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# Moosense pedometer activity and periestrual hormone profile in relation to oestrus in crossbred cattle

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ABSTRACT

The objective of the study was to establish the characteristics of pedometer activity in crossbred cows (20) and to determine the relationships between the pedometer activity and the serum concentrations of periestrual hormones. Animals in oestrus were detected by trained personnel based on oestrus behaviour by visual observation twice daily. Further oestrus was detected by pedometer activity and it was confirmed by serum progesterone and estradiol concentration. Individual animal daily activity data were collected and transformed into hourly activity in Excel sheet and arranged for statistical analysis. The relationship of pedometer activity with E2, E2: P4 and progesterone level on the day of oestrus was determined. Pearson correlation analysis was performed using the statistical package SPSS (SPSS Inc. USA). Activity count per hour (ACPH) increased from 248.51±22.46, 312.51±37.16, 323.52±49.24 and 423.42±47.77 from -3 day, -2 day and -1 day of proestrus, respectively to estrus day. After estrus, the activity decreased from 313.76±46.62 and 271.36±36.06 on the 1st day and 2nd day of metestrus, respectively. A similar trend was observed for serum E2 and E2: P4 which increased from 3 days before estrus to reach a peak level on the day of estrus (31.40±2.34 pg/ml) and declined after that to the basal level (2 pg/ml) after estrus. Contrary to this, P4 concentration showed a decreasing trend from –3 day proestrus to estrus (0.46±0.05) and then a gradual increase after estrus day. ACPH showed positive correlation with estradiol concentrations (r = 0.34; P = 0.0779) and E2:P4 ratio (r=0.50; P=2.077) but, negative correlation with progesterone concentration on day of oestrus (r=–0.73; P=2.74). In conclusion, our results suggested that the concentration of E2, E2:P4 ratio and P4 concentration during proestrus and on the day of estrus are the important factors contributing the behavioral manifestation of estrus in terms of ACPH in crossbred cows.

**Key words**: Crossbred cattle, Estradiol, Oestrus behaviour, Periestrual hormones, Progesterone

Oestrus detection since a long time has been an important component of a successful reproduction program. Further proper oestrus detection is important in detecting the time of ovulation and performing right time of insemination thus improving the conception rate. Therefore effective possibility to improve fertility performance is to intensify oestrus detection and this has been overcome by the new technology that is a pedometer. Detection and duration of oestrus by pedometer is very much similar to behavioural oestrus duration based on visual observation. Most of the studies have focused on improving the efficiency of estrous detection using pedometers (Rorie *et al.* 2002) rather than improving fertilization rates. However, various studies showed that the increase in steps is a promising tool for accurate detection of oestrus (Firk *et al.* 2002), a prerequisite for good insemination results. In the bovine, expression of estrus and ovulation is the result of highly synchronized hormonal milieu during the periestrual period (Beg *et al.* 2003, Galina and Orihuela 2007) in which estradiol is the key regulator that synchronizes the endocrinological and behavioural events to drive oestrus; resulting from the action of ovarian steroids on behavioural centres in the brain (Roelofs *et al.* 2010). This is the main hormone which is responsible for the initiation of behavioral oestrus. The rise in estradiol-17β concentrations which occurs almost simultaneously with the onset of oestrus activity (Stevenson *et al.* 1998) is responsible for the initiation of behavioral oestrus. During follicle development, estradiol concentration increases and secreted mainly from the dominant follicle (Staigmiller *et al.* 1982). Further changes in the progesterone level have a strong correlation with

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oestrus. The decrease in concentration of P4 to fairly low basal level allows the sudden release of gonadotropins (Arthur *et al.* 1992). Progesterone from the corpus luteum also controls oestrus by inhibiting GnRH and LH pulses, which reduces estradiol concentration (Smith and Jennes 2001). The level can be measured in blood plasma or milk. There is a sharp decline in milk progesterone from >10 to <3 ng/ml when pro-oestrus starts (Docke 1994).

