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Effects of a GnRH administration on testosterone profile, libido and semen parameters of dromedary camel bulls

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ABSTRACT

GnRH treatment has been suggested to increase testosterone levels temporarily and to stimulate libido in stallions, but its use has not fully ascertained in dromedary camels. The aim of this work was to study the effects of administering 100 μg of GnRH on testosterone profile, libido and semen parameters in dromedary camels. The same bulls were used as self-controls and experimental group. Blood samples were collected every 20 min (T0–T12) for 4 h, and semen collections were performed over a 2-hour period after T12. GnRH was administered immediately after T0. In GnRH-treated bulls, testosterone levels showed an upward trend, peaking after 140 min, and then slowly decreasing. GnRH administration also led to a decrease in mating time and an increase in spermatozoa concentration. Overall, it seems that administration of 100 μg GnRH might increase testosterone levels temporarily and enhance camel reproduction performance.

1. Introduction

The short breeding season and lack of libido of dromedary bulls are the major complaints in dromedary camel reproduction management. The seasonality of the animals, their libido, and their mating performances are, indeed, complex traits affected by management, environmental, nutritional, psychological, hormonal, and physical factors (Al-Qarawi, 2005; Stout, 2005; Fatnassi et al., 2014a). Depending on those factors, the duration of the breeding season varies from 2 to 6 months (El-Wishy, 1988); for instance, in the United Arab Emirates it lasts from October to late April (Tibary and Anouassi, 1997), while in Tunisia it is reported to be from December to March (Hammadi et al., 2008) or even April (Burgermeister, 1975). The libido of male dromedary camels generally peaks during the coolest, rainy months of the year, and then declines; some males lose libido early, while others maintain it for longer periods. However, the reasons for this variability are still not fully understood (Deen et al., 2003).

High testosterone levels are responsible for morphological and histological changes in the testicles and in the accessory sex glands, as well as for augmentation of the camel bull's libido and typical sexual behavior patterns (Yagil and Etzion, 1980; Tingari et al., 1984; Azouz et al., 1992; Pasha et al., 2013; Fatnassi et al., 2014b). Consequently, it has been reported that, outside the breeding season, the decline in libido is due to testosterone concentration falling below a critical level (Deen et al., 2005; Deen, 2008). Low testosterone concentration could be responsible not only for the failure of male libido, but also for increased reaction times, increased number of mounts per successful ejaculation, lower ejaculate volume and lower number of sperms per ejaculate (Al-Qarawi, 2005). The use of artificial insemination (AI) in dromedary camels may solve the problem of low reproductive performance due to male seasonality (Skidmore et al., 2013). However, mating failures also lead to low semen volume, a decreased concentration in spermatozoa and less ejaculates, which have the additional knock-on effect of reducing the number of frozen semen doses for artificial insemination (El-Hassanein, 2003).

Different treatments have been proposed to enhance the libido and mating ability of dromedary camel bulls. A testosterone propionate treatment has been used, but this treatment resulted in decreased testicular weight and size, reduction in sperm production and lower sperm motility (Al-Qarawi et al., 2001; El-Belely and Al-Qarawi, 2003). The administration of 100 μg of gonadorelin (GnRH analog) has been suggested to temporarily increase blood testosterone levels in stallions to stimulate their libido and mating ability (McDonnell, 1992; Stout, 2005). The effects of GnRH have also been previously investigated in

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dromedary camel bulls, but results are conflicting. It has been reported that continuous administration of 175 μg of gonadorelin induced a clinical improvement in the male camel's libido outside the breeding season (Moslah et al., 1992). However, endocrinological investigations into this therapeutic regimen showed that the high testosterone levels (above 20 ng/ml), reached during the first 4 days of the treatment, induced a negative feed-back on the hypothalamic–pituitary–gonadal axis, resulting in a decrease in the bulls' testicular diameter and in a significant drop in their testosterone levels (Quaranta et al., 2010).

It has been hypothesized that 100 μg of gonadorelin might temporarily raise plasma testosterone levels and libido in camel bulls, so the aim of this work was to characterize plasma testosterone levels for 4 h after this treatment. Semen collections were also carried out, between 4 and 6 h after GnRH administration, and mating behavior and semen parameters were evaluated.

2. Material and methods

2.1. Animals and housing

The trial was carried out at the end of the breeding season, from the 20th to the 29th of March 2013, in camels reared under an intensive system at the Arid Lands Institute's experimental station in Médenine, Tunisia (33° 30′ N, 10° 40′ E). Five clinically healthy male dromedary camels (*Camelus dromedarius*), with a mean body weight of 516 ± 25 kg and good body condition score (3.5 ± 0.3 arbitrary units; range from 0 to 5 (Faye et al., 2001)), were used for this study.

