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SERUM PROGESTERONE ANALYSIS BY COMMERCIALLY AVAILABLE EIA KIT TO MONITOR OVULATION AND CONCEPTION IN DROMEDARY CAMELS

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ABSTRACT

Commercially available human Enzyme Immuno Assay (EIA) kit was employed to analyse sequential serum progesterone levels subsequent to 22 matings given to 22 female dromedary camels. The serum progesterone levels at day 7, 9, 15 and 30 *post coitum* was found to be a good indicator of ovulation or its absence. The incidence of non-ovulatory coitus was 40.9% with resultant low (or below detectable limit) progesterone concentrations. The mean serum progesterone concentration of 2.92 ± 1.48 (n=7), 3.55±1.65 (n=3) and 2.41±1.21 (n=3) ng/ml was found at day 7, 9 and 15 *post coitum,* in ovulatory coitus, respectively. It was concluded that human EIA kits can be used for assay of the progesterone hormone in camels and such an assay can differentiate ovulatory and non-ovulatory coitus in camels.

Key words: Dromedary camel, enzyme immuno assay (EIA), female, serum progesterone

Progesterone is the most prevalent, naturally occurring progestogen and is secreted by luteal cells of the corpus luteum. This hormone is also secreted by placenta and adrenal glands (Reeves, 1978). The concentration of progesterone in blood and milk provides luteal function during the reproductive cycle. Progesterone concentration increases and decreases coinciding with the growth and regression of the corpus luteum. The mid-cycle rise of progesterone in plasma and milk in cows and buffalo result from luteinisation after ovulation and subsequent secretion by the corpus luteum. It decreases to follicular levels if implantation does not occur. If implantation occurs, progesterone levels remain elevated and increased progressively throughout the ensuing pregnancy (Batra *et al,* 1979).

The dromedary camel is an induced ovulator and the progesterone concentration *post coitum* is reported to be low (<1 ng/ml) during the first 4-5 days. It then increases steadily to reach a maximum of 4.5±1.5 ng/ml between days 7 and 11 *post coitum* and in absence of pregnancy decreases to <1 ng/ml from 10 to 13 days (Marie and Anouassi, 1987). This time course of progesterone secretion offers the opportunity to set up a very early pregnancy diagnosis based on plasma progesterone concentrations, with a period of application corresponding to 12-13 days after mating. This is of practical importance to raise the chances of fecundation when the male is not continuously present with the females, and as sexual activity is limited in the year for the male and the female (Marie and Anouassi, 1987). Commercially available enzyme immuno assay (EIA) kits are common for assay of progesterone in human medicine. Clinically, progesterone measurement is of use to monitor ovarian function, confirm ovulation, assess luteal phase defects, and to check the effectiveness of procedures for induction of ovulation (Abraham *et al,* 1974; Wu and Miassian, 1987). The EIA assay for milk progesterone on day 24 post insemination was reported to be 100% accurate for non-pregnancy and 77% for pregnancy in cows and buffaloes (Kaul and Prakash, 1994). The EIA for the progesterone hormone can be a practical method for the dromedary farmers and researchers because this method does not require radio-labelled molecules or expensive laboratory facilities. It can be performed in the field using a portable, battery powered plate reader. This method can be easily handled in areas where it is difficult to obtain radioactive elements and where equipment such as centrifuge and radioactivity counters are not available (Anouassi and Combarnous, 1991). The present study was

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aimed to use commercially available EIA kits for serum progesterone analysis to monitor ovulation and conception in dromedary camel.

Materials and Methods

Twenty-two adult female camels, having calved at least once, belonging to the herd of National Research Centre on Camel, Bikaner were used for the present experiment. The animals were apparently in healthy condition. They were maintained in semi-intensive management condition, i.e. allowed to graze in the rangeland and also provided with dry fodder @10 kg per day per animal.

