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## Bubaline Progesterone Concentrations in Serum, Fore-milk and Saliva During Different Phases of Oestrous Cycle

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### Abstract

Fluorimunoassay technique was used to determine the concentrations of progesterone in serum, fore-milk and saliva of 40 Nili-Ravi buffaloes during pro-oestrus, oestrus, met-oestrus and di-oestrus. Progesterone concentrations during oestrus were uniformly lower in all experimental body fluids; the highest concentrations were found during the mid luteal phase. Although the hormonal trend was similar but salivary progesterone concentrations in general were lower during all phases of oestrus cycle. There was a positive correlation between the concentrations of progesterone in serum with those of fore-milk ( $r = 0.82$ ) ( $P < 0.01$ ) and saliva ( $r = 0.75$ ) ( $P < 0.01$ ).

### Introduction

The low reproductive efficiency of buffaloes is widely accepted as a serious constraint to optimal production in this species (Shah *et al.*, 1989). The most common reproductive problem encountered in this species is the silent heat/sub oestrous and its incidence in Pakistan is about 33.3 percent (Samad *et al.*, 1987). Due to poor behavioral manifestations of oestrus (Ullah and Usmani, 1985), behavioral observations can not be used as available method for oestrus detection in buffaloes. The progesterone assay of blood and fore-milk has emerged as a useful tool to determine the appropriate time of insemination, monitoring of cyclicity and pregnancy diagnosis in cattle (Plotka *et al.*, 1967; Peters and Lamming, 1984) and buffaloes (Suri *et al.*, 1980; Arora and Pandey, 1982). Several workers have also reported a close correlation between progesterone concentrations in plasma and milk of bovines.

Salivary hormonal concentrations are also reported to have positive correlation with plasma (Walker *et al.*, 1979; Sorgo *et al.*, 1983) and it has been shown that the salivary contents of some steroids may be used to monitor cyclicity. This information is being used to monitor ovarian functions in women (Thirapatsukun *et al.*, 1978; Vining and McGinley, 1982) and cattle (Gao *et al.*, 1988; Kanchev *et al.*, 1988). Such information in buffaloes is lacking. The present study deals with the pattern and matched progesterone concentrations during different phases of oestrous cycle in serum, fore-milk and saliva samples of Nili-Ravi buffaloes.

### Materials and Methods

Experimental animals and sample collection: A total of 40 non-pregnant, lactating and cyclic Nili-Ravi buffaloes with normal reproductive tract were selected from the animals brought to the clinic of Department of Animal Reproduction or artificial insemination. The first oestrus of selected

buffaloes was missed for the collection of samples during different phases of oestrous cycle from the same animals. The serum, fore milk and saliva were taken at oestrus (day 1) met oestrus (day 3 following ovulation), mid luteal (day 10) and pro oestrus (day 19-20) phases of the oestrous cycle. Blood was collected by venepuncture from the jugular vein and serum was separated. Fore-milk samples were collected by hand strippings in clean sodium azide coated plastic vials. Saliva was collected in clean sterilized glass vials by giving gentle pressure on the middle of the dorsum of tongue. Before saliva collection, all food particles in the mouth were removed and it was washed with normal saline solution to remove the impurities. All samples were stored at  $-20^{\circ}\text{C}$  until assayed.

**Sample processing and progesterone assay:** Progesterone concentration was measured by direct 125 radioimmunoassay technique using kits provided by Diagnostic Products Corporation 5700 west 96th st. Los Angeles, CA 90045. All the laboratory work was done at Nuclear Institute of Agriculture and Biology, Faisalabad. The serum and fore-milk samples were processed according to the methods described by Singh and Puthiyandy (1980). The saliva samples were processed as described by Kanchev *et al.* (1988) with partial modification wherein salivary samples were not centrifuged in the present study.

**Statistical analysis:** Mean  $\pm$  SD values for progesterone concentrations in serum, fore-milk and saliva during different phases of oestrous cycle were calculated. A correlation (Steel and Torrie, 1980) was also computed between all experimental biological fluids in order to determine whether changes in the saliva concentration during different phases of oestrous cycle coincide with serum and fore-milk.

