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RELATIONSHIP BETWEEN PROGESTERONE CONCENTRATION THROUGHOUT MATING DAY AND PREGNANCY OF SHE-CAMELS.

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ABSTRACT

The present work was done to investigate the interrelationship between progesterone concentration in blood plasma and saliva of female - camels pre and post - mating day and pregnancy. Thirty healthy female camels were divided equally according to their age into three groups, G1(3-5y), G2(5-10y) and G3(10-20y). Blood samples were taken through one estrous cycle at day before mating (D-1), mating day (D0), 1st, 2nd, 4th and 8th day after mating. One sample of saliva per female was taken at day 8 post-mating for progesterone assay to compare between plasma and salivary progesterone concentrations. At days before the 4th day of mating, progesterone concentrations were undetectable in all groups (< 0.5 ng/ml). Beginning from day 4, all groups showed a similar detectable level of progesterone, the mean was (1.17, 1.20 and 1.22 ng/ml) for G1,G2 and G3, the progesterone mean significantly increased at the 8th day to reach (4.09, 4.54 and 4.58 ng/ml) for G1, G2 and G3, respectively. At 8th day, the mean of progesterone level for pregnant camels was (6.15, 5.74 and 5.73 ng/ml) for G1, G2 and G3, respectively. Whereas, in non-pregnant camels at 8th day the mean of progesterone level was significantly less (3.21, 3.34 and 3.42 ng/ml) for G1, G2 and G3, respectively. There was consistency between plasma and salivary progesterone concentrations, the mean salivary progesterone level in pregnant camels at 8th day was significantly higher for G1, G2 and G3, respectively (4.96, 4.63and 4.58 ng/ml) comparing with non-pregnant camels for the three groups (2.49, 2.64 and 2.73 ng/ml). However, this noticeable increase of plasma and salivary progesterone level in pregnant camels at 8th day post-mating could be considered as early diagnostic indicator for pregnancy in camels.

INTRODUCTION

Camels were reported to have low reproductive efficiency under normal conditions (**Kaufmann, 2005**). **Skidmore (2013**) indicated that the reproductive performance of camels is generally regarded to be low (40%), this could be due to the late age of reaching puberty (3 - 4 years), the long gestation period (13 months) and the relatively high incidence of abortions and non-conceptions possibly attributed to poor nutrition, poor

management, limited breeding opportunities for the females due to seasonality of breeding.

Camels reproduction displayed by different constrains as semen characteristics, long gestation period, late sexual puberty and maturity, limited breeding season and the mechanism of estrous cycle and ovulation of she- camel (**Deen, 2008; EL-Hassanien *et al*., 2010**). Dromedary camels are considered as seasonal breeding animals. In Egypt, breeding season has been prolonged from December to April (**Shalash, 1980; 1987**). **Zeidan, (1999**) reported low calving rate of camels approximately 41% in Egypt.

Breeders often tend to breed camels early in the season for better growth of calves (born between November to January) born to early bred females. The pregnancy rates during early season tend to be poor because of transition from summer anestrus and poor follicle growth during this period (**Sghiri and Driancourt, 1989**).

In managing any camelid herd efficiently there is a definite need to diagnose pregnancy as accurately and as soon as possible after mating so that if the camel is not pregnant she can be re-mated, re-inseminated or returned to an embryo transfer programme. There are several methods used to diagnosis pregnancy but it must be remembered that whatever method is used, a single pregnancy diagnosis is not sufficient to guarantee a birth, especially if done at a very early stage (i.e. before 40 - 50 days post mating). This is due in part to errors in diagnosis, but is also due to the high incidence of early embryo loss seen in these species. Further examinations should therefore be carried out at 3 - 4 months of gestation to ensure the pregnancy is developing normally (**Skidmore**, **2000**).

Studies on progesterone concentrations during pregnancy in the camelidae confirm that these species depend on ovarian progesterone throughout their pregnancy. Ablation of the CL-bearing ovary or administration of PGF2α or its analogue causes abortion or premature parturition at all stages of pregnancy, thus it would seem likely that the placenta either fails to secrete progesterone at all, or it does so in amounts insufficient to maintain pregnancy without help from the ovaries (**Sumar, 1988)**.

In the mated dromedary, serum progesterone concentrations increase from day 3 after ovulation to concentrations of around 3.4 ng/ml by day 8. If the camel is not pregnant concentrations rapidly return to basal levels of <1ng/ml by days 10 - 12, however, if she is pregnant the progesterone concentrations are maintained between 3 and 5 ng/ml for the first 90-100 days of gestation. According to some studies, progesterone levels then decrease slightly to 2 - 4 ng/ml where they remained until day 300. A further slight decrease then occurs over the

next 70 - 80 days followed by a rapid drop to values of <1 ng/ml on the day before, or the day of parturition (**Skidmore *et al*., 1996).**

The measurement of progesterone concentration in peripheral blood can thus be invaluable in the early detection of pregnancy. If a blood sample is taken between days 12 - 15 and the value is still high (i.e. >1.0 ng/ml) this would indicate that the camel is possibly pregnant. If the value has dropped to <1.0 ng/ml then the camel is definitely not pregnant.

