

## Seasonal Variations in Estradiol-17 $\beta$ , Progesterone and Cortisol levels during estrous cycle in Buffaloes\*

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### ABSTRACT

Variations in cyclic concentrations of Estradiol-17 $\beta$ , Progesterone and Cortisol were determined by RIA for one cycle each in five normal cycling buffaloes during summer, winter and rainy seasons. Overall mean values of progesterone were maximum in cold, followed by hot humid and warm seasons, with significant ( $P < 0.05$ ) differences between seasons. However, highest peak values ( $2.65 \pm 0.91$  ng/ml) were observed during hot-humid season. Estradiol-17 $\beta$  concentrations were higher on the day of estrus, with wide individual variations and remained low thereafter for most of the days of the cycle. Irrespective of the season, higher estradiol values were recorded during the cycle, particularly around day 3, 6, 10 and Day 3, possibly due to endocrinologically active dominant follicles of different waves in some buffaloes. Circulating cortisol concentrations fluctuated widely between animals ( $P < 0.05$ ) and were higher on the day of estrus, as compared to other days of the cycle, but the differences were not significant. No seasonal variations were recorded in cortisol concentrations.

**Key words:** Estradiol-17 $\beta$ , Progesterone, Cortisol, Estrous, Buffaloes

**E**stradiol-17 $\beta$  and progesterone are prominent gonadal hormones controlling reproductive and associated processes in an animal. These hormones change as events of an estrous cycle changes. Estradiol levels remain high in the presence of a large dominant follicle but the progesterone changes along with the changes in corpus luteum and remains low at the time of estrus. The cortisol is a metabolic hormone secreted from adrenal cortex. Changes in cortisol are due to several factors and stress is one of them. In buffaloes, estradiol-17 $\beta$  levels remain significantly higher during cooler months compared to hotter months (Rao and Panday, 1983). In a estrous cycle, the highest value of estradiol-17 $\beta$  has been reported on the day of estrus with a minor rise on day 4 and a more sustained on day 10 (Bachlaus *et. al.* 1979). On the other hand, progesterone levels have been reported to be minimum on the day of estrus with

a gradual rise upto day 15 followed by a sharp decline on day 2 before the next estrus (Bachlaus *et. al.* 1979; Chauhan *et. al.* 1983; Pahwa and Panday, 1983). Levels of progesterone and days of progesterone peaks vary from study to study. Seasons (Rao and Panday 1982) and nutritional stress coupled with high temperature (Kaur and Arora, 1984) do affect the progesterone levels of buffaloes. The level of cortisol has also been reported to show day to day variations (Rao and Panday, 1981) and also during the different seasons (Rao and Panday, 1983-84). The previous studies however, have been conducted in different group of animals during different seasons. Therefore, present investigation has been designed to study the seasonal variation in the level of estradiol, progesterone and cortisol in same group of animals that were maintained through out the year to avoid animal variations.

To study the influence of season on circulatory levels of hormones during estrous cycle five healthy, multiparous, non-lactating,

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normal cycling Murrah buffaloes were selected from NDRI buffalo herd. These animals were kept in loose paddock having brick flooring and asbestos roof over the manger. The animals were maintained as per standard feeding and managerial practices followed at the farm. The roughage consisted of a combination of green fodder, hay, wheat straw and silage depending on the availability at the farm. Free choice of fresh tap water for drinking was available to the animals throughout the day. Buffaloes were given shower bath during the hot hours of summer season. Buffaloes were maintained throughout the year and were not inseminated during the period of study. Estrus was detected by parading a vasectomised bull (teaser) twice daily each for 30 minutes at 7.00 AM in the morning and at 7.00 PM in the evening. The time of first detected estrus was taken as '0' hour. Signs of estrus expression were also observed by visual observations and recorded as suggested by Fraser (1968). The duration of estrous cycle in buffaloes was standardized to 21 days. For seasonal (hot-humid; August-September, Cold; December-January and Warm; April) study jugular blood samples were collected in heparinized vacutainer tubes, once daily at 0800 hr. The sampling was started 3 days prior to the occurrence of the expected estrus and continued further at specific intervals covering the entire estrous cycle till next estrus occurred. The samples were immediately carried to the laboratory in ice and were centrifuged at 3000rpm for 20 minutes to separate out plasma. Plasma was kept frozen at -20°C till analysis.