Thus by knowing pedometer activity on the day of oestrus and by quantifying estradiol and progesterone concentrations during periestrus improves the accuracy for oestrus detection. However, measuring these hormones is laborious, costly, time-consuming and requires sophisticated equipment and thus not suitable for field conditions. Circulating hormone concentrations during periestrus influences the estrusbehavior and intensity. Although the relationship among estrus behaviours and periestrus hormones has been studied, very little work has been carried out on the relationship among walking activity based on the pedometer activity and periestrus hormones in crossbred cows. Therefore, the present study was designed to evaluate the relationship among periestrual blood hormone concentration (E2 and P4) and pedometer activity in crossbred cattle to authenticate the accuracy of pedometer estrus.

## MATERIALS AND METHODS

The present study was conducted on Karan Fries cows (HF × Tharparkar) maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana (India).

*Experimental animals and general management:* Lactating cows (20), housed in a loose housing system of management were selected randomly for the study based on the behavioural signs and symptoms of oestrus. The average production of the animals during the 305 d lactation period was 3,988.42±278.21 with an average daily milk yield of 13.07 kg. The experimental animals were maintained in a loose housing system under group management practice.

*Measuring activity by pedometer:* The experimental animals were 40–60 days in milk at the time of attaching pedometer developed by IIT, New Delhi in collaboration with ICAR-NDRI, Karnal (Haryana). Wireless sensor network based precision animal management system known as Moosense was developed at ICAR-NDRI, Karnal (Sarangi *et al.* 2014). Moosense is a comprehensive monitoring solution meant to simultaneously monitor multiple animal management parameters such as ambient temperature and humidity, nutrient intake, and activity (Mohanty *et al.* 2010, Sarangi *et al.* 2014).

For daily recording of activity, a pedometer was tied on foreleg of cows at the metatarsal region. The pedometer recorded the number of steps a cow taken continuously 24 hr and send the activity on an hourly basis and these daily activities were collected by visiting the shed twice with the Mini Computer attached with the Base station (10:00 AM and 4:00 PM) from day zero up to 45 days for collecting daily biorhythm of activities from dairy cows. The pedometer has the capacity to store the activity data for 30 h. The battery was charged regularly after every week for 2 h. Pedometer oestrus or increase in a number of steps (measured by a pedometer) was calculated using a method based on the median number of steps as per Roelofs *et al.* (2005).

*Periestrual hormone profile during oestrus:* Approval of Institutes Animal Ethics Committee (IAEC) was obtained for blood collection in the experimental animals. Blood was collected on three days before oestrus and on the day of oestrus and two days after oestrus through jugular venipuncture.

*Hormone assay:* For confirmation of oestrus, estradiol and progesterone hornone assay was done in the sequencial serum sample collected during this period. Estradiol hormone (E2) was estimated to determine the peak level during the day of oestrus and decline in E2 during metestrus days. Progesterone (P4) level estimated for confirmation of oestrus with level below <1 ng/ml. The hormone was estimated using ELISA test kit (Uscn Life Science Inc. Wuhan, Hubei) as per manufacturer's instruction. The standard concentration of E2 and P4 hormone was calculated using Graph Pad PRISMs 3.0 software (GraphPadSoftware, USA).

*Statistical analysis:* The activity of the animals during experiments was collected every day from individual animals for collecting information about daily activity pattern analysis and was transformed to hourly activity in Excel sheet and arranged for statistical analysis. To determine the relationship of pedometer activity with E2, E2:P4 ratio and progesterone level on the day of oestrus Pearson correlation analysis was performed using the statistical package SPSS (SPSS Inc. USA). All means are presented as mean±SE unless otherwise stated. P values < 0.05 are considered for statistically significance.

## RESULTS AND DISCUSSION

Oestrus expression and behaviour is the outcome of various endocrinological events. Among all hormones, estradiol is the primary hormone for regulating oestrus drive and initiation of behavioural oestrus resulting from the action of ovarian steroids on behavioural centres in the brain (Roelofs *et al.* 2010). Concentrations of estradiol in plasma increased after concentrations of progesterone were less than 1 ng/ml. Concentrations of estradiol begin to rise from proestrus period, reaches its maximum level at 6–12 h before oestrus day and then sharply increase to its peak at the peak of LH (White *et al.* 2002). Further, it has been proved that in 50%, estradiol decreased by 5 h after LH peak and came to the basal level at 14 h (Chenault *et al.* 1975).