In the previous summer, each bull was kept in a single open-air paddock shaded by trees whereas, starting from October, they were put into single stalls (height = 3 m, length = 5 m and width = 3 m) with sand floors. The stalls were located far from the females' pen, thus preventing any visual or physical contact between the bulls and the dams. Feeding quantity and quality remained constant during the breeding season and throughout the whole experiment. The diet met the maintenance requirements, and water was made available once every two days (Laudadio et al., 2009).

During the breeding season, all bulls were used for semen collection and were normally divided into two groups (Group I: camel 504, 514, 515; Group II: camel 8, 808). They were well accustomed to this practice and to their husbandry system. To avoid any changes to normal management during the trial, the division between the two groups was maintained throughout.

2.2. Experimental design

The experiment lasted 9 days. On day 1, indwelling jugular catheters were inserted once slight sedation of the animals (5 mg/100 kg b.w. of Acepromazine, Combistress®, Belgium), surgical preparation of the neck, and local anesthesia (Lidocaine 2%, Unimed, Tunisia) had been conducted at the catheter insertion site. The catheters were regularly flushed with a heparin solution (20 I.U./ml) throughout the experimental period.

On days 2 and 3, blood and semen samples were collected from Group I and Group II, respectively, in order to obtain baseline testosterone measurements (self-control: SC).

On days 4 and 5, blood and semen samples were again collected from Group I and Group II, respectively. This time, 100 μg gonadorelin diacetate tetrahydrate (GnRH analog) (Fertagyl®, International Intervet) was injected through the catheter immediately after the first blood sample. To increase the number of observations, the experiment was repeated twice more, first on days 6 and 7, and then on days 8 and 9. GnRH was thus administered to each animal for a total of three times, once every 48 h, and blood and semen collections were performed after it.

2.2.1. Blood sampling procedure

In the self-control group (SC), blood samples were drawn at 20-minute intervals from 2.00 p.m. (T0) to 6.00 p.m. (T12), for a total of thirteen blood samples. In the experimental group (EG), GnRH was administered immediately after the first blood sample (T0, at 2.00 p.m.); the blood samples were then collected according to the same schedule. Blood samples were collected through the catheters using syringes and then drawn into Venoject® tubes (Terumo Europe, Leuven, Belgium) with lithium heparin, and kept in ice until plasma was separated, within 2 h of collection, by centrifugation at 4 °C for 15 min at 3000 rpm. Plasma samples were stored at −20 °C until analyzed for testosterone concentration.

2.2.2. Semen collection procedure

The first semen collection session took place within 10 min of T12 (the end of serial blood sampling) and the mating order of the males was randomized. Briefly, the first male began the semen collection procedure around 6.00 p.m., while each consecutive male started collection as soon as the previous procedure had been completed. All procedures were carried out over a 2-hour period after T12, from 6 p.m. to 8 p.m. A receptive female was led into the open paddock, near the males' stalls, and restrained in sternal recumbence. A bovine artificial vagina (IMV, France), with a water-jacketed tube located at the end of the collection funnel, was used for semen collection. When the operators were ready, the door of the male's stall was opened, the mating session began and was recorded by a video camera (Sony Camcorder digital video). The semen collection sessions were scheduled, according to a standard method, proposed by Padalino et al. (2015). Briefly, the semen collection was scheduled according to the following timings: 1) maximal latency time before mounting (the time from the moment the male exited the stall until he sat for the first time on the female for mounting): 15 min. 2) Maximal mating time (the time from first sitting on the female to his return to the stall = service/ejaculation time + standing over the female + walking around): 45 min. 3) Maximal time between two copulations: 30 min. 4) Maximal standing on/over the female time (the time when the male camel is near or over the female): 30 min. 5) Maximal walking around time (the time when the camel is walking in the semen collection pen, being uninterested in, and distant from, the female): 6 min. When a camel exceeded one of the above time limits, the session was ended.

2.3. Analysis of parameters

2.3.1. Testosterone analysis

Plasma testosterone concentrations were determined by radioimmunoassay (RIA) (Immunotech Beckman Coulter Company, Ref 1087, Marseille, France), a gamma counter was used for counting, and the resulting number was converted by way of a calibration curve to measure the hormone levels in unknown samples. Sensitivity was 0.04 ng/ml and intra- and interassay coefficients of variation were 7.4% and 11.1%, respectively.

2.3.2. Behavioral parameters

The semen collection videos were analyzed by filling out a “focal animal sampling” ethogram (Padalino et al., 2013). The durations of the following behavioral states were noted down: latency time, service/ejaculation time (ST) (time of copulation with the artificial vagina), standing near/over the female (SOF), walking around (W); consequently, total mating time was calculated by adding together ST, SOF and W. Moreover, occurrence of the following behavioral events was also recorded: number of mounts, sound emission, defecation, urination, tail flapping, blathering, *dulaa* extrusion, yawning, teeth grinding, neck touching, sniffing and *flehmen* (Fatnassi et al., 2014b). The frequency of behavioral events was expressed as number of events per minute (n/min). Finally, the male camel libido was scored (Padalino et al., 2013).