±0.4	$4.5^{abcde,A}$±2.0	5.1^{abcd}±2.0

[a-c] Values in the same row with different superscript letters differ significantly (P < 0.01)

[A,B] Values in the same column with different superscript letters differ significantly (P < 0.01)

*Time (0) is the time of injecting sub-fertile animals with 200 µg GnRH subcutaneously

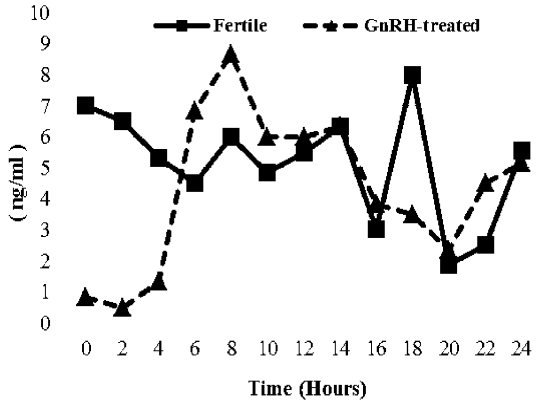

Fig. 1: Mean values for blood serum LH (ng/ml) in fertile and GnRH-treated sub-fertile bull camels throughout 24 hours following injection during the breeding season

the lowest was 1.9 ±0.7ng/ml. At the time of GnRH treatment, the mean base-line LH concentration was significantly lower (P< 0.01) in the sub-fertile group (0.8 ±0.3 ng/ml) compared to the fertile one (7±1.0 ng/ml). At six hours following the treatment, LH concentration reached a significant higher (P< 0.01) value in treated animals compared to the control ones with values being 6.8 ±0.8 and 4.5 ±1.3 ng/ml, respectively. Mean values of LH in sub-fertile camels remained in low levels until 4 hours following the treatment, thereafter it increased significantly (P< 0.01) reaching the highest value at 8 hours after GnRH administration (8.7 ±0.7 ng/ml), which was significantly higher (P < 0.01) than that of the control group (6 ±1.3 ng/ml). Then treated sub-fertile group exhibited a fluctuated but declining LH trend, which was similar in level to that of the control animals (Fig. 1). The results also revealed that, at the beginning of the breeding season, mean blood serum testosterone concentration was significantly low (P<0.01) in both fertile and sub-fertile animals with values 2 ±0.4 and 1.4 ±0.2 ng/ml, respectively. As the rut progressed, testosterone level began to increase significantly (P< 0.01) in the fertile group reaching the highest value at mid-February (5.0 ±0.1 ng/ml) compared to sub-fertile group (2.4 ±0.1 ng/ml), thereafter it began to decline towards the end of the breeding season returning to the basal level by mid-March. However, testosterone concentrations in sub-fertile animals remained in fairly constant basal levels throughout the breeding season (Table 2). On the other hand, blood serum testosterone increased significantly in sub-fertile camels following GnRH injection reaching a maximum value of 3.5 ±0.9 ng/ml at seven days after treatment, which was significantly higher (P<0.01) than that before treatment (2.1 ±0.3 ng/ml). Blood serum testosterone then exhibited similar trend in treated animals to that of the fertile ones towards

Table 2: Blood serum testosterone (ng/ml), prolactin (mIU/l) and estradiol 17-β (pg/ml) concentrations (Mean±SE) in fertile, sub-fertile and GnRH-treated sub-fertile camels during the breeding season