*Hormone profile during periestrual period and walking activity:* Activity count per hour (ACPH) in crossbred cows and estradiol concentration (pg/ml) during proestrus and oestrus days is depicted in Figs 1 and 2. Mean proestrus and oestrus activity count per hour found to be 294.85±32.68

(range 112–588.47) and 423.43±47.77 (range 149.75–875.29), respectively. Mean estradiol concentration (pg/ml) estimated to be 17.39±0.85 (range 12.49–25.44) and 31.40±2.34 (range 17.05–52.69) during proestrus and oestrus period, respectively. There is a relation between estradiol concentration and activity both in proestrus and oestrus days. Out of 20 animals, serum E2 estimated in serum samples collected in 3 animals showed a slight decrease in E2 concentration on the day of oestrus as compared to proestrus days. However, the activity was higher in these animals on the day of oestrus when compared to proestrus days. The difference in concentration may be due to individual variation and also may be due to an error in analyzing the sample. The maximum E2 concentration was lower in Holstein cows of both ultrasound performed and control group, i.e. 11.7±0.48 pg/ml and 10.2±0.48 pg/ml respectively (Roelofs *et al.* 2005). It has been reported that mounting other cow is the very next important oestrus behaviour after sniffing and chin resting being observed in the maximum percentage of oestrus cows. As 90–95% of the estrous periods mounting occurred during oestrus which acts as the best predictor for the time of ovulation (Roelofs *et al.* 2004, 2005). An important external indicator for an animal in oestrus is restlessness (Wendl *et al.* 1995). Based on the above fact ACPH can be included as useful oestrus behaviour for explaining the intensity of oestrus. Intensity and duration of estrus were positively related to circulating E2 concentration (Lopez *et al.* 2004).

However, it is not true for all as in an ovariectomized progesterone-primed cows revealed that those cows maintained on higher circulating E2 (12 vs.6 pg/ml) exhibited similar estrus intensity (Reames *et al.* 2011). Also supported by Lymio *et al.* (1999) and Roelofs *et al.* (2004) in Holstein cows where the intensity of the behavior, like maximum score or average estrus score, are not correlated with E2. In contrast to this it was found that higher circulating E2 (105.2 versus 85.6 pg/ml) respectively exhibited higher estrus intensity both in repeat breeder and

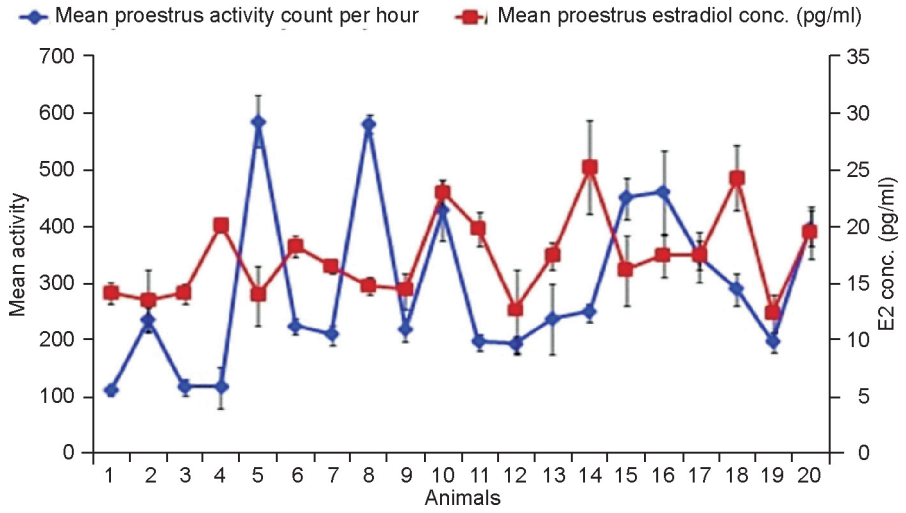


Fig. 1. Activity count per hour (ACPH) and estradiol concentration (pg/ml) during proestrus in crossbred cows

