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## Serum estradiol 17 $\beta$ and progesterone levels during different phases of estrous cycle in silent estrus Murrah buffaloes

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### Abstract

The present study was conducted in silent estrus buffaloes to estimate the concentration of estradiol 17- $\beta$  and progesterone. Seven silent estrus buffaloes were selected and their cyclicity was confirmed by ovarian ultrasonography for three cycles. Similarly, seven normal estrus buffaloes were kept as control. Blood samples were collected during the different phases of cycle for estimating estradiol 17 $\beta$  and progesterone concentration. The mean estradiol-17 $\beta$  concentration (pg/ml) in silent estrus buffaloes was 4.28 $\pm$ 0.08, 8.16 $\pm$ 0.11, 3.12 $\pm$ 0.23 and 2.80 $\pm$ 0.15 during proestrus, estrus, metestrus and diestrus, respectively. In the normal estrus animals, it was 6.28 $\pm$ 0.20, 16.36 $\pm$ 0.18, 4.60 $\pm$ 0.12 and 3.48 $\pm$ 0.21 during similar phases respectively. Similarly, progesterone concentration (ng/ml) was 0.54 $\pm$ 0.09, 0.36 $\pm$ 0.56, 0.90 $\pm$ 0.19 and 1.82 $\pm$ 0.06 during proestrus, estrus, metestrus and diestrus, respectively in silent estrus buffaloes, where as it was 0.82 $\pm$ 0.32, 0.72 $\pm$ 0.14, 1.32 $\pm$ 0.16 and 4.19 $\pm$ 0.20 in the normal estrus buffaloes. The mean serum concentration of estradiol-17 $\beta$  was significantly lower during estrus in silent estrus when compared to the normal estrus buffaloes. Similarly, the serum progesterone concentration was also significantly low during diestrus in silent estrus buffaloes.

**Keywords:** estradiol 17- $\beta$ , progesterone, buffalo, silent estrus

### Introduction

Buffalo plays a prominent role in gross domestic product contribution from agriculture sector of India, by contributing about 50 percent of total milk production and more than 20 percent of the meat production (Uppal, 2009) [17]. Problem of silent heat along with delayed maturity, poor estrus expression, irregular estrous cycle, seasonality in breeding, anestrus, reduced conception rate, extended post-partum interval, repeat breeding are some of the major problems in buffaloes (Madan,1990). Among these various factors which affect the reproductive efficiency, silent estrus is a major issue in buffaloes.

Silent estrus is a condition in which the buffaloes do not exhibit the behavioral symptoms of estrus, although the physiological symptoms of estrus are present (Cochran *et al.*, 2013) [16]. As a result of silent estrus, buffaloes become repeat breeders and fail to maintain the estrus regularity and cyclicity (Cho *et al.*, 2012) [5].

Silent estrus is mostly observed during the post pubertal period in heifers and early post-partum. Lower peak values of estradiol around estrus coupled with decreased progesterone concentrations was attributed to be the major reason responsible for a higher incidence of silent estrus during summer (Rao and Pandey, 1982) [12]. The estrogen level determines the intensity of estrus signs, which is low in high yielding dairy cows (Lopez *et al.*, 2004). Lower concentration of estrogen may be either due to higher metabolism and clearance with a high metabolic load (Sangsritavong *et al.*, 2002) [14] or sub-optimal follicular growth (Awasthi *et al.*, 2007) [2]. The probable cause of silent estrus is sub-optimal secretion of estradiol by mature follicles or higher threshold of estrogen in central nervous system to display the symptoms of estrus. Progesterone secreted from regressing CL of previous cycle potentiates the action of estrogen and seems to favour the manifestation of estrus in next cycle (Allrich, 1994) [1]. Thus, lack of progesterone priming results in sub-estrus. Such conditions have been frequently reported in dairy buffaloes especially in summer months (Singh *et al.*, 2013) [15] and may be the one of the reasons for prolonged calving interval in buffaloes.