The concentration of steroid hormones in a female animal is an indicator of reproductive status. Progesterone enters the saliva via passive diffusion from the salivary glands. The level of progesterone present in the saliva of the horse and man can be used to diagnose pregnancy by use of an Enzyme-Linked Immunosorbent Assay [ELISA] (**Smith, 2005; Kaufman and Lamster, 2002**). The need for a non-invasive pregnancy diagnosis test, the development of a reliable and accurate pregnancy diagnosis test for use on farm animals for the early diagnosis of pregnancy would enable the prompt rebreeding of nonpregnant animals and prevent the culling of pregnant animals in error.

The objective of this study is to investigate the relationship between plasma and salivary progesterone level of female – camels around mating day and early pregnancy diagnosis.

MATERIALS AND METHODS

1- Animals

The study was carried on female camels (*Camelus dromedaries*) that belonging to a private camel farm for camel breeding, located in Matrouh Governate, during the period from November 2018 until June 2020. A total of thirty healthy female camels at different ages were equally divided based on their ages into three groups, the age and body weight of groups were illustrated in Table (1).

Table(1): Numbers, age and body weight of female camels

Groups (no.)	Age (year)	Average of body weight (kg) Means±S.E	Range of body weight (kg)
G1(10)	3-5	430.50±6.78	395-465
G2(10)	5-10	486.90±6.23	460-515
G3(10)	10-20	537.90±5.42	517-563

Camels were fed per head twice daily (at 8 a.m. and 6 p.m.) of 7 kg of a forage mixture barley straw (*Hordeum vulgare*) and 3-4 kg of a commercial feed concentrate mixture (12% CP). Female groups were kept in three fenced sand pens with appropriate wide area for moving easily through the mating periods.

2- Samples

a-Blood samples were taken throughout one estrous cycle for all camels during the period of breeding season, female camels were noticed for showing estrous behavior: (frequent urination, restlessness, vaginal discharge and male acceptance), then estrus females were separated, at next day, females were naturally mated with fertile male, then they were being held to perform sampling, as it is described below.

Blood samples per female camel were taken via jugular vein as follows: at pre-mating day (D-1), mating day (D0) and 1^{st}, 2^{nd}, 4^{th}, 8^{th} days post-mating. Blood samples were collected in an EDTA tubes, plasma were harvested following centrifugation at 2000 rpm for 10 minutes, and stored under -20º until time of progesterone assay, plasma progesterone concentrations (ng/ml) were measured by specific radioimmunoassay (RIA) commercial kits of (Diagnostic Systems Laboratories) DIAsource ImmunoAssays® S.A, Louvain-la-Neuve – Belgium.

b- Salivary samples one salivary sample was taken per female at day 8 post-mating , it was performed by moving a plastic straw inside the camel's mouth, collecting saliva in a tube, then it was filtered, the clear liquid was taken, and stored under -20º for progesterone assay.

3- Statistical analysis

Data were statistically analysed by the General Linear Model (GLM), using **SAS (2002)**. Differences between means were then tested using **Duncan's** multiple range test **(1955).** Results are presented as least squares means and their corresponding standard errors.

RESULTS AND DISCUSSION

Plasma progesterone concentrations

Table (2) indicated that the three groups generally showed similar undetectable increase of progesterone concentrations (<0.50ng/ml) throughout the day before mating, mating day, day1, and day2 after mating. Progesterone concentrations were slightly increased significantly at day4 followed by greater significant increase at day 8 post-mating for the three groups. At day 8 groups 2 and 3 showed a higher close values of progesterone level (4.54±0.51 and 4.54±0.50 ng/ml), while group 1 showed slightly less value (4.09±0.48 ng/ml).

The S.E values were very small and much close into all groups from D-1 till 2^{nd} day, which reflected the similarity of the low progesterone concentrations in all groups at these days, and indicated that difference inside groups was minimal. Beginning from day 4, progesterone levels were increased above (1.0 ng/ml) and S.E increased inside every group, reflecting increasing variation between camels inside every group, similarly significant increase in values of progesterone concentrations as well as S.E values meaning more variation between

camels, which is due to ovulation after mating and formation of corpus luteum secreting significant amount of progesterone.