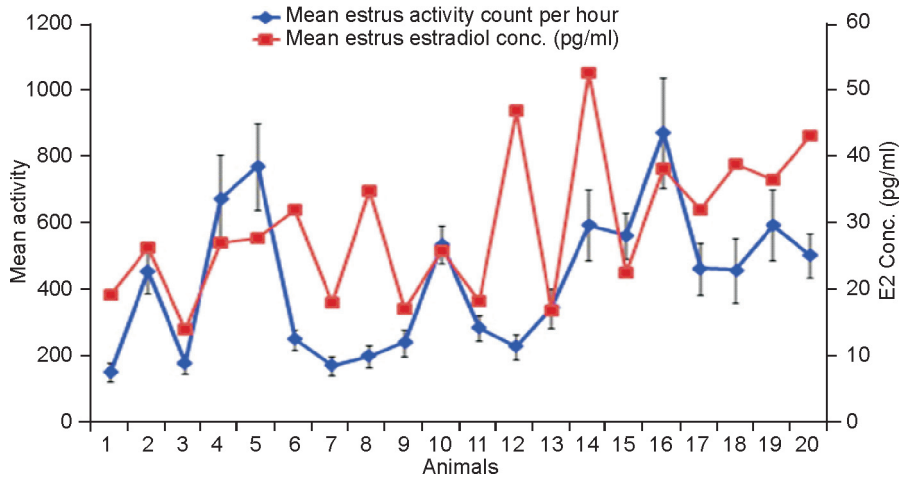


Fig. 2. Activity count per hour (ACPH) and estradiol concentration (pg/ml) during Oestrus in crossbred cows

Table 1. Activity count per hour (ACPH), Estradiol concentration, progesterone concentration, and E2:P4 ratio (Mean±SE) of different days for various phases of estrous cycle

| | Proestrus–3 day | Proestrus–2 day | Proestrus–1 day | Estrus | Metestrus1st day | Metestrus2nd day |
|---|---|---|---|---|---|---|
| E2 (pg/ml) | 15.06±1.54 | 18.14±1.68 | 21.99±1.71 | 31.40±2.34 | 24.21±1.93 | 19.07±1.48 |
| P4 (ng/ml) | 2.17±0.20 | 1.67±0.16 | 1.03±0.14 | 0.46±0.05 | 1.02±0.12 | 1.53±0.16 |
| E2:P4 | 6.93±2.21 | 10.81±1.35 | 21.21±14.43 | 67.89±15.15 | 23.52±6.76 | 12.45±2.45 |
| AverageActivity per hour | 248.51±22.46 | 312.51±37.16 | 323.52±49.24 | 423.42±47.77 | 313.76±46.62 | 271.36±36.06 |

E2, Estradiol; P4:Progesterone; E2 : P4, Estradiol : Progesterone

normal cows (Sood *et al.* 2015). However, some reports also suggested that the threshold estradiol level rather than circulating E2 is responsible for oestrus intensity (Allrich 1994). Furthermore, variation in estrus characteristics at an individual or herd level cannot be excluded (Bertilsson *et al.* . 1998). Mean ACPH on pro-estrus and estrus days reported to be 152.96±9.28 and 252.610±11.05 activity/h, respectively (Madkar 2013) and 371±91 during estrus when recorded with activity monitoring systems—heatime (Silper *et al.* 2015) was lower when compared to our study. The level of estradiol on proestrus and estrus days by Henricks *et al.* (1971) was less when compared with our study. The plasma estrogen concentrations were less than 10 pg/ml until the day before estrus, 15–25 pg/ml on the day before estrus. There were transient increases between 3 and 10 pg/ml during the first 3 days of proestrus. Further with the onset of estrus, the estrogen level became equal to that present on the day before estrus (15–25 pg/ml). However, it began to decrease within 2–5 h after the onset of estrus. E2 concentrations were correlated with milk production (r=–0.57; P<0.0001) and it was reported that higher producers had lower E2 concentrations than lower producers (6.8±0.5; n=31vs 8.6±0.5 pg/ml; n=40; P=0.01) (Lopez *et al.* 2004). The other factor which may affect the decrease in walking activity is milk yield. Lopez *et al.* (2004) reported that cows with higher milk yield had a lower serum E2 concentration on the day of estrus. In another study on estrus events, each 1 kg increase in milk yield was associated with a 1.6% decrease in walking activity (Lo´pez-Gatius *et al.* 2005b). Thus from the figure it is clear that cows activity during oestrus is related to the concentration of E2, with maximum average activity during both proestrus and oestrus has highest E2 concentration.