## Materials and methods

### Animal selection

The study was performed with seven silent estrus and seven normal cycling pluriparous graded Murrah buffaloes at Post Graduate Research Institute in Animal Sciences, Kattupakkam, Chennai. The seven silent estrus animals were identified and selected based on the visual observations and farm records. The cyclicity of these buffaloes was confirmed by regular trans-rectal ovarian ultrasonography at an interval of 3 days for three consecutive estrous cycles. All the buffaloes were maintained with similar feeding and managerial conditions during the entire study.

### Blood Sampling

Blood collection was done during proestrus, estrus, metestrus and diestrus periods. Blood samples were collected in sterile, heparinized vacutainers through jugular vein puncture from all these buffaloes as per the standard protocol. The samples were transported to laboratory in ice box and the serum was separated by centrifugation at 3000 rpm for 15 min and stored at  $-20^{\circ}\text{C}$  until analysis.

### Hormonal Estimation

The stored serum samples were utilized for the estimation of estradiol 17- $\beta$  and progesterone concentration by Enzyme Linked Immuno Assay (ELISA) using the Calbiotech, Inc (CBI) estradiol (E2) ELISA Kit and Progesterone ELISA Kit, respectively at the Department of Veterinary Gynaecology and Obstetrics, Madras Veterinary College, Chennai as per the kits protocol.

## Results and Discussion

### Serum oestradiol-17 $\beta$ profile

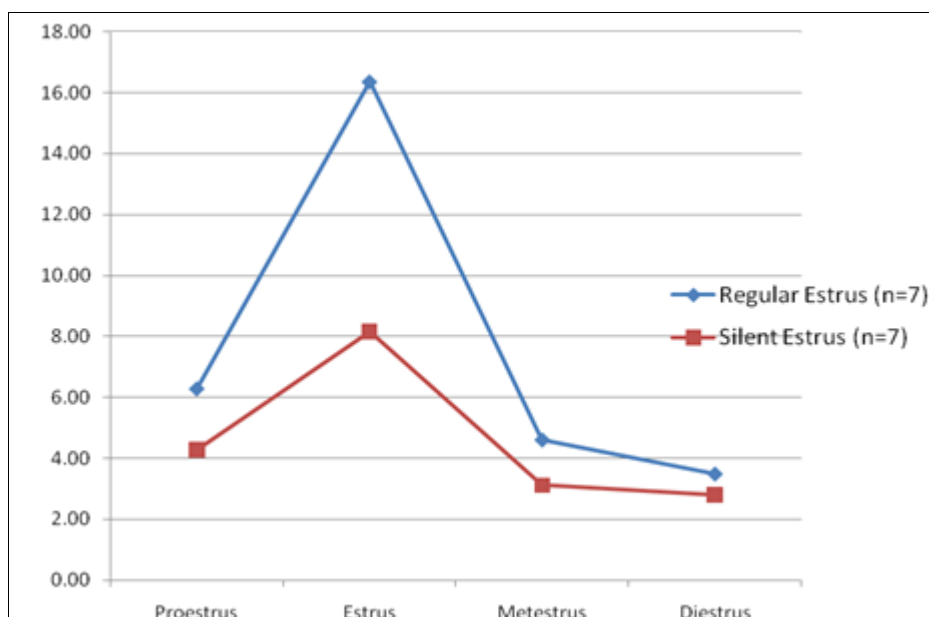
In the silent estrous group, the estradiol-17 $\beta$  concentration was  $8.16\pm 0.11$  pg/ml during estrus and  $2.80\pm 0.15$  pg/ml during diestrus phase (Table 1). During proestrus and metestrus the estradiol concentration was  $4.28\pm 0.08$  and  $3.12\pm 0.23$  pg/ml, respectively in the silent estrus buffaloes. In the normal estrus group, the mean estradiol 17 $\beta$  concentration was lowest during the diestrus phase ( $3.48\pm 0.21$  pg/ml) and increased to a maximum of  $16.36\pm 0.18$  pg/ml during the estrus phase (Figure 1). During proestrus and metestrus, the

estradiol concentration was  $6.28\pm 0.20$  and  $4.60\pm 0.12$  pg/ml, respectively in the normal estrus buffaloes.