		December		January		February			March		
Item	Group	1-Dec	15-Dec	1-Jan	15-Jan	1-Feb*	7-Feb	15-Feb	1-Mar	15-Mar	Average
Testosterone											
(ng/ml)	Fertile	$2.0\pm0.4^{de,A}$	$2.3\pm0.1^{cd,A}$	$3.0\pm0.5^{bc,A}$	$4.9\pm0.1^{a,A}$	$5.0\pm0.1^{a,A}$	$4.7\pm0.3^{a,A}$	$5.1\pm0.1^{a,A}$	$3.6\pm0.2^{b,A}$	$1.3\pm0.2^{e,B}$	3.6 ± 0.2^{A}
	Sub-fertile	$1.4\pm0.2^{b,B}$	$1.2\pm0.1^{b,B}$	$1.3\pm0.1^{b,B}$	$1.9\pm0.2^{ab,B}$	$2.4\pm0.2^{a,B}$	$2.1\pm0.3^{ab,C}$	$2.4\pm0.1^{a,C}$	$1.3\pm0.2^{b,B}$	$1.3\pm0.1^{b,B}$	1.7 ± 0.2^{C}
	GnRH-treated	$1.3\pm0.1^{c,B}$	$1.4\pm0.2^{c,B}$	$1.6\pm0.1^{c,B}$	$1.5\pm0.1^{c,B}$	$2.1\pm0.6^{b,B}$	$3.5\pm0.9^{a,B}$	$2.7\pm0.8^{ab,B}$	$3.1\pm0.9^{a,A}$	$2.8\pm0.9^{ab,B}$	2.2 ± 0.1^{B}
Prolactin											
(mIU/l)	Fertile	$273.3\pm6.7^{e,B}$	$250.0\pm5.8^{a,C}$	$93.3\pm8.8^{c,C}$	$93.3\pm13.3^{c,B}$	$86.7\pm3.3^{c,C}$	$86.7\pm6.7^{c,C}$	$93.3\pm6.7^{c,C}$	$96.7\pm8.8^{c,C}$	$153.3\pm17.6^{b,C}$	136.3 ± 3.9^{C}
	Sub-fertile	$290.0\pm5.8^{ab,A}$	$280.0\pm11.5^{bc,B}$	$280.0\pm11.5^{bc,A}$	$286.7\pm6.7^{abc,A}$	$233.3\pm13.3^{d,B}$	$280.0\pm11.5^{bc,A}$	$266.7\pm17.6^{bcd,A}$	$246.7\pm6.7^{cd,A}$	$323.3\pm14.5^{a,A}$	276.3 ± 5.8^{A}
	GnRH-treated	$246.7\pm24.0^{bcd,C}$	$300.0\pm11.5^{a,A}$	$256.7\pm23.3^{abc,B}$	$283.3\pm16.7^{ab,A}$	$300.0\pm0.1^{a,A}$	$226.7\pm17.6^{cde,B}$	$190.0\pm10.0^{e,B}$	$196.7\pm8.8^{de,B}$	$186.7\pm6.7^{e,B}$	243.0 ± 6.6^{B}
Estradiol											
(pg/ml)	Fertile	73.3±13.3	46.7±6.7	66.7±17.6	53.3±24.0	53.3±6.7	60.0±0.0	53.3±6.7	60.0±20.0	33.3±6.7	55.6±4.3
	Sub-fertile	60.0±20.0	53.3±6.7	46.7±13.3	86.7±37.1	53.3±24.0	53.3±6.7	33.3±23.3	46.7±6.7	30.0±10.0	51.5±6.1
	GnRH-treated	60.0±0.0	53.3±13.3	53.3±6.7	46.7±13.3	60.0±0.0	46.7±13.3	66.7±6.7	53.3±6.7	53.3±6.7	54.8±2.7

[a-c] Values in the same row with different superscript letters differ significantly (P < 0.01)

[A-C] Values in the same column with different superscript letters differ significantly (P < 0.01)

* Day of injecting sub-fertile animals with 200 µg GnRH subcutaneously during the breeding season

