Original Research

Physiological and Endocrinological Characterization of Mid-Cycle Oestrus in Crossbred Cows

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Abstract

To study the follicular, luteal and endocrinological turnover during Mid-cycle oestrus (MCO) in comparison with true oestrus (OES) in crossbred cattle. A total of 36 animals were categorized into two groups viz. OES (n = 18) and MCO (n = 18). The diameter of the dominant follicle (DF) and corpus luteum and peri-follicular blood flow parameters were documented ultrasonographically on the day of OES and MCO. Plasma progesterone and oestradiol concentrations were analysed on day 9 of OES group and on the day of MCO. There was no significant difference in the diameter of DF between OES and MCO, but the diameter of CL was significantly larger in MCO than in OES. The values of Doppler pulse duration and pulsatility index of peri-follicular blood flow were significantly higher in MCO than OES, which was indicative of an ovulatory status of DF in MCO. In MCO, the oestradiol secreted by the DF over rode the low progesterone and induced the animal to exhibit oestrus-like signs during the mid luteal phase of the cycle.

Key words: Blood Flow and Endocrine Changes, Crossbred Cattle, Mid-Cycle Oestrus, True Oestrus, Follicle and Corpus Luteum

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Introduction

Efficient oestrus detection is a key factor that determines the success of breeding program in a dairy herd. Inspite of progress in the development of oestrus detection aids, errors occur leading to setback in reproductive management of dairy cows. Nebel *et al.* (1987) reported that insemination of cows not in oestrus was a problem in 30 per cent of the herds and emphasized that errors in detection of oestrus must be considered as a significant cause of low conception rates in problem herds. Anoestrus, prolonged oestrus and silent oestrus are the widely discussed aberrations of oestrus expression pattern in cattle. However in

the recent year's occurrence of mid-cycle oestrus (MCO) i.e. exhibition of oestrus-like signs during the mid-luteal phase of the oestrous cycle is found to be emerging as a serious aberration in the crossbred cows (Sood *et al.,* 2009, Satheshkumar *et al.,* 2014). The animals with MCO exhibit signs similar to that of true oestrus (OES) like restlessness, vaginal discharge, mounting etc. Transrectal gynaeco-clinical examination also revealed moderate to good tonicity of uterine horns simulating the OES (Satheshkumar *et al.,* 2014). These factors mislead the farmers and the veterinarians equally in diagnosing the OES. Number of inseminations per conception tends to increase when animals are inseminated repeatedly during MCO, thereby falsely projecting the incidence of repeat breeding condition. Thus, it is evident that MCO is also equally limiting the reproductive outcome in cattle. However, this aberration is not being considered seriously in the dairy industry which is evident by very few direct reports in *Bos taurus* (Williamson *et al.,* 1972, Heersche and Nebel, 1994) and crossbred (*B.taurus* × *B.indicus*) cows (Sood *et al.,* 2009, Satheshkumar *et al.,* 2014). Perusal of previous reports revealed that the incidence of MCO is in increasing trend among the crossbred cattle. Identifying the causative factors of MCO is the need of the hour in order to differentiate it from OES and in developing protocols to control the same.

It was hypothesized that the development of dominant follicles (DF) during the luteal phase of oestrous cycle and their related hormonal interactions with corpus luteum (CL) is responsible for the occurrence of MCO. The present report places on record, the ovarian and endocrinological changes associated with MCO in comparison with OES in crossbred cattle. In addition the incidence of MCO and its impact on fertility of cattle were also reported.

Materials and Methods

Crossbred cattle brought for artificial insemination (AI) with the history of oestrus signs to the Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Thanjavur, Tamilnadu were utilized for the study. Apart from the routine transrectal examination, ultrasongraphic screening of ovaries was also carried out to confirm the reproductive status of the animal before being assigned for AI. Cows with oestrus signs were considered to be in OES if the CL was $\leq$ 15 mm in diameter and atleast one DF (> 9 mm diameter) was present in one of the ovaries. Cows were considered to be in MCO if the CL was >15 mm in diameter and all the other parameters exist as that of OES (Kayacik *et al.,* 2005).