Activity count per hour (ACPH), estradiol concentration, progesterone concentration and E2:P4 ratio of different days for various phases of the estrous cycle is presented in Table 1 and Figs 3, 4 & 5. Estradiol concentration starts increasing from three days before oestrus i.e. 15.06±1.54, 18.14±1.68 and 21.99±1.71 pg/ml on –3 day, –2 day and –1 day of proestrus with maximum level on day of oestrus, i.e. 31.40±2.34 pg/ml and then decreasing after day of estrus, i.e. 24.21±1.93 and 19.07±1.48 pg/ml on 1st day and 2nd day of metestrus. The trend for E2:P4 ratio was almost similar to those recorded for plasma E2, with the minimum ratio on –3 day proestrus (6.93±2.21) and maximum ratio on the day of estrus (67.89±15.15). A similar trend was seen for pedometer activity when recorded during proestrus period till metestrus. ACPH started increasing from 248.51±22.46, 312.51±37.16, 323.52±49.24 and 423.42±47.77 from 3 day, –2 day and –1 day of proestrus to estrus day. The maximum activity count was found during estrus. After estrus, the activity decreased from 313.76±46.62 and 271.36±36.06 on 1st day and 2nd day of metestrus, respectively. When comparing the P4 concentration, the level showed a decreasing trend from –3 day proestrus to estrus. Serum progesterone concentration was lowest at the day of estrus showing a gradual increase in the concentration to estrus. So, keeping in view of the above finding it is evident that activity count is dependent on estradiol concentration of animal. The graph clearly indicated that the activity has a positive relationship with estradiol concentration and E2:P4 ratio, whereas the negative relationship with progesterone concentration. The result was in accordance with the study of Mondal *et al.* (2006) where plasma E2 estimated in blood samples collected daily throughout estrus cycle in mithun cows and showed that the concentration increased from day –6 of the cycle and reached a maximum level on the day of estrus (day 0) and decreased thereafter to a basal level on day 3 of the cycle exhibiting minor fluctuation thereafter. During proestrus, a preovulatory follicle develops on one of the two ovaries. Under the influence of pituitary gonadotrophins, the follicle secretes large amounts of estrogens, which in turn, causes plasma concentrations of the E2, to reach peak levels (Schallenberger *et al.* 1985). The onset of estrus behaviour (estrus, heat) and the surge release of LH are coincident with the peak in plasma estradiol (Rajamahendran and Taylor 1991). Plasma concentrations of progesterone were very low during estrus in crossbred cows as also reported for other bovines, and this low progesterone concentration is a necessary prerequisite to the expression of estrus because progesterone is clearly inhibitory to estrus behaviour (Waldmann *et al.* 2001). Contrary to this it was reported that the pedometer score lags behind the behavioural score and E2 maximum (Lyimo *et al.* 1999). This could be expected since the pedometer was read after the activity had increased. The variation in result may be due to the difference in blood sampling intervals. In the above study, the sample was taken 4 times/24 h, which were more precise for peak E2 hormone assay and activity.