Table(2): Plasma progesterone levels (ng/ml) of groups as (Means±S.E)

Group	Days					
	D-1	D 0	D 1	D 2	D 4	D 8
G1	$0.20^{c} \pm 0.02$	$0.43^{c} \pm 0.02$	$0.43^{c} \pm 0.03$	$0.45^{c} \pm 0.03$	$1.17^{b} \pm 0.12$	$4.09^{a} \pm 0.48$
G2	$0.19^{c} \pm 0.02$	$0.43^{c} \pm 0.03$	$0.43^{c} \pm 0.03$	$0.45^{c} \pm 0.05$	$1.20^{b} \pm 0.13$	$4.54^{a} \pm 0.51$
G3	$0.20^{c} \pm 0.02$	$0.44^{c} \pm 0.03$	$0.44^{c} \pm 0.03$	$0.44^{c} \pm 0.04$	$1.22^{b} \pm 0.13$	$4.58^{a} \pm 0.50$

Means with different superscripts letters in the same row are differed significantly at (P <0.05),

Kamoun and Jemmali (2014) reported that progesterone level rise after a successful mating This significant rise was ≥ 2.96 ng/ml two days after mating in all females, which disagree with the current study. **Skidmore *et al*., (1996**) reported that serum progesterone concentration increase from 3rd day after ovulation to concentrations of around (3.4 ng/ml) by 8th day. If the camel is not pregnant concentrations rapidly return to basal levels of (<1ng/ml) by days (10 – 12) after mating, these results agree with the results of this study.

Plasma progesterone levels of pregnant and non-pregnant camels

Table (3) illustrated means of progesterone values for pregnant and non-pregnant camels, means of days (D-1, D0, D1and D2 after mating) showed increasing but undetectable values of progesterone in all groups.

Table(3): Plasma progesterone levels (ng/ml) of pregnant and non-pregnant camels as (Means±S.E)

Days	Case	Groups		
		G1(3/10)	G2(5/10)	G3(5/10)
D-1	Non.	$0.17^{g} \pm 0.15$	$0.13^{g} \pm 0.18$	$0.13^{g} \pm 0.18$
	Preg.	$0.25^{gf} \pm 0.23$	$0.26^{gf} \pm 0.18$	$0.27^{gf} \pm 0.18$
D0	Non.	$0.38^{egf} \pm 0.15$	$0.35^{egf} \pm 0.18$	$0.35^{egf} \pm 0.18$
	Preg.	$0.55^{egdf} \pm 0.23$	$0.52^{egdf} \pm 0.18$	$0.52^{egdf} \pm 0.18$
D1	Non.	$0.38^{egf} \pm 0.15$	$0.35^{egf} \pm 0.18$	$0.35^{egf} \pm 0.18$
	Preg.	$0.55^{egdf} \pm 0.23$	$0.52^{egdf} \pm 0.18$	$0.52^{egdf} \pm 0.18$
D2	Non.	$0.41^{egf} \pm 0.15$	$0.32^{egf} \pm 0.18$	$0.33^{egf} \pm 0.18$
	Preg.	$0.56^{egdf} \pm 0.23$	$0.58^{egdf} \pm 0.18$	$0.55^{egdf} \pm 0.18$
D4	Non.	$0.99^{d} \pm 0.15$	$0.84^{edf} \pm 0.18$	$0.86^{ed} \pm 0.18$
	Preg.	$1.58^{c} \pm 0.23$	$1.57^{c} \pm 0.18$	$1.59^{c} \pm 0.18$
D8	Non.	$3.21^{b} \pm 0.15$	$3.34^{b} \pm 0.18$	$3.42^{b} \pm 0.18$
	Preg.	$6.15^{a} \pm 0.23$	$5.74^{a} \pm 0.18$	$5.73^{a} \pm 0.18$

Numbers between brackets refer to the pregnant camels in each group.

Means with different superscripts letters in the same column are differed significantly at (P <0.05),

Non. = Non-pregnant, Preg.= Pregnant

At day 4 post mating, pregnant camels in the three groups showed similar progesterone mean values that exceeded (1.0ng/ml), means were (1.58, 1.57 and 1.59 ng/ml) for G1, G2 and G3, respectively. By 8th day post mating, plasma progesterone mean values increased significantly,

pregnant camels of G1 showed the higher mean value (6.15 ng/ml) comparing with G2(5.74ng/ml) and G3(5.73ng/ml), this higher value of G1mean may be due to small number of pregnant camels of this group(3/10), whereas G2 and G3 had more number of pregnant animals (5/10).

For non-pregnant camels in the three groups, progesterone levels at day 4 showed limited rise values, but not overreach (1.0ng/ml), while at day 8 after mating progesterone means increased significantly for non-pregnant camels of the three groups comparing progesterone means of day4, G1(3.21ng/ml), G2 (3.34 ng/ml) and G3(3.42 ng/ml).

