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Concentration of serum estradiol, progesterone and cortisol during biological stress period of normal cyclic and silent Oestrous crossbred cows of Assam

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Abstract
The present study was planned to compare the concentration of serum estradiol, progesterone and cortisol level during biological stress period of normal cyclic and silent oestrous cycles of crossbred cows of Assam. Jugular blood samples were collected from ten normal cyclic and ten silent oestrous cows on day 0, 10, 17 and 20 of cycles for the present study. These 3 hormones were estimated by using ELISA kit manufactured by LDN GmbH& Co.KG, Germany, by solid phase enzyme-linked immunosorbent assay. Both estradiol and progesterone concentrations in serum were varied significantly ($P< 0.01$) between days of oestrous cycles of normal and silent oestrous cows. Lowest level of progesterone with concomitant highest level of estradiol was recorded on the day of oestrus. On the other hand, highest concentration of progesterone was found on the day 10 of cycles with lowest concentration of estradiol on day 17 of cycle. The mean levels of cortisol varied significantly between days of cycles with highest level on the day of oestrus.

Keywords: Cattle, cortisol, normal oestrous cycle, progesterone, silent oestrous cycle, estradiol

Introduction
Serum Progesterone plays an essential role in various reproductive functions, including regulating the length of the estrous cycle, maintaining pregnancy. Progesterone is high during the luteal phase and in pregnancy, however the levels decline if the animal fails to conceive. Progesterone has been implicated in inhibiting estrogen secretion and ovulation (Echternkamp *et al.* 1973 and Henricks, 1971) [11, 12]. Estradiol in plasma in the cycling cow and heifer play role in expression of estrus and ovulation (Akbar *et al.*, 1974; Chenault *et al.*, 1975 and Dobson, 1978) [2, 6, 9]. Cortisol is considered to be the indicator of biological stress (Cole and Cupps, 1977, Rao and Pandey 1981, Lyimo *et al.* 2007) [7, 17, 14] where high milk production and manifestation of reproductive cycles might be associated with oxidative stress (West 2003) [23]. Research information in regards to simultaneous modulation pattern of these 3 hormones in high yielding crossbred cows during normal and silent oestrous cycles. Therefore, the present study was carried out to compare the modulation of these 3 hormones in serum in normal cyclic and silent oestrous crossbred cows of Assam during oestrous cycle.

Materials and Methods
Ethical approval
All applicable institutional ethical guidelines for care and use of animals were followed.
Blood samples were collected from 10 normal cyclic and 10 silent oestrous cows on day 0, 10, 17 and 20 (day 0 of the next cycle) of oestrous cycle and serum was separated. Serum progesterone, oestradiol and cortisol were estimated by using ELISA kits manufactured by LDN GmbH & Co.KG, Germany, by solid phase enzyme-linked immunosorbent assay. The statistical analysis of the experimental data was carried out using standard software version (SPSS20).

Results and Discussion

The mean concentrations of serum estradiol, progesterone and cortisol in normal cyclic and silent oestrous cows on day 0, 10, 17 and 20 have been presented in Table 1. The mean serum estradiol concentration in silent oestrous and normal oestrous cows on different days of oestrous cycle *i.e.* on day 0, 10, 17 and 20 were 40.13 ± 0.64 & 39.34 ± 1.11; 24.24 ± 2.00 & 20.00 ± 0.82; 18.12 ± 1.74 &14.52 ± 0.79; and 40.15 ± 0.73 & 38.35 ± 0.68 pg / ml, respectively. Progesterone level were 0.33 ± 0.03 & 0.32 ± 0.04; 4.74 ± 0.41 & 4.47 ± 0.41; 3.67 ± 0.21 & 3.08 ± 0.33 and 0.43 ± 0.02 & 0.42 ± 0.02 ng / ml, respectively while cortisol level were 29.58 ± 0.83 & 28.48 ± 1.08; 25.46 ± 0.93 & 24.28 ± 0.67; 22.77 ± 1.22 & 21.51 ± 0.78 and 30.08 ± 0.76 & 27.62 ± 0.99 nM / L, in silent oestrous and normal oestrous cows on different days of oestrous cycle *i.e.* on day 0, 10, 17 and 20, respectively.