Ovarian examination

The camels were restrained in sternal recumbancy and were sedated with 3-4 ml of inj. Xylaxin i.v. The rectal examination was performed in sternal recumbency as described previously (Vyas and Sahani, 2000). The operator was same for all examinations. The she camels were mated with virile male twice at an interval of 48h.

Blood collection

The blood samples were collected through jugular venipuncture immediately after mating (day 0), 7, 9, 15 and 30 days post mating. The serum samples were separated and stored at –20°C till assay. The animals were subjected to rectal palpation on day 60 for pregnancy diagnosis. A total of 110 blood samples were collected from 22 matings given to female camels.

Progesterone assay

EIAgen progesterone kit that employs direct solid phase enzyme immunoassay for the quantitative measurement of progesterone in human serum or plasma (Adaltis Italia S.p.a. via Cristoni, 1240033 Casalecchio di Reno, Bologna, Italy) was used for assay of progesterone in camel serum.

The solid phase enzyme immunoassay for progesterone is a competitive type immunoassay wherein horseradish peroxidase-labelled progesterone (HRP-Progesterone) competes with progesterone present in the serum sample for a fixed and limited number of antibody sites immobilised on the wells of the microstrips. Once the competitive immunoreaction has occurred, the wells are washed and the HRP-progesterone fraction bound to the antibody in the solid phase is measured by adding a chromogen/substrate solution, which is converted to a blue compound. After 15 minutes of incubation, the enzymatic reaction is stopped with sulphuric acid, which also changes the solution to a yellow colour. The absorbance of the solution, photometrically measured at 450 nm, is inversely related to the concentration of progesterone present in the sample. Calculation of progesterone content in the sample is made by reference to a calibrator curve.

The mean absorbance of calibrators and samples (A) were estimated at 450 nm using a ELISA reader (Anthos htII of M/S Anthos Labtec Instruments A-5022 Salsburg). The absorbance of the chromogen blank (Ac) was subtracted from all the means. The corrected mean absorbance obtained by the corrected mean absorbance of the zero calibrator (Ao) and multiplied by 100

$$B/Bo \times 100 = \frac{A\text{-}Ac\_ \times 100}{Ao\text{-}Ac}$$

The B/Box100 versus the respective progesterone concentrations were plotted on logit-log graph paper. The concentration of the progesterone in the samples was determined by interpolation from calibration curve.

The precision of the assay as mentioned by the manufacturer was as follows, for serum samples with progesterone concentrations ranged between 1-20 ng/ml (3.18-63.6 nmol/l) the within and between assay coefficients of variation were found to be 5.8-7.5% and 9.7-12.5% (min.- max.), respectively. The lower limit of detection was defined as the progesterone concentration given by the mean absorbance of the zero calibrator minus 3 standard deviations and was assessed to be approximately 0.16 ng/ml (0.51 nmol/L).

Results and Discussion

The serum progesterone concentration at 0, 7, 9, 15 and 30 days *post coitum* is shown in table 1. The serum progesterone values were below detectable limits at day 0 (mating) in all serum samples. The concentration increased to ≥ 1.0 ng/ml in camels (at S.N. 1 to 13) indicating ovulation. Progesterone concentration remained ≥ 1.0 ng/ml up to 30 d *post coitum* in camels at S. N. 1, 2, 8, 11, 12, 13 (Figs 1 and 2). These animals were diagnosed as pregnant and were also showing "tail cocking" (lifting of tail, remained stand still when approached by male camels or camel attendants). In J 77 (S.N. 9 and Fig 4) the progesterone level lowered below detectable limit on day 30 after rising ≥ 1.0 ng/ml at day 9, 15 *post coitum* indicating early embryonic death. This camel was showing "tail cocking" from 12 to 24 days *post-coitum*. The progesterone level in camels at S. N. 3, 4, 5, 6, 7 decreased below detectable limit or very low (S.N. 10) on day 15 *post coitum* indicating that ovulation did occur in these animals but fertilisation

Table 1. Serum progesterone (ng/ml) at the time of and subsequent to coitus in female dromedaries.

