

# Oestradiol-17 $\beta$ profiles throughout Pregnancy and postpartum period associated with first estrus postpartum, in Sudan she Camel

***(Camelus dromedarius)***

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## **Abstract**

The present study was designed to investigate Oestradiol-17 $\beta$  in she-camel during pregnancy and postpartum period associated with first estrus postpartum.

A total of 20 she camels were used for the purpose of this study. To investigate Oestradiol-17 $\beta$  during pregnancy, 8 pregnant she camels were used and blood samples were collected at 2 weeks intervals started at 2 months of pregnancy till parturition. To study Oestradiol-17 $\beta$  profiles during postpartum period, 12 she camels were used, blood samples were collected every week starting from parturition till estrus signs were detected by observation.

The profile of estrogen concentrations in the peripheral serum during pregnancy remained remarkably steady at average range of 26.5 pg/ml then increased gradually after 8 months of gestation to reach 109.6 pg/ml at ten months of gestation. And then rose steeply and steadily during last stage of gestation (>10 months) to reach a peak of 275.8 pg/ml at end of gestation. During postpartum, estrogen peaks in first estrus cycle after parturition with mean level of 49.9 pg/ml and the estrus cycle observed between the 4<sup>th</sup> – 12<sup>th</sup> weeks after parturition.

## **Introduction:**

The endocrine hormone profiles that occur in the peripheral blood during pregnancy, at parturition and during postpartum period have been studied in many large domestic animal species (reviewed by Wagner et al, 1974.)

Serum estradiol -17 $\beta$  have been observed to increase steeply in the last few days before birth in the several species, including the cow ( Peterson et al. 1975), ewe ( Bedford et al. 1972 ) and pig ( Robertson and King 1974). The mare forms an interesting contrast to these other species – serum estrogen concentration

rise to very high values in the middle of gestation but decline steadily during the final 2-3 months (Cox 1975; Nett et al 1975).

There are only few published reports of estrogen production during pregnancy in one-humped Camel. Elias et al.(1984) measured oestradiol-17B concentration in peripheral plasma samples recovered at monthly intervals during pregnancy and noted that the concentrations remained relatively constant at 50-100 pg/ml for the first 10 months of gestation before rising abruptly to a peak during the twelfth month. Agarwal et al .(1987) however, described a steady increase in plasma oestradiol-17B concentrations throughout pregnancy, from a mean of only 20pg/ml during the second and third months to 450pg/ml in final month of gestation.

There have been no reports to date of concentration of oestradiol-17B first peak during postpartum period associated with first estrus postpartum.

In the present study existing radioimmunoassay (RIA) was valued for she camel serum and used to determine the levels of oestradiol-17B during pregnancy and postpartum period associated with first estrus postpartum.

## **MATERIEALS AND METHODS**

### **1. Animals:**

A total of 20 dromedary she-camels (Arabia and Rashidi) between 6 to 8 years old at the Camel Research Center, Faculty of Veterinary Medicine (Eight were pregnant, five non-pregnant as control and 5 during the postpartum period added to the 8 delivered pregnant) were employed to execute the objectives of this study.

All animals were fed through the natural ranged vegetation and forest from 8:00 AM to 3:00 PM. After they are back from pasture and when in the barn, she camels were receiving Abu 70 (Sorghum bicolor) or alfalfa. Water and salt lick were provided ad-libitum. She camels were milked twice a day in the morning (7: 00 am) and evening (4:00 pm). After parturition; the neonates (huwar) were fed by suckling their mother naturally.

Routine examination for brucellosis and vaccination against the brucellosis, anthrax, haemorrhagic septicemia and rabies are practiced annually. All She

camels had dewormer drench as a preventive measure to control internal parasites. She camels were also sprayed with acaricidal drugs two times, one time and another after two weeks to eliminate external parasites. The barns were cleaned daily.

The barn constructed with iron bars, partially roofed with mat. Animal are grouped according to age and pregnancy status.

### **2. Study area:**

This study was carried out at Camel Research Center (CRC), Faculty of Veterinary Medicine, University of Khartoum. The location of this center is about four km north to the Faculty of Veterinary Medicine at Shambat, North Khartoum. The average rainfall per year is 176 mm. The maximum rainfall is between July and September. Temperature is very high and it exceeds 45°C during the days of summer months (March to June).

### **3. Methods**

#### **3.1. Blood sampling**

Blood samples were collected from the jugular vein by vacutainer tube. Serum was immediately separated in centrifuge (2000g for 10 minutes) and stored in eppendurf tube at -20°C until assayed to determine the levels of estrogen by Radio Immuno Assay.

#### **3.2. Pregnancy diagnosis:**

In the present study we used two methods to detect pregnancy:

##### **a) Rectal palpation:**

She camels were rectally examined for pregnancy diagnosis 2 months after mating.

