

**BLOOD PLASMA PROGESTERONE CONCENTRATIONS IN TWO DIFFERENT VEINS AND COMPARISON OF PROGESTERONE CONCENTRATIONS AND RECTAL PALPATION FINDINGS TO DETERMINE OVARIAN CYCLICITY IN THE NILI-RAVI BUFFALO (*Bubalus bubalis*)**

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**ABSTRACT**

This study comprised of two experiments. The first experiment was aimed at determining progesterone concentrations in the blood samples collected from tail and jugular veins of pluriparous cyclic buffaloes. Plasma progesterone concentrations were  $0.19 \pm 0.36$  and  $0.12 \pm 0.22$  in early luteal phase (N=4) and  $1.60 \pm 0.72$  and  $1.35 \pm 0.47$  ng/ml in mid luteal phase (N=3) from tail and jugular veins, respectively. No difference ( $P > 0.05$ ) was found in progesterone concentrations in blood taken from the two sites. In the second experiment, cyclic or non-cyclic status of buffalo ovaries was determined by measuring plasma progesterone concentrations and palpation of ovaries per rectum. The reported anoestrous buffaloes (not showing heat symptoms for  $\geq 6$  months postpartum) were palpated rectally on two occasions, at eleven days interval, to monitor their ovarian activity. Blood samples were collected from jugular or tail vein. The buffaloes having no corpus luteum (N=3) on any ovary at both occasions were declared as true anoestrous, while those having a palpable corpus luteum (N=3) on one of the ovaries on any one occasion were termed as suboestrous. True anoestrous buffaloes had progesterone concentrations  $< 0.25$  ng/ml on both occasions. The cyclic or suboestrous buffaloes had progesterone concentrations  $> 1.0$  ng/ml at one of the occasions. The results of this study indicated that progesterone concentration of blood plasma and rectal palpation of ovaries could be used for determining ovarian cyclicity in Nili-Ravi buffaloes.

**Key words:** Buffalo, progesterone, tail vein, jugular vein, rectal palpation, ovarian cyclicity.

**INTRODUCTION**

Reproductive efficiency of buffaloes is hampered by inherent late maturity and prolonged inter calving interval (Singh *et al.*, 2000). The longer calving interval is due to the long service period, which is affected by various factors, including post partum anoestrus. Anwar *et al.* (2003) recorded 35% incidence of anoestrus in buffaloes maintained under rural managemental conditions in Pakistan.

Anoestrus in the buffalo has been categorized in two type's i.e. true anoestrus, where no ovarian activity and no outward signs of oestrus are observed and suboestrus, where ovarian activity occurs, but outward heat signs are not manifested. Measuring progesterone level in blood can differentiate between these two conditions. In true anoestrus, blood progesterone level remains constantly low (less than 0.25 ng/ml: Perera, 1984). While in suboestrus condition the progesterone level would be different at luteal and follicular phases.

Progesterone can be measured in milk, blood and faeces for monitoring ovarian activity. For measuring progesterone in blood, it is usually collected from jugular vein which may become difficult under field

conditions, where animals cannot be properly restrained. In such animals, an alternate site for blood collection is needed. The present study was aimed at comparing plasma progesterone levels obtained from tail and jugular veins of normally cycling buffaloes to determine whether any site can be used for blood sampling. Attempts were also made to measure plasma progesterone levels in true anoestrous and suboestrous buffaloes and to match these values with state of ovaries monitored through rectal palpation.

**MATERIALS AND METHODS**

**Comparison of progesterone level in tail and jugular veins**

Seven non-pregnant, cyclic buffaloes, in their second to third lactation, maintained at the National Agricultural Research Centre, Islamabad, Pakistan were selected for this study. A teaser bull was used twice-daily to monitor oestrous activity. Ten ml blood was separately collected from a dorsal tail vein and a jugular vein of each buffalo in a heparinized plastic test tube, plasma was separated by centrifugation at 3000 rpm for 10 minutes and stored at  $-20$  °C until progesterone assay.

### Progesterone concentration in true anoestrous and suboestrous buffaloes

Six buffaloes, which had not shown oestrous signs for more than 6 months after calving, were selected for this study. Ovaries in all six buffaloes were examined twice through rectal palpation at 11 days interval. Ten ml blood was collected by jugular venipuncture from each buffalo at both palpations, plasma was harvested and stored as described above. The buffaloes with no corpus luteum on their ovaries at both palpations and less than 0.25 ng/ml of plasma progesterone at both samplings (Perera *et al.*, 1984) were termed as true anoestrus. The buffaloes with a corpus luteum on one of the ovaries and progesterone values more than 0.25 ng/ml at one of the occasions were considered suboestrous females.