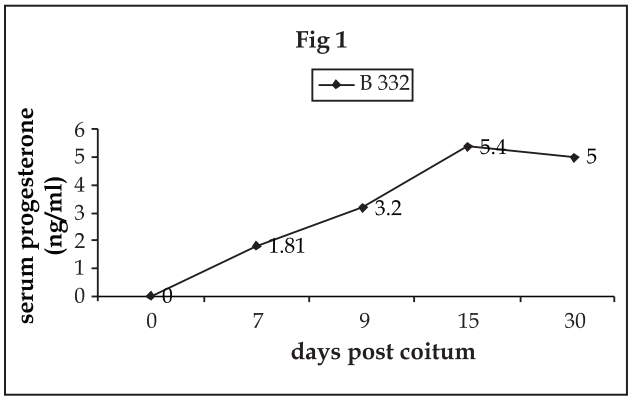
S. N.	Animal No.	0 Day	7 Day	9 Day	15 Day	30 Day	P/NP/O/NO/EED
1	B 332	ND	1.81	3.2	5.4	5.0	O,P
2	B 439	ND	1.0	2.9	2.2	2.5	O, P
3	B 585	ND	7.0	0.5	ND	ND	O, NP
4	J 139	ND	5.0	7.7	ND	ND	O, NP
5	B 463	ND	2.1	ND	ND	ND	O, NP
6	B 425	ND	1.5	1.7	ND	ND	O, NP
7	B 505	ND	2.0	ND	ND	ND	O, NP
8	J 389	ND	0.71	1.25	2.5	2.0	O, P
9	J 77	ND	0.70	1.1	0.90	ND	O, NP, EED
10	J 153	ND	ND	7.0	0.25	0.32	O, NP
11	B 551	0.29	0.3	0.29	1.1	1.9	O, P
12	B 483	ND	ND	ND	2.25	4.8	O, P
13	B 467	ND	ND	ND	2.5	1.3	O, P
14	B 583	ND	ND	0.7	0.27	ND	NO, NP
15	K 107	ND	ND	ND	ND	ND	NO, NP
16	J 167	ND	ND	ND	ND	ND	NO, NP
17	B 565	ND	ND	ND	ND	ND	NO, NP
18	B 563	ND	ND	ND	ND	ND	NO, NP
19	J 141	ND	ND	0.5	ND	ND	NO, NP
20	J 129	ND	ND	ND	ND	ND	NO, NP
21	B 559	ND	ND	0.37	ND	ND	NO, NP
22	J 405	ND	ND	ND	ND	ND	NO, NP

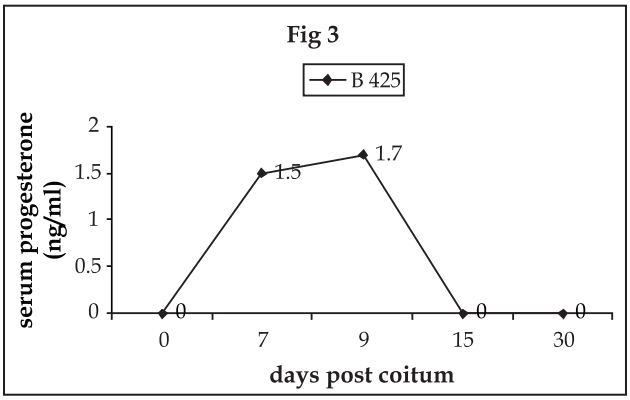
and/or nidation failed to occur (Fig 3). These camels showed "tail cocking" only for 2-3 days between days 10 to day 15 *post coitum*. In camels at S. N. 14 to 22, the progesterone concentration remained below detectable limits or very low on day 7, 15, 30 *post coitum*, indicating that ovulation did not occur in these animals; these camels did not show "tail cocking."