The results of the current study agree with those of **(Bravo *et al.*, 1996**) in pregnant llamas and alpacas, as progesterone was detectable 4 days after breeding and was maintained > 2 ng/ml throughout pregnancy. While the current results disagree with those of (**Kamoun and Jemmali, 2014**) who reported, in Camillus dromedaries, slightly higher figures during pregnancy in the first half than the second half 4.37±1.38 ng/ml vs. 3.70±0.96 ng/ml. Moreover, the results match also with (**Zhao *et al.*, 1998**) who reported higher serum progesterone concentrations in Bactrian camel (3.06±0.49 to 8.51±4.80 ng/mL) throughout most of gestation.

Salivary progesterone of pregnant and non-pregnant camels

Table 4 presented collective data of salivary progesterone mean at day 8 for the three groups, and salivary progesterone mean at day 8 for pregnant and non-pregnant camels in all the groups.

Group1showed the least mean of salivary progesterone (3.23 ng/ml), G2 and G3 had closed salivary progesterone means, for G2 (3.63 ng/ml) and G3 (3.65 ng/ml).

Pregnant camels from G1 showed higher significant progesterone mean (4.96 ng/ml), while means of pregnant camel of G2 (4.63 ng/ml) and G3 (4.58 ng/ml) had closed and non-significant means.

Non-pregnant camels of the three groups showed lower non-significant means comparing of pregnant camels, salivary progesterone means were (2.49, 2.64and 2.73 ng/ml) for G1, G2 and G3, respectively.

Table (4): Salivary progesterone levels (Mean±S.E) (ng/ml) of pregnant and non-pregnant camels

Groups	Progesterone level (D8)	Case	Progesterone level (D8)
G1	$3.23^{bc} \pm 0.43$	Non.	$2.49^{c} \pm 0.23$
		Preg.	$4.96^{a} \pm 0.57$
G2	$3.63^{bac} \pm 0.47$	Non.	$2.64^{c} \pm 0.37$
		Preg.	$4.63^{ba} \pm 0.61$
G3	$3.65^{bac} \pm 0.45$	Non.	$2.73^{c} \pm 0.33$
		Preg.	$4.58^{ba} \pm 0.63$

Means with different superscripts letters in the same column are differed significantly at (P <0.05),

Non.=Non-pregnant , Preg.=Pregnant

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العلاقة بين مستويات البروجستيرون خلال يوم التزاوج

وبداية حدوث الحمل فى النوق

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تهدف الدراسة الى بحث العلاقة بين مستويات البروجستيرون فى بلازما الدم وفى اللعاب للنوق قبل و بعد يوم التلقيح و بداية حدوث الحمل . استخدم للدراسة عدد ثلاثين ناقة، تم تقسيمها تبعا للعمر الى ثلاث مجموعات متساوية فى العدد، تم أخذ عينات الدم بيوم قبل التزاوج و فى يوم التزاوج و بعده بيوم و بعده بيومين و بعده بأربعة أيام و بعده بثمانية أيام. و تم أخذ عينة لعاب من الحيوانات كلها فى اليوم الثامن بعد التزاوج. تم تقدير البروجستيرون فى عينات بلازما الدم وعينات اللعاب.

وجد ان مستويات البروجستيرون فى بلازما الدم للمجموعات كلها فى الايام قبل اليوم الرابع كانت تركيزات ضئيلة جدا (اقل من 0,5 نانوجرام/مل) . بدءا من اليوم الرابع بعد التزاوج اظهرت الحيوانات زيادة فى مستويات البروجستيرون ، كان المتوسط (1.17 ، 1.20 ، 1.22 نانوجرام / مل) لـ G1 و G2 و G3 ، وزاد متوسط البروجسترون بشكل ملحوظ في اليوم الثامن ليصل إلى (4.09 و 4.54 و 4.58 نانوجرام / مل) لـ G1 و G2 و G3 على التوالي. في اليوم الثامن ، كان متوسط مستوى هرمون البروجسترون للنوق الحوامل (6.15 ، 5.74 ، 5.73 نانوجرام / مل) في G1 و G2 و G3 على التوالي ، بينما في النوق غير الحوامل في اليوم الثامن كان متوسط مستوى البروجسترون أقل معنوياً (3.21 ، 3.34 و 3.42 نانوجرام/ مل) لـ G1 و G2 و G3 على التوالي.

كان هناك تناسق بين تركيزات البروجسترون في البلازما واللعاب ، وكان متوسط مستوى البروجسترون في النوق الحوامل في اليوم الثامن أعلى معنويا في G1 و G2 و G3 على التوالي (4.96 و 4.63 و 4.58 نانوجرام / مل) مقارنة بالنوق غير الحوامل في المجموعات الثلاثة (2.49 و 2.64 و 2.73 نانوجرام / مل). من هذه الزيادة الملحوظة في مستوى البروجسترون فى اللعاب والبلازما في النوق الحوامل في اليوم الثامن بعد التزاوج يمكن اعتبارها كمؤشر تشخيصى مبكرا لحدوث الحمل في النوق.