**Table 1:** Mean serum Estradiol 17 $\beta$  concentration (pg/ml) during different phases of estrous cycle in normal estrus and silent estrus graded Murrah buffaloes

Estradiol 17- $\beta$ (pg/ml)	Stages of Estrus Cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Normal estrus (N=7)	$6.28\pm 0.20$	$16.36\pm 0.18$	$4.60\pm 0.12$	$3.48\pm 0.21$
Silent estrus (N=7)	$4.28\pm 0.08$	$8.16\pm 0.11$	$3.12\pm 0.23$	$2.80\pm 0.15$

The mean serum oestradiol-17 $\beta$  concentration (pg/ml) did not show any significant difference ( $p>0.05$ ) among the two groups of silent estrus and normal estrus buffaloes during proestrus, metestrus and diestrus. However, the mean serum Oestradiol-17 $\beta$  concentration during estrus phase significantly differed among the groups ( $8.16\pm 0.11$  vs  $16.36\pm 0.18$  pg/ml). These results showed that, plasma estradiol concentration started increasing during proestrus, reached maximum value during estrus and declined during mid luteal phase which is in accordance with the reports of Batra and Pandey (1982/83) and Samad *et al.*, (1988) [13] where, the estradiol- 17 $\beta$  concentration increased after luteolysis and reached its peak value during estrus. After attaining the peak level, estradiol started decreasing and thereafter, estradiol fluctuated at lower levels throughout the entire luteal phase, except during metestrus where a little increase was observed, which could have been due to second wave of follicular growth. Buffaloes have been reported to have two or three waves cycle of follicular development, with the second wave occurring during days 10 to 11 of the cycle (Barnselli *et al.*, 1997) [4]. The proestrus rise of estradiol may be associated with triggering of LH release by positive feedback on hypothalamo-hypophyseal axis (Batra and Pandey, 1982; 1983) [3]. Proestrus rise in estradiol after withdrawal of progesterone is considered to be a prerequisite event for the initiation of behavioral estrus and preovulatory LH surge in most livestock (Mondal *et al.*, 2010) [11] since these values are lower in the silent estrus buffaloes, it could be cause for the poor estrus expression when compared with the normal estrus buffaloes.



**Fig 1:** Comparison of mean serum estradiol-17 $\beta$  concentration (pg/ml) in silent estrus and normal estrus buffaloes

### Serum Progesterone profile

The mean circulating plasma progesterone concentration ranged from  $0.72\pm 0.14$  to  $4.19\pm 0.20$  ng/ml (Table.2) during

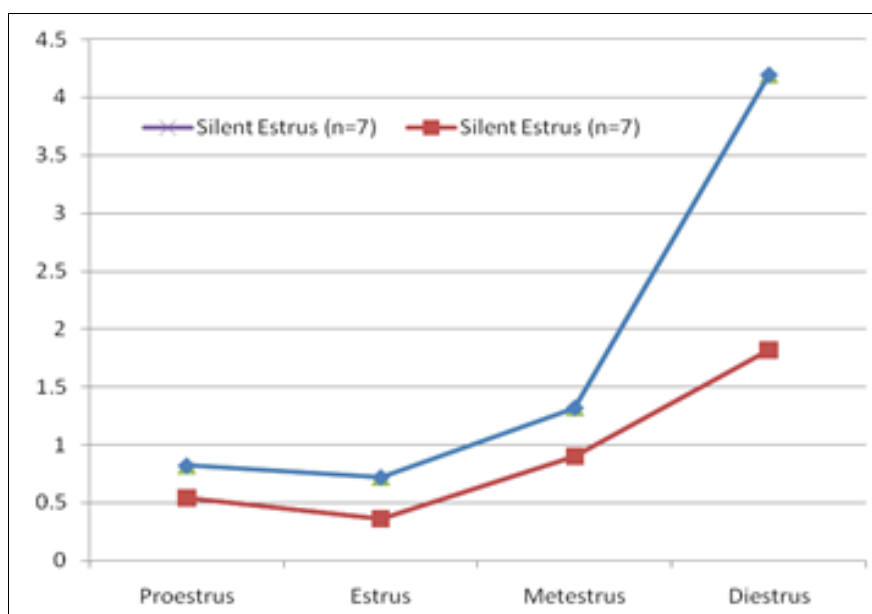
the entire phase of estrous cycle in normal estrus/control group. In the silent estrus group it ranged from  $0.36\pm 0.56$  to  $1.82\pm 0.06$  ng/ml.