#### Assay Systems

**Estradiol - 17 $\beta$ :** Estradiol - 17 $\beta$  in blood plasma was determined by RIA technique as described by Echterkamp *et al.* (1976) with minor modifications.

#### Progesterone:

Progesterone in blood plasma was determined by RIA procedure described by

Kamboj and Prakash (1993).

#### Cortisol:

Cortisol in blood plasma was determined by RIA technique described by Henericks *et al.* (1984) and Sujata (1987) with minor modifications.

#### Recovery of Purified hormones:

Recovery of estradiol, progesterone and cortisol varied from 96.9-105.7%, 97.9-101.1% and 95.0-98.4%, respectively. Intra-assay and inter-assay coefficient of variations were 6.67-10.54, 9.23-10.25 for Estradiol; 6.97-11.52, 8.54-10.39 for progesterone and 5.43-10.54, 10.20-11.25 for cortisol, respectively. The data was subjected to appropriate statistical analysis to draw scientific inferences. Means and SE were calculated, least square analysis performed and correlations were worked out as described by Snedecor and Cochran (1967).

#### Estradiol - 17 $\beta$ :

The level of estradiol- 17 $\beta$  was high on day of estrus and declined to a very low level by day 1. These levels were maintained at low for most of the remaining days of estrous cycle in all the three seasons. However, at certain occasions, estradiol -17 $\beta$  was very high and this pattern was observed in all three seasons. A perusal of individual animal data indicated that these elevations were due to sudden rise in estradiol- 17 $\beta$  levels in one or two animals of the group at a time is evident from data in Table 1. Appearance of sharp peaks of high magnitude of estradiol- 17 $\beta$  during the luteal phase of the cycle has not been reported earlier. However, Bachlaus *et al.* (1979) reported that a minor rise occurs on day 4 and a more sustained on day 10 of the cycle. This is in accordance with Oanell (1987) who suggested a two wave pattern of follicular growth in buffaloes, one from day 3 to day 13 and another from day 9 to the end of the cycle. Similar values of estradiol- 17 $\beta$  during the estrous cycle in three seasons obtained in the present study do not agree with the findings of Rao and Pandey (1983) who

reported significantly higher concentrations of estradiol-17 $\beta$  during cooler months compared to hotter months.

#### Progesterone:

Progesterone levels during estrous cycle have been presented in Table 1. Levels of progesterone from the day of estrus onwards up to day 3 remained low, thereafter increased continuously with a little variation at certain occasions. After attaining a peak between days 12-15, the levels rapidly declined and the minimum level was reached 1-2 day before the next estrus. The pattern of change in progesterone levels during the estrous cycle was similar in all the three seasons; however, the mean levels were highest in cold followed by hot-humid and warm seasons. Similar pattern of change in progesterone level during estrous cycle has been reported by several workers (Chauhan *et al.*, 1983; Pahwa and Pandey, 1983), but values varied from worker to worker. The levels of progesterone observed in the present investigation were lower than those reported by Raizada *et al.* (1977) and Sanwal *et al.* (1980) during the luteal phase. However, these levels were similar to those reported by Chauhan *et al.* (1983). Seasonal variation in progesterone levels as observed in present study was also reported by Rao and Pandey (1982) and Kaur and Arora (1984). These workers also reported highest concentration of progesterone during cold season and lowest during warm or hot season when the temperatures started rising or were high.

#### Cortisol:

On different days of estrous cycle though the values fluctuated but remained similar in the three seasons. The findings indicate that in spite of great fluctuations on different days of the cycle, values were higher at/or around estrus. The pattern of change in cortisol levels is in agreement with Rao and Pandey (1981, 1983-84).

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Singh *et al.*

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