Also out of 20 estrus cows in which P4 level was <1 ng/

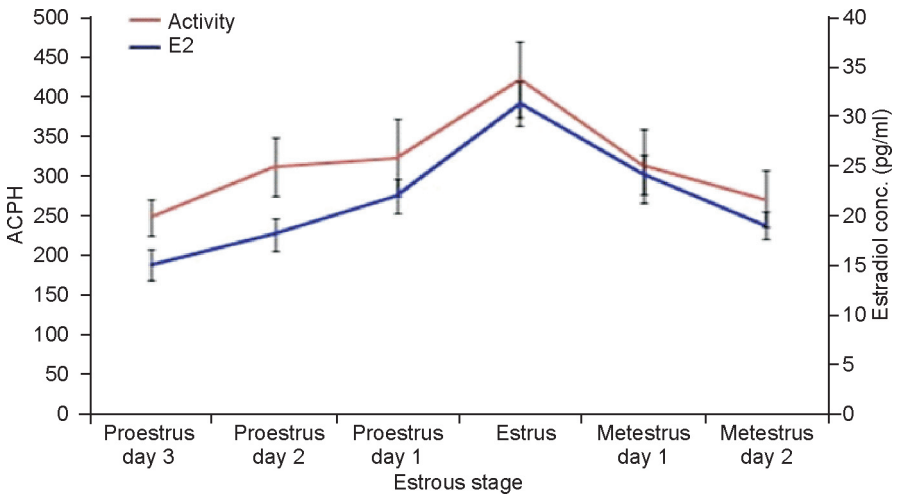


Fig. 3. Average activity count/hour and estradiol concentration (pg/ml) at different estrous cycle stages in crossbred cows

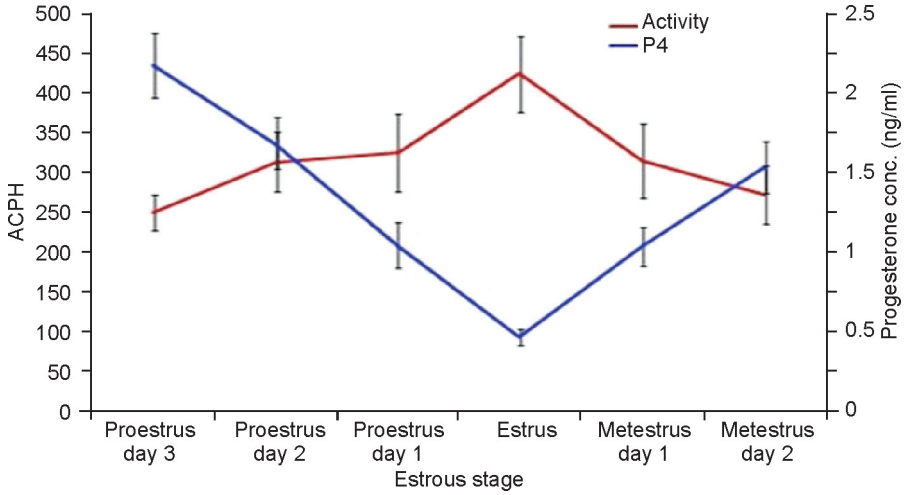


Fig. 4. Average activity count/hour and progesterone concentration (ng/ml) at different estrous cycle stages in crossbred cows

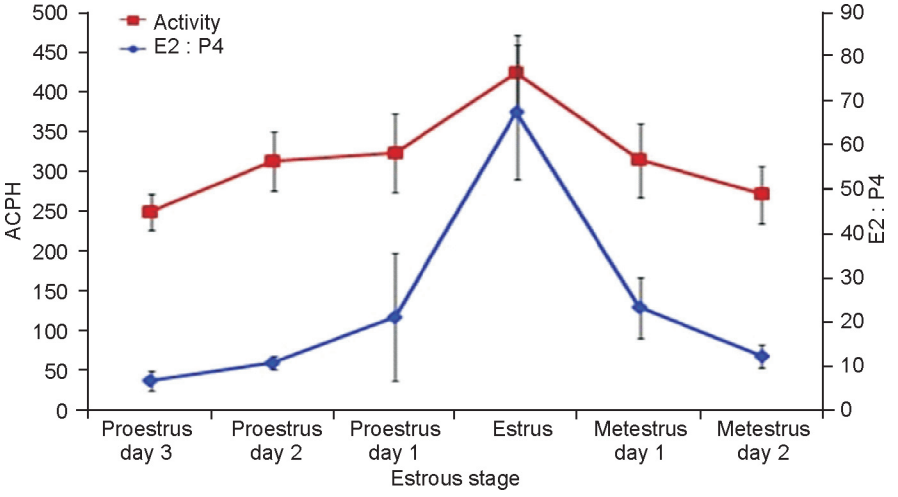


Fig. 5. Average activity count/hour and E2:P4 ratio at different estrous cycle stages in crossbred cows