**Table 2:** Mean serum progesterone concentration (ng/ml) during different phases of estrous cycle in normal estrus and silent estrus graded Murrah buffaloes

Progesterone (ng/ml)	Stages of Estrus Cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Normal estrus (N=7)	$0.82\pm 0.32$	$0.72\pm 0.14$	$1.32\pm 0.16$	$4.19\pm 0.20$
Silent estrus (N=7)	$0.54\pm 0.09$	$0.36\pm 0.56$	$0.90\pm 0.19$	$1.82\pm 0.06$

The progesterone values were found to be lowest during estrus phase ( $0.72\pm 0.14$ ) and increased to  $1.32\pm 0.16$  during the metestrus period and latter on the values peaked during the diestrus phase ( $4.19\pm 0.20$ ) in normal estrus expressing

buffaloes (Figure 2). Whereas in the silent estrus group, the plasma progesterone concentration were found to be lower when compared with the normal estrus buffaloes at the corresponding phases of estrous cycle.



**Fig 2:** Comparison of mean serum progesterone concentration (pg/ml) in silent estrus and normal estrus buffaloes

The progesterone concentration were lowest during estrus ( $0.36\pm 0.56$ ), increased to  $0.90\pm 0.19$  and it peaked during the diestrus phase ( $1.82\pm 0.06$ ). The mean plasma progesterone concentration during diestrus period was significantly low in silent estrus buffaloes when compared with the normal estrus buffaloes at the similar phase of cycle.

In the present study, in both the groups, the plasma progesterone concentration was lowest during the proestrus and estrus phase, increased during early luteal phase to a maximum concentration during mid-luteal phase. These results are in agreement with earlier observations in buffaloes (Mondal *et al.*, 2003a and Mondal *et al.*, 2004) [7, 10] with minimum level on the day of estrus with gradual increase to the higher levels during luteal phase and then declining to basal level at next estrus. The decline in progesterone levels towards the end of the cycle and a sharp rise during luteal development suggests an active and functional corpus luteum. Similarly in cattle and buffalo that exhibiting silent estrus, progesterone level was lowest during estrus phase and increased to maximum level during luteal phase (Mondal *et al.*, 2003c; Mondal *et al.*, 2003d) [8-9].

### Conclusion

The findings of present study provide the circulating levels of estradiol  $17\beta$  and progesterone during estrous cycle in silent and normal estrus expressing buffaloes which can be used for

clinical and experimental interpretations. Although some basic information is now available on various hormones related to estrus expression in buffaloes, information on pulsatile release of gonadotropins and steroid hormones status, control of receptors for different sex hormones, the close relationship between hormones, follicular recruitment and growth and development patterns could be essential for better understanding of silent estrus in buffaloes. Such information would help to solve some of the problems specific to buffaloes like silent estrus with poor expression of behavioral estrus. Further research studies are needed especially at cellular and molecular level for better understanding of silent estrus in buffaloes.

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