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EFFECT OF BLOOD SAMPLING METHOD DURING A MATING TIME IN MALE CAMELS (DROMEDARY CAMELS)

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Abstract

This study was to shed light on levels of testosterone and cortisol hormones in male dromedary camels during the mating time, by collection the blood samples using the manual method and compared with the remote-controlled blood sampling (RBS) method, to assess whether either these methods had affected changes in the concentration of the hormones or not. The blood samples were collected from fifteen adult male camels, via two experiment with one-day intervals: first experiment by manual method during three periods (pre-mating, mating, and post-mating) one hour between each period, and the second experiment by the RBS in the same protocol. The serum testosterone and cortisol concentrations of all animals were determined via ELISA technique. The result which appearance a significant difference in the mating time used RBS compared with manual method. These findings might be due to the withdrawal of blood remotely which could cause a reduction of excitement in animals using the manual blood sampling at the presence of veterinarians, so it was considered as an ideal method to measure hormonal concentrations, especially in experiments which need accurate results.

***Key words*:** camels, cortisol, mating, remote-controlled blood sampling, testosterone

Introduction

The breeding season of dromedary camels is at the coldest winter months of the year (Padalino *et al*., 2015). In this season, these camels become very aggressive towards other males and humans and their handling is thus considered very difficult and the copulation begins with foreplays and such behaviors are disappeared after rutting (Fatnassi *et al*., 2014). Most researchers have adopted different procedures to determine the blood testosterone level in order to clarify the characteristics of reproductive phenomena, reach an understanding of physiological status, and also determine libido and sexual behaviors in these animals (El-Bahrawy *et al*., 2015). The cortisol hormone has been similarly used as an indicator of stress in dromedary camels (El-Khasmi *et al*., 2015) and in other animals (Tajik *et al*., 2016), the serum cortisol which increases during acute stress is largely made up of free cortisol; therefore, its concentration can be influenced by stress and physical stimulation of dromedary camels when they are exposed to stressors which also cause deregulation of sexual hormones (Majchrzak *et al.,* 2015; Dickens and Romero 2013). Collection of blood samples to measure hormones can be invasive, and values can potentially be confounded by handling stress which may lead to alternative measurements of hormone levels (Johnstone *et al.,* 2012). In this respect, remote-controlled blood sampling (RBS) is a powerful device to take blood samples and consequently give important information on animal health status, like hormonal access (Fonss and Munksgaard, 2008), yet the traditional or manual blood sampling method has its own restrictions and it cannot be possibly used due to male camel aggression during this period. The same restrictions can also lead to stress reactions even in animals accustomed to the given procedure (Hopster *et al.,* 1999) which results in increased levels of cortisol due to the activation of hypothalamic stimulation-pituitary-adrenal axis during this sampling (Sapolsky *et al.,* 2000). It is clear that the RBS device placed on animals will be more convenient than the manual blood sampling method, especially when a series of blood samples are needed (Goddard *et al.,* 1998). Accordingly, the present study aimed to verify the levels of testosterone and cortisol hormones in the breeding season of male dromedary camels during the mating time. To this end, blood samples were taken using the traditional method and compared with the levels of these hormones using the RBS device,

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to assess whether either the given method had affected changes in the concentration of the hormones or not, and to improve understanding of reproductive mechanisms and development.

Materials and Methods

The present study was conducted in the bio-molecular laboratory of the College of Biotechnology at the University of Al-Qadisiyah, Iraq. The blood samples were then collected from camel herds in southern of Iraq, during the rutting season (December-February/2017-2018), with intervals. There were a Thirty-five of adult males (8-10 years of age) with good bodily conditions. Animals' age was also determined based on dental formula described by Ibrahim *et al.,* 2009.

Experimental protocol

All samples were collected by two experiments with a one-day interval from a total numbers of the male camels marketed from dromedary camels which were clinically healthy.

First experiment

The blood samples (5 ml) were collected from the jugular vein by the manual (traditional) method via syringe (gauge 18) in three periods. The first period was before the male camel's exposure to estrus she-camel (pre-mating), the second period occurred one hour following the first period during the copulation time (mating period), and the third period took place after one hour from copulation (post-mating). In this experiment, the researchers had great difficulty in blood sample collection due to the camels became very dangerous and often unfriendly, so the camels were frequently motion and kicking. They also lost the jugular vein was puncture and occasionally the needle and the syringe were broken.