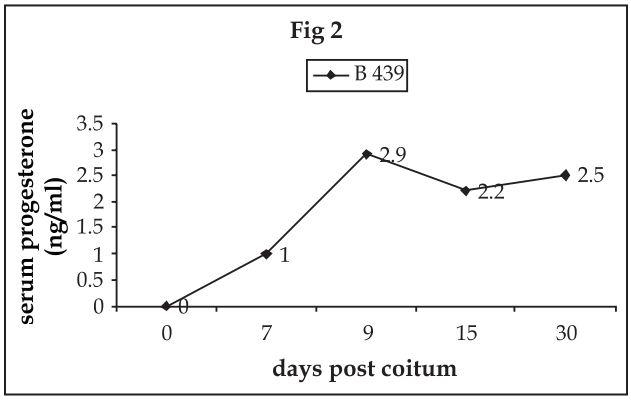
The progesterone concentration ≥ 1.0 ng/ml was observed on *a*- day 7 *post coitum* in 7 camels (S. N. 1 to 7; mean 2.92±1.48 ng/ml, range 1.0 to 7.0 ng/ml); *b*- day 9 *post coitum* in 3 camels (S. N. 8 to 10; mean 3.55±1.65 ng/ml, range 1.1-7.0) and *c*- day 15 *post coitum* in 3 camels (S. N. 11 to 13; mean 2.41±1.21 ng/ml, range 0.9-5.4)

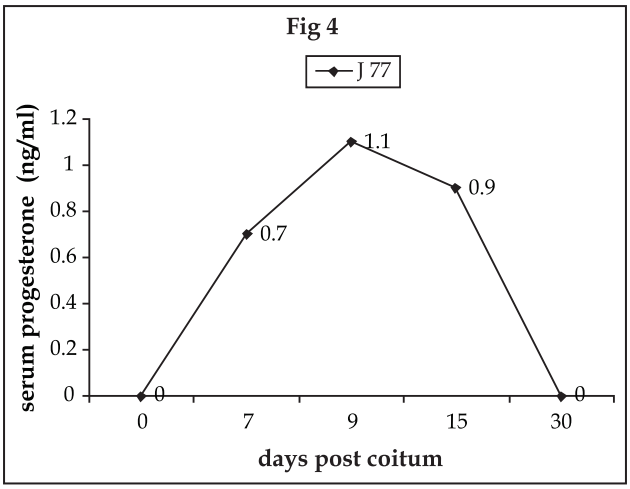
It has been reported that corpus luteum in dromedary secrete enough progesterone to have blood level ≥ 1.0 ng/ml at day 7 *post coitum* (Marie and Anouassi, 1987) and ovulation occurs 36 h after the ovulatory stimulus (Williamson and Payne, 1978). Therefore, it appeared that in 7 camels (S. No. 1-7) occurred between 0-2 days of first mating, 3 camels (S. No. 8-10) ovulation occurred between day 2-4 of first mating and 3 camels (S. No. 11-13) ovulation occurred on ≥ day 4 of first mating. It can be safely concluded that ovulation occurred as a result of first mating in 7 camels (S. No. 1-7), and as a result of second mating (48 h after first mating) in 3 camels (S. No. 11-13). But it is not sure whether 1st or 2nd mating resulted in ovulation in 3 camels (S. No. 8-10).

Marie and Anouassi (1987) used radio-immuno assay reported that the progesterone concentration *post coitum* remained low during the first 4-5 days, then increased steadily to reach a maximum of 4.5±1.5 ng/ml between days 7 and 11 and then in absence of pregnancy decreased to ≥ 1.0 ng/ml from 10 to 13 days. Similar results were reported by Agarwal and Khanna (1998) and Vyas *et al* (1998) using radio-immuno assay. Similarly, Saleh *et al* (2000) also used EIA kits and reported 2.78±0.5, 5.56±0.42 and 5.42±0.37 ng/ml mean serum progesterone in early, mid and late pregnancy in dromedary camel. Mahamat *et al* (1997) reported conclusive but higher values of progesterone indicating diagnostic potential of EIA in pregnancy diagnosis of the dromedary camel. Deen *et al* (2001) reported inconclusive results of EIA in superovulated and hCG administered dromedary females. The use of anti-fluorescein coupled to a magnetic solid separation of fluorescein progesterone anti-progesterone complex in their









Figs 1,2,3,4. *Post-coitum* serum progesterone (ng/ml) levels in camels- pregnant (Figs 1-2), ovulatory but non-pregnant (Fig 4) and ovulatory but early embryonic death (Fig 3).

procedure instead of enzyme substrate used by other previous and present study might be the reason for inconclusive result.