### Progesterone assay

Progesterone estimation in blood plasma was made by using Coat-A Count RIA kits (Diagnostic Products Corporation, USA). According to the procedure described by Rodbard and Lewald (1970), series of marked antibody coated polypropylene tubes were prepared with 100µl of standard or plasma sample. One ml of I<sup>125</sup> labeled progesterone was added to each tube. Tubes were covered and incubated for 3 hours at room temperature (25°C) and decanted until removal of visible moisture. Tubes were counted for one minute in a gamma counter. Sensitivity of test was 0.03 ng/ml, while specificity of test was 100 percent for progesterone.

### Statistical analysis

Paired t-test was used to compare progesterone values in blood obtained from tail and jugular veins (Mendenhall, 1983).

## RESULTS AND DISCUSSION

Plasma progesterone concentrations in tail and jugular veins were quite low (< 0.2 ng/ml, Table 1). However, at mid luteal phase the level of progesterone in both sampling sites was higher (>1 ng/ml) compared to the early luteal phase. Progesterone concentration in blood plasma of true anoestrous and suboestrous buffaloes at 11 days interval has been presented in Tables 2 and 3.

Usually jugular vein is used for blood sampling in large animals but tail vein has also been used for the collection of blood for hormone assays in earlier studies (Schallenberger *et al.*, 1990; Heyman *et al.*, 2002) in buffaloes and cows. Many a times, blood samples have to be collected from buffaloes maintained in rural areas where proper restraint facilities are not available, and jugular vein is not approachable; hence, an alternate

vein was explored. In this context, dorsal tail vein was found approachable easily. Progesterone concentration in the blood taken from jugular and tail veins did not differ significantly in the present study (Table 1). So, tail vein could be used for blood sampling for progesterone measurement in the buffalo.

**Table 1: Comparison of plasma progesterone levels obtained from tail and jugular veins of Nili-Ravi buffaloes**

State of oestrous cycle	No. of buffaloes	Plasma progesterone concentration (ng/ml) in	
		Tail vein	Jugular vein
Early luteal phase	4	0.19 ± 0.36	0.12 ± 0.22
Mid luteal phase	3	1.60 ± 0.72	1.35 ± 0.47

Means in the same row do not differ (P>0.05).

In the second part, plasma progesterone measurement in blood samples taken eleven days apart was used to differentiate between true anoestrous and suboestrous buffaloes. Qureshi *et al.* (1998) recorded an incidence of 51.5 % silent oestrus in buffaloes under field conditions and it was confirmed by repeated milk progesterone assay. In the present study, buffaloes which were declared as anoestrous on the basis of rectal findings, showed progesterone concentration less than 0.25 ng/ml on both the sampling occasions. Perera *et al.* (1984) and Qureshi *et al.* (1998) also observed constantly low milk progesterone levels in anoestrous buffaloes.

In suboestrus buffaloes, plasma progesterone concentration was greater than 1 ng/ml on one of the occasions. In these buffaloes, there was a difference in progesterone concentration at day 1 and 11. This is because of positive correlation between numbers of active luteal cells and plasma progesterone level. Kamonpatana *et al.* (1979) observed change in progesterone concentration in milk at day 1 and 11 in cyclic buffaloes. Although the suboestrous buffaloes in the present study were not showing any external signs of oestrus but their ovaries were cyclic and progesterone concentration confirmed the cyclicity. Chauhan *et al.* (1983) used progesterone assay along with rectal palpation to diagnose and treat suboestrous buffaloes.

In the present study, suboestrous and true anoestrous state of buffaloes has been diagnosed with progesterone level measurement and a corpus luteal diagnosis by rectal palpation of ovaries, with equal efficiency (Tables 2 and 3). It indicates that although progesterone measurement in blood confirms the cyclic state of buffalo, but in the absence of progesterone measurement facilities, rectal palpation of ovaries can be used to assess the cyclic or acyclic state of buffaloes.

**Table 2: Plasma progesterone concentrations in true anoestrous buffaloes at 11 days interval**

Buffalo No.	Plasma progesterone concentration at day 1 (ng/ml)	Rectal palpation finding	Plasma progesterone concentration at day 11 (ng/ml)	Rectal palpation finding
1	0.09	No CL	0.09	No CL
2	0.05	No CL	0.01	No CL
3	0.00	No CL	0.08	No CL

**Table 3: Plasma progesterone concentrations in suboestrous buffaloes at 11 days interval**

Buffalo No.	Plasma progesterone concentration at day 1 (ng/ml)	Rectal palpation finding	Plasma progesterone concentration at day 11 (ng/ml)	Rectal palpation finding
1	0.49	No CL	4.94	CL
2	0.41	No CL	1.32	CL
3	2.87	CL	0.15	No CL

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