Incidence of Mid-Cycle Oestrus

A retrospective analysis of the cases (n = 5640) presented to the AI unit of the institution between June 2014 to June 2016 was carried out in order to record the incidence of MCO among the crossbred cattle. The mean day of expression of MCO (Day 0 - oestrus) was also recorded. A total of 36 animals were selected

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and categorized into two groups based on the diagnosis viz. Group I: OES (n = 18) and Group II: MCO (n = 18). These animals were subjected for thorough investigation as follows-

Ultrasonographic Characterization of Ovarian Structures

All the animals were screened using a real time ultrasound scanner (Esaote, MyLab30 Vet Gold, Italy) equipped with a 7.5-MHz transrectal linear probe to observe the follicular and luteal characteristics on the day of OES and on the day of MCO.

Biometry of Dominant Follicle and Corpus Luteum

Using B-mode of the scanner, ovaries were scanned on latero-medial and dorso-ventral planes (Zeitoun *et al.*, 1996) to ensure complete and accurate study of the ovarian structures. Follicle and luteal images were frozen at the maximum cross-sectional area and the internal ultrasound caliper was utilized to measure the length and width of these structures. The diameter (mm) was determined by taking the mean of their length and width (Savio *et al.*, 1988).

Peri-Follicular Blood Flow of Dominant Follicle

Peri-follicular blood flow mapping of the DF was conducted in various transverse sections using the Power Doppler-mode of the scanner on the day of OES and on the day of MCO. Animals were secured properly in the chute without any sedation before imaging (Altermatt *et al.*, 2012). The sample cursor was set at a width of 1mm and pulsed Doppler was performed on the "brightest and largest" blood vessel in the follicular wall (Siddiqui *et al.*, 2009, Ginther, 2007). Doppler spectrums or waveforms were obtained for at least three consecutive cardiac cycles. Two velocity parameters viz. peak systolic velocity (PSV) and end diastolic velocity (EDV) were recorded using inbuilt calipers (meters per second; m / s). Pulsatility index (PI) was automatically arrived with the software of the instrument. Doppler pulse duration (DPD) i.e. the duration of a cardiac cycle was recorded by measuring the linear distance between two subsequent EDV points (milli seconds; ms). DPD value is indirectly proportional to the pulse frequency (Satheshkumar *et al.*, 2012a). The mean values of these measurements were calculated for the statistical analyses.

Progesterone and Oestradiol Assay

In the OES and MCO groups, the circulating progesterone (P_4) and oestradiol (E_2) concentrations were assessed on day 9 (mid luteal phase) of the cycle and on the day of expression of MCO respectively. Plasma was separated by centrifuging the blood sample (5 mL) collected from jugular vein and stored at -20° C until assayed. The concentrations of both the hormones were measured with solid-phase radio immuno assay kit (Coat – A – Count, Immunotech SAS, France) and the radioactivity was counted in ^{125} I (STRATEC, Germany) gamma counter. The sensitivity of the P_4 assay was 0.05 ng / ml and intra-assay co-

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efficient variation was 6.5 and the sensitivity of E_2 assay was < 6 pg/ml and intra-assay co-efficient variation was ≤ 12.1.

Statistical Analysis

Data on follicular and luteal parameters, follicular blood flow parameters and plasma P_4 and E_2 concentrations in both the OES and MCO groups were analyzed by Student's *t*-test with completely randomized design (Snedecor and Cochran, 1994). SPSS.10.0® software was used for analysis of data.

Results

Incidence of Mid-Cycle Oestrus

Retrospective analysis showed that, among the 5640 crossbred cattle screened during the period, 3210 (56.9%) were in OES and 496 (8.8%) animals were in MCO. The rest of the animals (34.3%) were in different stages or reproductive status during the examination. Out of the 496 MCO animals, 339 (68.3%) were recorded as repeat breeders. The MCO was evinced on the mean day of 8.3 ± 2.3 (range: day 5 - 12).

