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Short Communication

SERUM ESTRADIOL AND PROGESTERONE PROFILE BEFORE AND AFTER ESTRUS INDUCTION WITH CIDR+ $PGF_{2\alpha}$ PROTOCOL IN SILENT ESTRUS BUFFALOES

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SUMMARY

Seven silent estrus graded Murrah buffaloes maintained at Post Graduate Research Institute on Animal Sciences, Kattupakkam were selected for the study based on the farm records, visual observation and trans-rectal ultrasonography. All the selected buffaloes were subjected for estrus induction with CIDR and $PGF_{2\alpha}$ protocol. The CIDR was inserted per-vaginally on day 0, $PGF_{2\alpha}$ was administered on day 8, CIDR was removed on day 9 and estrus signs were recorded on day 11 and 12. Blood sampling were done during the various phases of the estrous cycle before and after estrus induction. The serum samples were subjected for estradiol and progesterone estimation. The mean estradiol concentration during proestrus, estrus, metestrus and diestrus were 4.28±0.28, 8.16±0.32, 3.12±0.21 and 2.80±0.30 (pg/ml), respectively before estrus induction; 5.11±0.34, 10.44±0.36, 3.97±0.27 and 2.93±0.23, respectively after estrus induction. Similarly, the mean progesterone concentration during proestrus, estrus, metestrus and diestrus in silent estrus buffaloes were 0.54±0.28, 0.36±0.32, 0.9±0.21 and 1.82±0.30 ng/ml, respectively before estrus induction. Similarly, the mean serum progesterone concentrations were 0.89±0.10, 0.56±0.12, 1.14±0.14 and 3.50±0.41 ng/ml during proestrus, estrus, metestrus and diestrus, respectively after estrus induction. From the present study, it was inferred that both the estradiol and progesterone concentrations were higher during estrus after estrus induction with CIDR and PGF2Alpha protocol in silent estrus buffaloes.

Keywords: Buffaloes, Estradiol, Estrus induction, Progesterone, Silent estrus

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Buffalo plays an important role in gross domestic product contribution from Indian agriculture, by contributing about 50 per cent of total milk production and more than 20 per cent of the meat production (Uppal, 2009). Among the various reproductive problems, poor estrus expression with silent estrus is a major contributing factor affecting their reproductive efficiency. Silent estrus is a condition in which the buffaloes do not exhibit the behavioral signs of estrus, although the physiological signs are present. As a result, most of the buffaloes become repeat breeders and fail to maintain the regular estrus and cyclicity. Silent estrus is usually observed during the post pubertal and early post partum period. Lower peak values of estradiol around estrus along with progesterone level were described to be the major cause for an increased incidence of silent estrus (Rao and Pandey, 1982).

Based on these facts, the present study was carried out to determine the levels of estradiol and progesterone at different phases of estrous cycle before and after estrus induction with CIDR- PGF2α combination protocol in silent estrus buffaloes.

Source of buffaloes: Seven healthy, pluriparous, silent estrum graded Murrah buffaloes below 10 years of age maintained at Post Graduate Research Institute in Animal Sciences, Kattupakkam, Chennai were utilized for this study. Seven regular cycling buffaloes were included as control. These buffaloes were maintained with similar feeding and managemental conditions during the entire study period. The regular and silent estrus buffaloes were selected based on the visual observations and farm records. The cyclicity of these buffaloes was confirmed by rectal palpation and regular trans-rectal ultrasonography.

Synchronization of estrus: The selected silent estrus buffaloes were synchronized with (Controlled Internal Drug Release) CIDR+ PGF2α protocol. The CIDR was inserted per-vaginally on day 0, $PGF_{2\alpha}$ was administered on day 8, CIDR was removed on day 9 and estrus signs were recorded on day 11 and 12. The trans-rectal ovarian ultrasonography was performed for one entire cycle after estrus induction with CIDR+PGF2α on alternate days.

Blood Sampling and Hormonal estimation: Blood collection was carried out during proestrus, estrus, metestrus and diestrus in all the silent estrus buffaloes before and after estrus induction with CIDR+PGF2α protocol. Blood samples were collected from jugular venipuncture and the serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20 °C until analysis. The stored serum samples were utilized for the estimation of estradiol and progesterone concentration by Enzyme Linked Immuno Sorbent Assay (ELISA) using the Calbiotech, Inc (CBI) kit in the Department of Veterinary Gynaecology and Obstetrics, Madras Veterinary College, Chennai.

Serum estradiol-17β profile: The result (Table 1)

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revealed that the estradiol concentration was non-significantly lower in silent estrus buffaloes before estrus induction during all the phases of the estrus cycle except metestrus where it was significantly lower ($p < 0.05$). After estrus induction, the estradiol concentration increased significantly during estrus and metestrus and increased non-significantly during proestrus and diestrus phase.

The present finding is in agreement with Chaudhary *et al.* (2015) and Saxena *et al.* (2017) who reported a significant increase in the estradiol concentrations on the day of estrus after estrus induction in silent estrus buffaloes. These results showed that the serum estradiol concentration started increasing during proestrus, reached maximum value during estrus and declined during mid luteal phase (Table 1) which is in accordance with the report of Batra and Pandey (1983) where, the estradiol-17β concentration increased after luteolysis during proestrus and reached its peak value during estrus. After reaching the peak level, estradiol starts declining and fluctuate at low levels throughout the entire luteal phase, except during metestrus. The estradiol rise during proestrus may be associated with increased LH release by positive feedback on hypothalamo-hypophyseal axis. Proestrus rise in estradiol after withdrawal of progesterone is considered to be a prerequisite event for the onset of estrus behavior and preovulatory LH surge in most livestock (Mondal *et al.*, 2010).