Second experiment

Following one day after the first experiment, the blood samples were taken remotely in the same periods by the RBS device (Fonss and Munksgaard. 2008) which was made locally with a simple modification (Fig. 1) to reducing the weight, size and facilitate its fixation on the camel's neck using installation belts to prevent animal sensitivity and reduce nervousness. All blood samples were further allowed to clot for two hours at room temperature and then centrifuged for 20 minutes at 3000 rpm. The supernatants were also collected carefully, and then placed in the eppindroph tub before storage at -20°C until hormonal analysis.

Fig. 1: (a) a chart of the RBS device (modified): 1-Restraint rings, 2-Motor base, 3- Receiver, 4-batteries (6 volt), 5-Plastic crosslink, 6-Test tube, 7-Pipes, 8-Peristaltic pump, 9-Cannula, 10-Pipes, 11-Motor of pump, 12-Bulb let, 13-Antenna, 14-Power switch; (b) a chart of the remote control: 1-Cover, 2-Battery (9 volt), 3-Control switch, 4-Power switch, 5-Power light, 6-Antenna, 7-Transmitter; (c): the RBS device (modified); (d) Remote Control.

Hormonal assay

The serum testosterone and cortisol levels of all animals were determined in the bio-molecular laboratory of the College of Biotechnology at the University of Al-Qadisiyah using the enzyme-linked immunosorbent assay (ELISA) in adult male camels, validated and accredited by male dromedary camel's serum (all samples were analyzed in duplicate). The results were then expressed in ng/ml in which both kits (MyBioSource Company, TESTO Elisa kit, Camel Testosterone ELISA Kit - MBS107991 and CORT Elisa kit, Camel Cortisol ELISA Kit- MBS082766/ USA) were also processed, according to the manufactory protocol.

Statistical analysis

All the values were expressed as mean ± standard error (SE) and subjected to analysis using two-way analysis of variance (ANOVA). The significance level among different parameters was calculated at $p<0.05$. The software used was the IBM SPSS program package (Version 23) (McDonald. 2014).

Results

First experiment

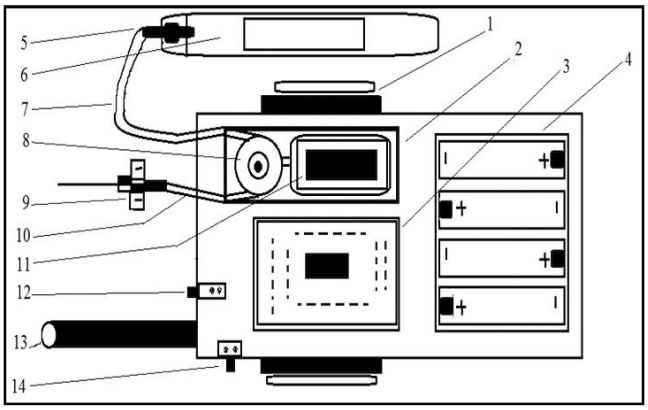
The findings revealed that the mean ± SE of the serum testosterone concentration in pre-mating, mating, and post-mating periods were 14.1438 ± 0.5833, 26.2675 ± 0.8523 and 24.037 ± 1.0071 ng/ml of; respectively, yet the serum cortisol in these periods were 44.8725 ± 5.0133, 32.4775 ± 1.9738, and 40.7688 ± 3.0897 ng/ml; respectively (Table 1 and Fig. 2).

Second experiment

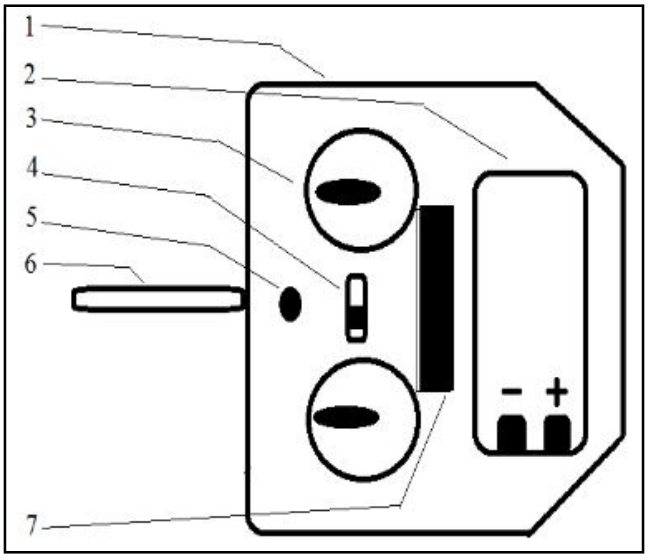
Using the RBS device, the concentration values of the serum testosterone concentration were reported by 16.8925 ± 0.7008, 32.8563 ± 1.3292, and 29.8519 ± 1.0736 in pre-mating, mating, and post-mating periods respectively. However, the means of serum cortisol concentration in three periods, such values were 30.1918 ± 3.0299, 21.4425 ± 1.8629, and 22.4013 ± 1.8645 ng/ml; respectively (Table 1 and Fig. 2).