Both oestradiol and progesterone concentrations in serum varied significantly ($P< 0.01$) between days of oestrous cycle of both types of cyclic cows of the present study, but no significant variation was noticed between the groups. The estradiol was significantly highest with lowest serum progesterone level on the day of oestrus of cycles while reversal variation of steroids being highest concentration of oestradiol with lowest progesterone was recoreded on day 0 and 17 of cycles. Similar cyclic variation of steroids were also reported by (Dorbrwolski *et al.* 1973, Kang *et al.* 1994, Shukla *et al.* 2000, Bhuyan *et al.* 2004, Deka *et al.* 2007 and Bonia and Goswami (2011) [10, 13, 19, 5, 8, 4]. Serum progesterone level above 2ng/ml at mid luteal phase could be considered as normal as reported by Nakao *et al.* (1983a) [16]. In agreement to the present result Stevenson (1997) [20] also opined that the presence of corpus luteum associated with increase concentration of progesterone when estrogen level was varied towards mid luteal stages in cows.

Table 1: The concentration (mean ± s.e.) of serum estradiol, progesterone and cortisol in normal cyclic and silent oestrous cows on different days of cycles

Parameter	Types cows	Days of oestrous cycle			
		Day 0	Day 10	Day 17	Day 20
Estradiol (pg / ml)	Normal cyclic	$39.34^{\mathbf{A}} \pm 1.11$	$20.00_{ad}{}^{B} \pm 0.82$	$14.52_{\mathbf{ad}}{}^{\mathbf{C}} \pm 0.79$	$38.35_{a}{}^{A} \pm 0.68$
	Silent oestrus	$40.13^{\mathbf{A}} \pm 0.64$	$24.24_{bc}{}^{B} \pm 2.00$	$18.12_{\mathbf{bc}}{}^{\mathbf{C}} \pm 1.74$	$40.15_{ab}{}^{A} \pm 0.73$
Progesterone (pg / ml)	Normal cyclic	$0.32^{\mathbf{A}} \pm 0.04$	$4.47^{\mathbf{B}} \pm 0.41$	$3.08^{\mathbf{C}} \pm 0.33$	$0.42^{\mathbf{A}} \pm 0.02$
	Silent oestrus	$0.33^{\mathbf{A}} \pm 0.03$	$4.74^{\mathbf{B}} \pm 0.41$	$3.67^{\mathbf{C}} \pm 0.21$	$0.43^{\mathbf{A}} \pm 0.02$
Cortisol (nM/L)	Normal cyclic	$28.48_{abc}{}^{A} \pm 1.08$	$24.28_{ab}{}^{B} \pm 0.67$	$21.51_{b}{}^{C} \pm 0.78$	$27.62_{a}{}^{A} \pm 0.99$
	Silent oestrus	$29.58_{ab}{}^{A} \pm 0.83$	$25.46_{a}{}^{B} \pm 0.93$	$22.77_{ab}{}^{C} \pm 1.22$	$30.08_{ab}{}^{A} \pm 0.76$

Means with different subscripts (a,b,c) within a column and superscripts (A,B,C) within a row differ significantly ($P< 0.01$).

The analysis of variance revealed that means level of serum cortisol was significantly higher ($P< 0.01$) among different days of oestrous cycle in silent oestrus and normal cyclic cows. In the present study the mean concentration of cortisol in serum ranged from 22.77 to 30.08 nM / L in silent oestrous crossbred cows and the values ranged from 21.51 to 28.48 nM / L in normal oestrous cows. The highest concentration was recorded on day of oestrus and lowest value was recorded on day 17 of oestrous cycle in both the silent and normal oestrous cows. The pattern of changes of cortisol levels in both types of oestrous cycle was in agreement with the findings of Rao and Pandey (1981) [17], Singh *et al.* (2008) [19], Bonia and Baishya (2011) [3] and Bonia and Goswami (2011) [4]. The variation of levels of cortisol at oestrus might be due to biological stress factors like restlessness with wide range of body activities (McDonald, 1980) [15]. Tanaka *et al.* (2008) [21] observed that there was relationship between milk production and oxidative stress markers concentration in plasma with concomitant variations of estrogen, progesterone and cortisol during the time of onset of oestrus (Walker *et al.*, 2008) [22] and the low levels of this hormone in serum during luteal and follicular phases of cycles of the present study might be reflection of adaptation to the environment and some mechanism to prevent high metabolic heat (Adeyemo *et al.*, 1981) [1].

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