### Results

Progesterone concentrations in serum, fore-milk and

concentrations in oestral buffaloes were  $0.23 \pm 0.06$ ,  $0.49 \pm 0.03$  and  $0.08 \pm 0.06$  in serum, fore-milk and saliva samples, respectively. While the respective values at mid luteal phases were  $4.42 \pm 0.14$ ,  $4.22 \pm 0.10$  and  $2.28 \pm 0.08$ . The hormonal trend in serum, fore-milk and saliva during all phases of oestrous cycle was similar but relatively low levels were recorded in saliva. The correlations between the concentrations of progesterone in serum and those in fore-milk and saliva were  $r = 0.82$  ( $P < 0.01$ ) and  $r = 0.75$  ( $P < 0.01$ ), respectively.

	Saliva	Milk	Serum
Oestrus	$0.08 \pm 0.03$	$0.49 \pm 0.18$	$0.23 \pm 0.03$
Met-oestrus	$0.61 \pm 0.17$	$0.80 \pm 0.13$	$1.44 \pm 0.23$
Di-oestrus	$2.28 \pm 0.51$	$4.22 \pm 0.64$	$4.43 \pm 0.92$
Pro-oestrus	$0.27 \pm 0.08$	$1.45 \pm 0.38$	$0.34 \pm 0.09$

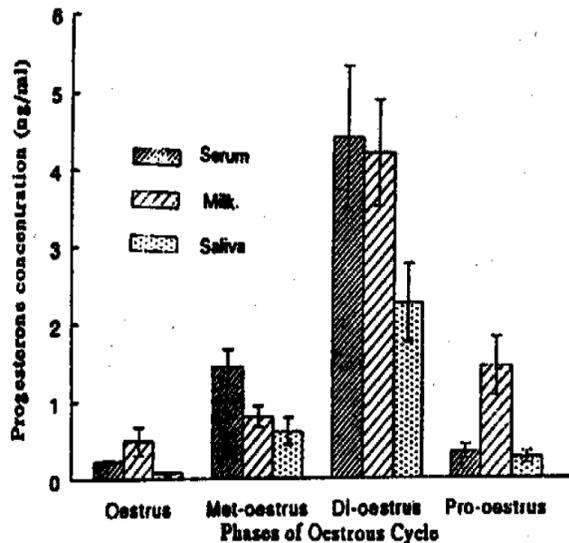


Fig. 1: Progesterone concentration (mean  $\pm$  sd) in serum, milk and saliva of buffaloes during different phases of oestrous cycle (n = 40)

### Discussion

The hormonal trend and serum progesterone concentrations reported in this study are similar to those found by others (Arora and Pandey, 1982; Chiesa *et al.*, 1983; Singh *et al.*, 1984; El-Sobhy *et al.*, 1987). The milk progesterone trend and concentration coincides with those of Taha *et al.* (1984), Murray *et al.* (1990) and Tariq *et al.* (1990). The milk progesterone concentrations during the luteal phase in the present study are lower than that of serum, which is in contrast with the findings of Bulman *et al.* (1978), who reported 3 to 4 times higher concentrations in milk than that of serum. This variability in concentration may be due to variable fat contents of individual milk samples (Arora *et al.*, 1980). The difference may also be attributed to the origin of sample (Singh and Puthiyandy, 1980) as fore-milk was used in this study compared to whole milk, skimmed

milk or milk fat. The positive progesterone correlation serum and milk samples in the present study is also supported by the findings of Heap *et al.* (1974), Hoffmann *et al.* (1976) and Pope *et al.* (1976), who reported that temporal pattern of progesterone concentration was similar in both fluids. The author of this paper is unable to trace any previously published work on buffalo salivary progesterone concentrations and its correlation with serum and milk during different phases of oestrus cycle. We found a similar progesterone trend and positive correlation between serum, milk and saliva samples. A similar progesterone trend and close correlation among above mentioned biological fluids in cows is previously reported by Gao *et al.* (1988) and Kanchev *et al.* (1988), proving the equal usefulness of salivary progesterone concentration for monitoring the ovarian functions. Similarly, Schramm *et al.* (1990) favoured the use of saliva as an alternate of serum for monitoring the reproductive status *in situ*. The results obtained in the present study show that progesterone determination in saliva can be used for evaluation of ovarian function in the buffaloes. However, pregnancy rate after inseminating the buffaloes on the basis of salivary progesterone level at the time of insemination and throughout the pregnancy to establish the basal levels for pregnancy diagnosis needs further investigations.

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**Qureshi *et al.*: Progesterone, buffalo, saliva, milk, serum**

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