Discussion

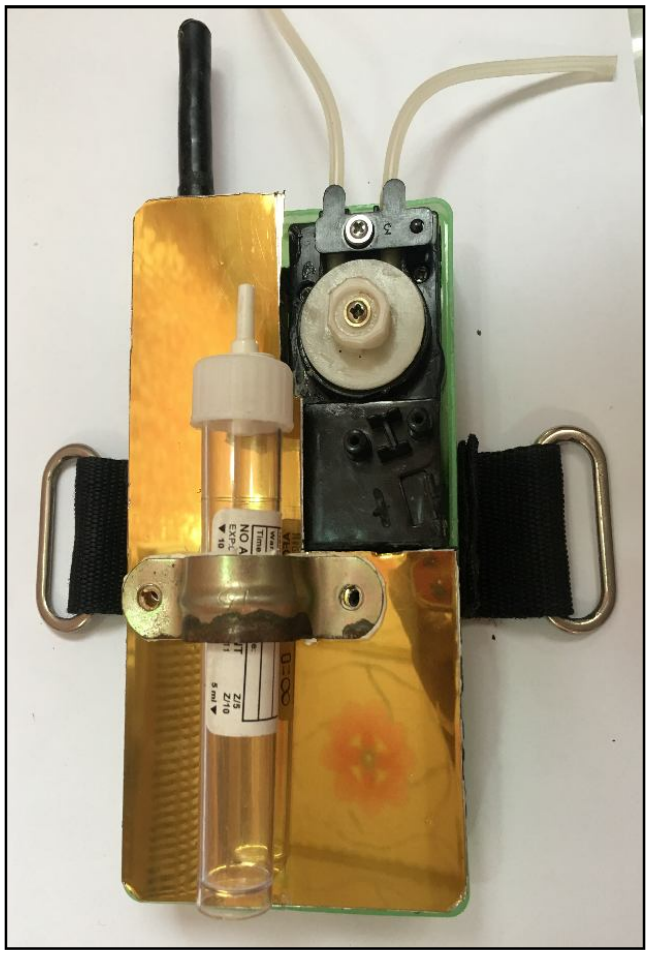
The present data obtained from the manual method of blood sampling (first experiment) indicated that the serum testosterone level increased in the mating time



(a)



(b)



(c)

(d)

compared with those in the pre-mating and post-mating ones (Table 1 and Fig. 2). The given rise may be due to interactions with libido in male camels, these findings were in agreement with (Aubè *et al*., 2017). The testosterone hormone acting as a positive regulator of sexual desire has been also reported in many animals like horses, camels (Stout, 2011), monkeys (Phoenix *et al*., 1977) and in humans (Podlasek *et al*., 2016). That were confirmed by studies of (Goldey and Van Anders, 2015) in which recorded decrease the testosterone level in men had led to reduced libido; moreover, increase in the activity of enzymes synthesizing the testosterone hormone was greater in the breeding than the non-breeding season (El-Kon *et al*., 2011). The activation of the hypothalamus-pituitary-adrenal axis and this can lead to a suppression of luteinizing hormone release because of the dysregulation of homeostasis (stress) and triggers an adaptive stress response (G¹dek-Michalska *et al*., 2013). Our study results were discrepancy with concluded of (Exton *et al*., 2001) that orgasm in humans did not acutely affect testosterone levels in the blood. It was also argued that sex in men did not have any effects on testosterone levels but rather it could positively influence the production of testosterone (Dabbs and Mohammed, 1992. Yet, a statistically significant increase in serum testosterone concentration in our study during mating time by using both sampling methods were in consonance with those experiments in men in which plasma levels of testosterone were compared and examined before and after mating periods, yet in the second experiment, the

Table 1: Concentrations of testosterone and cortisol hormones (ng/ml) using the manual method and the RBS device in three periods.