the end of the breeding season. The average values of blood serum testosterone were significantly higher (P<0.01) in the fertile group (3.6 ±0.2 ng/ml) compared to the sub-fertile one (1.7±0.2 ng/ml). However, the average testosterone concentrations increased reaching an intermediate value (2.2±0.1 ng/ml) in sub-fertile camels following GnRH injection. Concerning blood serum prolactin profile, the results indicate that, at the beginning of the breeding season, mean blood serum prolactin concentration was significantly higher (P < 0.01) in sub-fertile than in the fertile ones with values 290 ±5.8 and 273.3 ±6.7 mIU/l, respectively. As the rut progressed, prolactin levels began to decrease significantly (P<0.01) in the fertile group reaching the lowest significant value during February (86.7±6.7 mIU/l). Then it began to elevate towards the end of the breeding season returning to a higher level of 153.3 ±17.6 mIU/l by mid-March (Table 2). On the other hand, prolactin concentrations in sub-fertile animals remained in fairly constant high levels throughout the breeding season. However, following GnRH treatment, sub-fertile camels showed a significantly lower (P<0.01) prolactin concentration (190 ±10 mIU/l) at mid-February and remained in low levels even by the end of rut reaching a minimum value of 186.7±6.7 mIU/l by mid-March. The average values for prolactin concentration was significantly higher (P<0.01) in the sub-fertile group (276.3 ±5.8 mIU/l) compared to the fertile group (136.3 ±13.9 mIU/l) this value reached an intermediate value (243.0 ±6.6 mIU/l) in the sub-fertile group following GnRH treatment. In the meantime, neither day of the breeding season nor the reproductive status had significant effects on blood serum estradiol profile. However, a marked increase in mean estradiol concentrations was observed at the peak of the breeding season (February) in sub-fertile camels with values being 60 and 66.7±6.7 pg/ml at the 1st of February and mid-February, respectively. These values markedly decreased in sub-fertile camels following GnRH injection reaching low level (33.3 ±3.3 pg/ml) at mid-February (Table 2).

DISCUSSION

The elevated basal LH level in blood serum of the fertile group of camels during the breeding season is similar to earlier investigations [16]. However, the observed low LH concentration below the normal base-line level in the sub-fertile group prior to GnRH treatment is in conformity with previous results [17] in sexually inactive dromedary camels. Monitoring the responsiveness of the pituitary gland to a single or repetitive dose of exogenous GnRH with the subsequent release of LH and FSH has been reported in stallions [8]. The fluctuated LH releasing pattern observed in the present investigation in sub-fertile camels following GnRH treatment is similar to previous findings in bulls [18]. Meanwhile, the pattern of blood serum testosterone profile observed in the fertile camels is in agreement with previous reports [19]. The elevated testosterone levels during breeding season may be attributed to an increase in sensitivity of Leydig cells to LH or enhanced secretions of LH from the pituitary gland or both [20]. Moreover, the pattern of blood serum prolactin profile observed in the fertile camels is in agreement with previous observation in dromedary camels [12] who reported that serum prolactin levels decrease significantly when transiting from the non-breeding season into the rutting season. However, if prolactin levels are elevated it may, then, considered a causative factor of the reduced fertility and libido in the male camel [13]. On the other hand, although a marked increase was observed at the peak of rut in sub-fertile camels, the pattern of blood serum estradiol profile observed in the fertile ones is in agreement with reports [21].

Results of the present study indicated that sub-fertile camels were suffering hyperprolactinaemia during the breeding season. Since prolactin has an anti-gonadotropic action at the gonadal level [22], hyperprolactinaemia reduced the synthesis and secretion of gonadotropins from the pituitary which decreased their level in blood serum [13]. In males, a higher level of LH is important to stimulate the androgen-secreting interstitial tissues and, hence, testosterone production [23]. Therefore, in the present study, the decreased LH level observed in sub-fertile camels due to hyperprolactinaemia was followed by a subsequent reduction in testosterone concentration during the breeding season. Similar findings have been reported in male rats where induced hyperprolactinaemia caused a prolonged suppression of gonadotropins concentrations in both serum and pituitary tissue [22] and decreased testosterone production [24]. On the other hand, the reduction in prolactin levels in GnRH-treated sub-fertile camels were accompanied by increased basal LH levels and testosterone concentration in serum. This reflects the efficiency of the treatment in setting the reproductive endocrine mechanism in sub-fertile camels during the breeding season. Stimulatory therapy with GnRH has been reported to successfully induce normal libido and fertility in goats [7], enhance sexual activity in stallions outside the breeding season [8] and stimulate libido and sexual activity in camels [10, 11].

CONCLUSION

The present study demonstrates that treating sub-fertile camels with exogenous GnRH successfully altered the abnormal reproductive hormones pattern by enhancing and stimulating the synthesis and release of gonadotropins from the pituitary and, thus, enhancing the pituitary-gonadal axis resulting in increased testosterone production.

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