##### **b) Chemical method:**

Urine samples were collected from females 120 – 150 days after mating to conduct Cuboni test for pregnancy confirmation. The test is preformed as follows:-

To 15 ml of urine 3 ml of concentrated hydrochloric acid is added and heated in a water bath for 10minutes and then cooled under a tap. To this 18

ml of benzene is added and shaken vigorously for half minute. The supernatant (mainly benzene) is collected in another tube and 3 to 10 ml of concentrated sulfuric acid is added and the mixture heated in a water bath at 80°C for 5 minutes, and then allowed to cool. Positive (pregnant) test is indicated by the appearance of a dark, only green fluorescent color in the lower sulfuric acid layer and a negative (non-pregnant) is characterized by absence of fluorescent color and presence of a brownish color.

### 3.8.1 Estradiol (E2) RIA kit (PR) IMK-481

For the direct determination of  $17\beta$  – estradiol (E2) in serum measurement range 10 ~ 2000pg/ml, 0.04~7.35 nmol/L.

The kit was purchased from Department of Isotope, China Institute of Atomic Energy, Bijing, 102413.

### 3.8.2 Principle of the method

This procedure for the direct determination of serum E2 is based on the competitive binding principle of RIA. Standards and patient samples are preincubated with E2 anti-body.  $^{125}\text{I}$  - E2 then competes with E2 in the standards and the patient samples for a fixed and limited number of E2 antibody sites. After incubation, separation of bound from free is achieved by the PR method. The antibody bound fraction is precipitated and counted. Patient sample concentrations are read from a calibration curve.

### 3.8.3 Contents of kit (100 test pack)

One vial of  $^{125}\text{I}$  - E2 derivative solution (10ml, red)

One vial of E2 antiserum solution (10ml, blue)

Seven vials of E2 standards, lyophilized. The standards contain respectively 0, 10, 30, 100, 300, 1000 and 2000 (pg/ml). equivalently: 0, 0.04, 0.11, 0.37, 1.10, 3.68 and 7.35 (nmol/L). At least 10 minutes before use, reconstitute each vial by adding 1 ml distilled water (except standard A with 2.0 ml distilled water). Vortex vigorously.

One bottle of precipitating solution (P. R) (before use the reagent should be thoroughly mixed).

### 3.8.4 Assay procedure

Bring all reagents to room temperature (except the precipitating solution)

(E2 has a strong tendency to adsorb to untreated plastic surfaces. It is important to use containers made of glass rather than plastic for both tracer and patient samples.)

1. Label assay tubes in duplicate: T (total counts), NSB (nonspecific binding), A (maximum binding), B through G. label additional tubes, for samples.

2. Pipet 200 $\mu\text{l}$  of the zero standard A into the NSB and A tubes, and 200 $\mu\text{l}$  of B through G or samples into the prelabelled tubes (see table 2.1).

3. Dispense 100 $\mu\text{l}$  E2 antiserum into all tubes except the NSB and T tubes. Vortex.

4. Incubate for 2h at room temp. (18 - 22°C)

5. Dispense 100 $\mu\text{l}$   $^{125}\text{I}$  - E2 derivative into all tubes. vortex

6. Incubate for 1h. at room temp. (18 -22°C)

7. Dispense 500 $\mu\text{l}$  precipitating solution into all tubes. vortex. (except the T tubes)

Vortex at room temp. for 10 minutes. Centrifuge. Discard the supernatant and count.

8. Incubate for 10 minutes at room Temperature.

9. Centrifuge for 20 minutes at 2000xg, discard the supernatant, then Count in r-counter.

### 3.8.5 Calculation of result

Results can be calculated using log it -log plotting: log it -log plotting:

1) Calculate the mean counts for the zero standard (B0).

2) Divide Counts of each standard (B) by the mean counts for the zero standard (B0) and multiply by 100.

3) Plot the B/B0 % values for each standard against the E2 concentration on log it -log graph paper.

4) Read of the E2 concentration from the logit -log plot. .

It is recommended that every laboratory establish its own normal range for its own population, since variations may be obtained from laboratory, from region to region.

**Data analysis**

The data were analyzed using statistical package social science (SPSS) computer system

**Results**

1 Hormone profiles

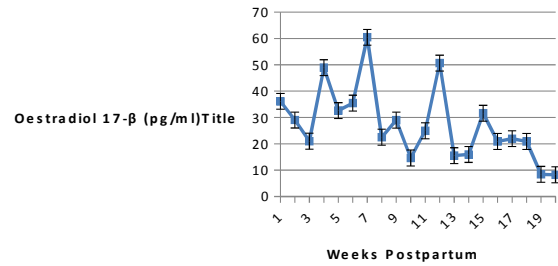
**1.2 Oestrogen profiles during pregnancy period:**

Oestrogen concentrations in the peripheral serum of the 8 she camels throughout pregnancy are shown in Fig. 2. Mean serum concentration of estradiol 17β remained remarkably steady at average range of 26.5 pg/ml then increased gradually after 8 months of gestation to reach 109.6 pg/ml at ten months of gestation. And then rose steeply and steady during last stage of gestation (>10 months) to reach a peak of 275.8 pg/ml at end of gestation.