Periods	Testosterone (mean±SE)		Cortisol (mean±SE)	
	Manual	RBS devic	Manual	RBS device
Pre-mating	14.1438±0.5833 ^{Aa}	16.8925±0.7008 ^{Ba}	44.8725±5.0133 ^{Aa}	30.1918±3.0299 ^{Aa}
Mating	26.2675±0.8523 ^{Ab}	32.8563±1.3292 ^{Bb}	32.4775±1.9738 ^{Ab}	21.4425±1.8629 ^{Bb}
Post-mating	24.037±1.0071 ^{Ab}	29.8519±1.0736 ^{Bb}	40.7688±3.0897 ^{Aa}	22.4013±1.8645 ^{Bb}

^{A,B} Difference within a row ($p<0.05$).
^{a,b} Difference within a column ($p<0.05$).

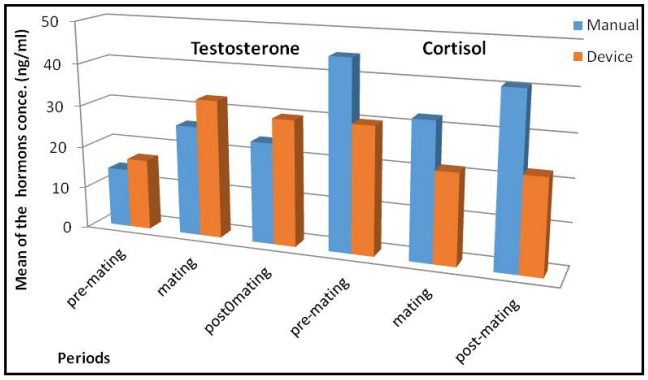


Fig. 2: Patterns of serum testosterone & cortisol concentrations in camels (ng/ml) suing the manual method and the RBS device. Significant differences were indicated ($p<0.05$) in mean comparisons between the three periods.

RBS device as another method employed for blood collection which was applied on the same camels after one day following the first experiment to record the results in the same periods of libido. These findings confirmed the relationship between testosterone hormone levels and mating efficiency. On the other hand, the concentration of this hormone was highly significant ($p<0.05$) in a way that it had elevated greater compared with the manual method (Table 1 and Fig. 2), The manual method might have caused stress or fear in animals, so the mean testosterone level in the pre-mating, mating and post-mating periods were significantly lower than those in the second experiment. The reason for given elevation was significantly reduced tension, stress, and fear in animals induced by veterinarians. So, the withdrawal of blood in routine methods by syringe or cannula may lead to the secretion of some hormones like cortisol, that may cause a reduction of secretion in the testosterone hormone during intercourse. That testosterone hormone could influence libido in male camels, one needs to assess the stress state when measuring free cortisol in serum (Peeters *et al.*, 2011). In this regard, the cortisol hormone level was elevated in all three periods using the manual method compared with the second experiment in which this level had declined because cortisol had been increased in response to physiological stress and fear which was in line with studies such as (Chen *et al.*, 2015 and Erickson *et al.*, 2003). According our result, we suggested the cortisol can be blocked by the testosterone actions and cause less testosterone which influences behaviors like mating since, but finding of (Tajik *et al.*, 2016) it was argued that the serum cortisol did not change significantly due to stress in the sheep and cattle; however, (Chen *et al.*, 2015) showed a significant increase which did not consistently change. The findings of this study were in line with other research investigations like (Gordon *et al.*, 2007) in which it was indicated that there were numerous interactions between stress and reproductive functions, and stress lead to stimulation of the hypothalamic-pituitary-adrenal axis might have reduced fertility in horses, caused by stress. In addition to the cortisol hormone in most mammals as well as fish and amphibians, corticosterone in reptiles and birds also plays an important role in all stasis as they are involved in the regulation of the hypothalamic-pituitary-adrenal axis (Haase *et al.*, 2016).

Conclusion

There was a statistically significant difference between the concentrations of the testosterone hormones during the mating period and intercourse. Using the RBS device which led to reduced cortisol concentrations, the testosterone levels increased. These findings might be due to the withdrawal of blood remotely which could cause a reduction of excitement in animals using the manual blood sampling at the presence of veterinarians, so it was considered as an ideal method to measure hormonal concentrations, especially in experiments which need accurate results.

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