The present study revealed high incidence of non-ovulatory coitus. Previous researchers have also reported incidence of inovulation after coitus in dromedary females (Marie and Anouassi, 1987; Vyas *et al,* 1998). These findings are suggestive of use of exogenous hormonal (hCG or its analogues) preparation as ovulatory stimulus adjunct to coitus to increase the chances of ovulation and subsequent conception, especially, in repeat breeding cases.

The present study concluded that human EIA kits can be used for assay of the progesterone hormone in camels and such an assay can differentiate ovulatory and non-ovulatory coitus and conception in dromedary camel.

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Reference

Abraham GE, Marcoulis GB and Marshall JR (1974). Evaluation of ovulation and corpus luteum function using measurement of plasma progesterone. Obstetrics and Gynaecology 4L:522-525.

Agarwal SP and Khanna ND (1998). Early pregnancy diagnosis through serum progesterone estimation in camels (*Camelus dromedarius*). Indian Veterinary Journal 75: 131-132.

Anouassi A and Combarnous Y (1991). Development of radioimmuno assay and enzyme immuno assays for luteinizing hormone in dromedaries (*Camelus dromedarius*). Journal of Reproduction and Fertility 93: 409-414.

Batra SK, Arora RC, Bachlaus NK and Pandey RS (1979). Blood and milk progesterone in pregnant and non-pregnant buffalo. Journal of Dairy Science 62:1390-1393.

Deen A, Mal G and Sahani M.S (2001). Applicability of commercial progesterone analysis kits standardised on human serum/plasma for progesterone analysis in camel. Journal of Camel Practice and Research 8:221-226.

Kaul V and Prakash BS (1994). Accuracy of pregnancy/ no pregnancy diagnosis in zebu and crossbred cattle and Murrah buffaloes by milk progesterone determination

post insemination. Tropical Animal health Production 26:187-192.

Mahamat H, Olah-Mukani W, Mboloi MM, Guya SO and Krombartis GE (1997). Pregnancy diagnosis in the dromedary camel (*Camelus dromedarius*) based on a competitive progesterone enzyme linked immuno assay (ELISA). Journal of Camel Practice and Research 4: 203-205.

Marie M and Anouassi A (1987). Induction of luteal activity and progesterone secretion in the non-pregnant one–humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility 80:183-192.

Reeves JJ (1987). Endocrinology of reproduction. In: Reproduction in Farm Animals, Ed E.S.E. Hafez. 5th Edition, Lea & Febiger, Philadelphia USA.

Saleh MA, El-Sokkary GH and Abdel-Eazik AR (2000). Circulating steroids and proteins in Egyptian oasis pregnant camel (*Camelus dromedarius*). Journal of Camel Practice and Research 7:9-13.

Vyas S, Agarwal SP, Rai AK and Khanna ND (1998). Monitoring ovulation and conception employing blood progesterone estimation in female *Camelus dromedarius*. Indian Journal of Animal Production Management 15: 33-34.

Vyas S and Sahani MS (2000). Real-time ultrasonography of ovaries and bruding of one humped Camel (*Camelus dromedarius*) during the early postpartum Period. Animal Reproduction Science 59:179-184.

Williamson G and Payne WJA (1978). An introduction to Animal Husbandry in the Tropics. Longman, London United Kingdom.

Wu CJH and Miassian SS (1987). The integrated luteal progesterone: an assessment of luteal function. Fertility and Sterility 48:937-940.