The estradiol concentration on the day of estrus increased in the estrus induced silent estrus buffaloes. The differences in concentrations of estradiol in buffaloes induced with synthetic progestins may be due to an increased LH pulse frequency which in turn may stimulate ovarian follicular development and further increase in estradiol secretion. The estrogen concentration determines the intensity of behavioral estrus signs and since the estradiol concentrations are lower in buffaloes with silent estrus, it could be a cause for the poor estrus expression when compared with the silent estrus buffaloes. 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 in buffaloes (Priya *et al.*, 2014).

Serum progesterone profile: In the silent estrus buffaloes, the mean circulating plasma progesterone concentration during various phases of estrous cycle ranged from 0.36±0.13 to 1.82±0.30 ng/ml (Table 2). After estrus induction in silent estrus buffaloes, the mean serum progesterone concentration ranged from 0.56±0.12 to 3.50±0.41 ng/ml. There was a significant increase ($p<0.01$) in the mean serum progesterone levels in the silent estrus buffaloes before and after estrus induction in proestrus and metestrus phase. Whereas during estrus and diestrus, the progesterone levels increased non-significantly. The progesterone levels were found maximum during the diestrus phase of cycle both before and after estrus induction.

The mean serum progesterone concentration in silent estrus buffaloes before and after estrus induction were lowest during estrus phase and started increasing during the metestrus and reached its peak value during

Table1

Mean serum Estradiol-17β concentration (pg/ml) before and after estrus induction with CIDR+PGF2α during different phases of estrous cycle in silent estrus buffaloes

Estradiol 17-β(pg/ml)	Stage of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Before estrus induction (n=7)	4.28±0.27	8.16±0.13	3.12±0.20	2.80±0.30
After estrus induction (n=7)	5.11±0.34	10.44±0.36	3.97±0.26	2.93±0.22
t value	-1.88NS	0.98*	-2.50*	-0.33NS
P value	0.08	0.04	0.02	0.74

**t < 0.01, *t < 0.05 and NS t > 0.05

Table 2

Mean (±SE) serum progesterone concentration (ng/ml) before and after estrus induction during different phases of estrous cycle in silent estrus buffaloes

Progesterone (ng/ml)	Stage of estrous			
	Proestrus	Estrus	Metestrus	Diestrus
Before estrus induction (n=7)	0.54±0.27	0.36±0.13	0.9±0.20	1.82±0.30
After estrus induction (n=7)	0.89±0.10	0.56±0.12	1.14±0.14	3.50±0.41
t value	11.33**	1.05NS	7.69**	-1.36NS
P value	0.00	0.31	0.00	0.19

**t < 0.01, *t < 0.05 & NS t > 0.05

diestrus and latter on started decreasing as the buffaloes progress into proestrus (Table 2). The mean serum progesterone concentration after estrus induction was 0.56±0.12 ng/ml during estrus. This finding was in close agreement with Chaudhary *et al.* (2015) who reported the mean progesterone concentration did not differ significantly before and after (0.45 ± 0.04; 0.36 ± 0.02 ng/ml) estrus induction with similar estrus induction protocol during estrus phase. These findings were also well in accordance with Fanning *et al.* (1992), who reported a non-significant difference in progesterone concentration on the day of estrus with progesterone + PGF2α combination. Similar values were also reported by Saxena *et al.* (2017) during estrus phase before and after (0.63±0.07 and 0.58±0.08 ng/ml) progesterone based estrus induction procedure in buffaloes.

However, higher mean serum progesterone concentrations of 3.36±0.50 ng/ml (Butani *et al.*, 2011) and 2.89±0.14 to 3.22±0.19 ng/ml (Chaudhary *et al.*, 2015) in subestrus postpartum Surti buffaloes, 2.90±0.46 ng/ml (Dugwekar *et al.*, 2008) in Jafarabadi buffaloes, and 4.70±1.27 ng/ml (Tiwary, 2010) in cyclic Murrah buffaloes have also been documented.

The lack of progesterone priming could be a cause for silent estrus in the buffaloes (Allrich, 1994) which could be corrected by the application of CIDR for a short duration (7-12 d) which stimulates a negative feedback effect in the hypothalamus and pituitary and can inhibit the release of gonadotropines (Barile, 2012) and this inhibition ceases after CIDR withdrawal and excess amount of gonadotropins are released. This in turn stimulates the growth and maturation of follicles and induction of estrus (Cerri *et al.*, 2009) that leads to formation of strengthened CL which is responsible for increased secretion of progesterone which could be the reason for the higher concentration of circulatory progesterone during mid-luteal phase in estrus induced buffaloes.

The present study quantifies the circulating level of estradiol and progesterone during various phases of estrous cycle before and after estrus induction with CIDR+PGF2α protocol in silent estrus buffaloes, which could be utilized to achieve précised estrus identification and better conception rate in silent estrus buffaloes. Hence, estrus induction with CIDR+PGF2α protocol can be recommended for estrus induction at field condition.

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