ml only one cow showed lower activity. Hence, this can be due to individual variation which showed activity lower as compared to others. Further results of oestrus detection varied depending on the used threshold value, the number of cows, housing and treatment of cows and the utilised method of time series analysis (Firk *et al.* 2002). So by considering activity for estrus detection, the detection rate of the device in oestrus detection is 95% after being confirmed by serum P4 level. Accuracy was as low as 49% (Pulvermachr and Wiersma 1991) and over 90% (Roelofs *et al.* 2005). The detection of oestrus depends upon different threshold values. Further results are highly dependent on the number of cows investigated and also on managemental conditions of the farm (Firk *et al.* 2002).

*Correlations between hormone concentrations and ACPH during proestrus, oestrus and metoestrus period:* The correlation coefficients between hormone concentrations and ACPH during proestrus (3 days before oestrus), oestrus and metoestrus period (2 days after the end of oestrus) are presented in Table 2. ACPH had positive correlation with estradiol concentrations (r = 0.34; P = 0.0779) and E2:P4 ratio (r = 0.50; P = 2.077), but negative correlation with progesterone concentration on day of oestrus (r=–0.73; p=2.74). Similarly for proestrus days ACPH had significant positive correlation with estradiol concentrations (r = 0.26; P = 0.0417) and E2:P4 ratio (r=0.43; P=0.0007), but negative correlation with progesterone concentration on day of oestrus (r=–0.69; P=1.52). Further, the result was similar for metestrus days with ACPH had a positive correlation with estradiol concentrations and E2:P4 ratio, but a negative correlation with progesterone concentration. The positive correlation of estradiol concentrations and ACPH in crossbred cows in the present investigation is in agreement with the study of Mondal *et al.* (2006) and Lyimo *et al.* (1999) for oestrus behaviour in mithun cows. This indicated that estradiol hormone is important contributor for expressing estrus activities. Similarly, the positive association of activity and E2:P4 ratio at oestrus days also reflect the importance of ratios for day exhibiting behavioral estrus signs in cows. Further, the negative correlation of ACPH with progesterone concentration on the day of proestrus and oestrus indicated that the change in concentration of this hormone changes the ACPH, i.e. on the day of proestrus. P4 level was higher so activity was less but on oestrus as the P4 level decreases the activity increases. Negative correlation exists between P4 and estrus intensity (Waldmann *et al.* 2001). Furthermore, P4 depresses heat signs during estrus which is dose dependent, also P4 blocks the estrus inducing action of E2 (Davidge *et al.* 1987).

The detection rate of the pedometer in oestrus detection is 95% which becomes more reliable after being confirmed by serum P4 level. Pedometer activity has a strong relationship with serum periestrual hormone profile in deciding the oestrus in crossbred cattle. Further the concentration of E2, E2:P4 ratio and P4 concentration during proestrus and on the day of estrus are the important factors contributing to the behavioral manifestation of estrus in terms of ACPH in crossbred cows. Thus pedometer based on activity is very accurate in oestrus detection and may prove to be a promising tool for improving fertility performance.

Table 2. Correlation of hormone concentrations and ACPH during proestrus, oestrus and metoestrus period

| | Correlation coefficient | P value |
|---|---|---|
| Estradiol concentration on proestrus | 0.26 | 0.0417 |
| Progesterone concentration on proestrus | –0.69 | 1.52 |
| Ratio E2: progesterone on proestrus | 0.43 | 0.0007 |
| Estradiol concentration on day of oestrus | 0.34 | 0.0779 |
| Progesterone concentration on day of oestrus | –0.73 | 2.74 |
| Ratio E2: progesterone on day of oestrus | 0.50 | 2.077 |
| Estradiol concentration on metestrus | 0.20 | 0.009 |
| Progesterone concentration on metestrus | –0.65 | 0.812 |
| Ratio E2: progesterone on metestrus | 0.45 | 1.05 |

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