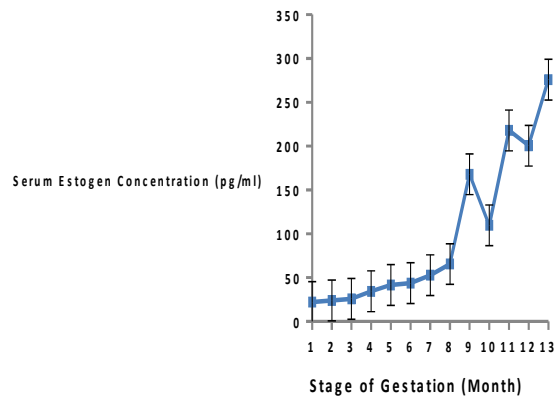
**1.3 Oestrogen levels during postpartum period**

Oestrogen peak levels during postpartum period associated with first postpartum oestrus phase are shown in Fig. 3 Oestrogen peaks in first oestrus cycle after parturition with mean level of 49.9pg/ml and the oestrus cycle observed between the 4<sup>th</sup> – 12<sup>th</sup> weeks after parturition.

Fig. 3 Oestradiol levels during Postpartum Period



ation of Estrogen (mean ± sem) in serum of eight prgnant She camels during gi



**Discussion:**

In the present study, serum oestradiol – 17 β concentrations showed a first rise that began around early stage of pregnancy recorded a concentration of 16 – 28 pg/ml for first four months then increased gradually after 8 months of gestation. This result accords with those of Skidmore *et al* (1996). The increase in rate of secretion of oestrogen could be ovarian or placental in origin. The latter possibility seems more likely. The time when oestrogen concentrations in the blood begin to increase towards the end of pregnancy varies between animal species. In the pregnant ewe, maternal plasma oestradiol - 17β concentration show an explosive rise

in the 24 – 48h period before lambing (Challis 1997), whereas in the mare, plasma oestrogen concentrations show a steady decline from a peak at around Day 240 of the 340 – day gestation period (Cox 1975; Nett *et al.* 1975). On the other hand, in the cow (Edquist *et al.* 1973; Robertson 1974), llama (Leon *et al.* 1990) and camel, once maternal oestrogen concentrations have begun to increase they remain elevated until the time of birth (Skidmore *et al.* 1996). The similar observations were the finding in the present study. The marked rise in oestrogen concentrations measured in late stage of pregnancy (>12 months of gestation) in the present study coincided with a gradual decline in serum progesterone concentrations over the same period accords with those of Skidmore *et al.* 1996.

In addition, the increased production of oestrogen coincides with the spurt in fetal growth and the substantial increase in the volume of fetal fluids noted to occur between 9 months and 12.5 months of gestation in the dromedary (El- Wishy *et al.* 1981). This highlights the like hood that placental oestrogens are important for fetal growth in camelids. Hence, Rawlings and Ward (1976) and others have proposed that the pre-partum rise in oestrogens concentration which occur in the blood of sheep, cattle and other domestic species, is likely to constitute an important component of the physiological indicators for parturition. Certainly, the increased concentrations of oestrogen, combined with the commencing fall in progesterone concentrations, will help greatly to overcome the 'progesterone block' on myometrial activity present throughout gestation. In addition, as demonstrated in mares carrying gonadectomized fetal (Pashen and Allen 1979) fetal oestrogens are necessary in this species to stimulate the synthesis of PGF $2\alpha$  in the myometrium which will, in turn, stimulate, together with oxytocin, the powerful myometrial contractions associated with birth (Rossdale *et al.* 1979).

Postpartum resumption of ovarian function and follicular activity is highly variable in camels (Tibary and Anouassi, 1997). The first postpartum estrus was reported to be delayed for one year (Yasin and Wahid, 1957; Matharu, 1966; Willianson and Payne, 1978; Wilson, 1989) and irrespective of date of birth, until the next breeding season (Musa and Makawi, 1989). In another study, a variable number of mature follicles on the left ovary of non suckled animals

were observed at 39 – 42 days postpartum; three she camels were mated but none conceived (Elias, 1990). No growth of mature follicles (Ahmed 1990) and an interval of 4.5 – 10 months for first postpartum estrus (Evans and Powys, 1979) were reported in lactating dromedaries. In the present study, the estrus signs like straddling of the hind legs, raising the tail, frequent urination and submissive behavior towards and approaching male were shown by animals. Therefore, it was observed that, the periods of postpartum were variable, from one month for first postpartum estrus in non suckling animals to three months in lactating and suckling animals. Also in this study was observed that, the Rashidi breeds have less postpartum periods than Arabian. Further trails on large number of animals are required to study the influence of nutrition, suckling and lactating as well as age and breed of camels on the postpartum period.

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## Conclusion

Finally, the results of this study had provide basic information on some hormone profile during pregnancy and postpartum periods and suggested that oesteradiol - 17 $\beta$  may be utilised as good indicators to predict the time of parturition.

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