Ultrasonographic Characterization of Ovarian Structures

The follicular and luteal characteristics on the day of OES and MCO were presented in Table 1. Statistical analysis revealed that there were no significant differences in the diameter of DFs between OES and MCO, but the diameter of CL was significantly (P<0.01) larger in MCO than in OES.

Table 1: Mean diameter of dominant follicle and corpus luteum during OES and MCO in crossbred cattle

	Type of oestrus		Significance
	OES	MCO	
Dominant follicle (mm)	11.9 ± 1.5 [b]	10.9 ± 1.1 [b]	NS
Corpus luteum (mm)	10.2 ± 1.4 [a]	19.3 ± 1.0 [b]	**

*Values within the row with different superscripts differ significantly; ** P < 0.01; NS: Not-significant*

Peri-Follicular Blood Flow of Dominant Follicle

The peri-follicular blood flow parameters of the DF on the day of OES and MCO were represented in Table 2.

Table 2: Mean values of peri-follicular blood flow parameters of the dominant follicle during OES and MCO in crossbred cattle

Parameters	Dominant follicle		Significance
	OES	MCO	
PSV (m / s)	0.1 ± 0.0 [a]	0.2 ± 0.0 [a]	NS
EDV (m / s)	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	NS
DPD (ms)	875.8 ± 39.5 [a]	1207.1 ± 25.7 [b]	**
PI	0.6 ± 0.1 [a]	1.1 ± 0.1 [b]	**

*Values within the row with different superscripts differ significantly; * P < 0.01; NS: Not-significant*

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There were no significant differences in PSV and EDV values between them, but the DPD was significantly ($P<0.01$) lengthier in MCO when compared to OES, which was indicative of low pulse frequency in the former category. The PI value was also significantly ($P<0.01$) higher in DF of MCO than OES indicative of higher blood flow resistance in the former group.

Plasma Progesterone and Oestradiol Concentrations

The plasma concentrations of P_4 and E_2 of OES and MCO groups were shown in Table 3. The P4 concentration was significantly ($P<0.05$) lower and E_2 concentration was significantly ($P<0.05$) higher in MCO than the OES group.

Table 3: Mean plasma concentration of progesterone and oestradiol during mid-luteal phase of normal cycle (OES) and on the day of MCO in crossbred cattle

Hormones	Day 9 - OES	Day of MCO	Significance
Progesterone (ng/ml)	4.6 ± 0.5^{b}	2.6 ± 0.2^{a}	*
Oestradiol (pg/ml)	8.1 ± 1.4^{a}	10.1 ± 2.0^{b}	*

Values within the row with different superscripts differ significantly * $P < 0.05$

Discussion

Williamson *et al.* (1972) reported that 7.3 per cent of exotic cows showed standing oestrus within 21 days of insemination. Sood *et al.* (2009) and Satheshkumar *et al.* (2014) recorded an incidence rate of 2.4 and 6.2 per cent in crossbred cows which was lesser than the present finding (8.8%). Perusal of the reports revealed an increasing trend in the incidence of MCO among the crossbred cattle.

Even though there was no significant difference in the diameter of DF between the two groups, the diameter of CL was significantly larger in MCO than in OES. Perusal of the data revealed presence of naturally occurring matured CL during the mid-luteal phase in MCO and regressed CL during OES. Practically, assessment of CL size by transrectal or ultrasonographic examination before the insemination might help the veterinarians to distinguish between OES and MCO. The oestrus behavior and its associated signs like intensity of uterine tone and nature of vaginal discharge are dependent on and positively correlated to the concentration of E_2 in the circulation (Lyimo *et al.*, 2000, Noakes, 2001). Sah (2002) reviewed the cases of gestational oestrus and concluded that if the P_4 level decreased to intermediate level which was sufficient enough to maintain pregnancy but not enough to suppress LH pulse, it led to maturation of DF and thereby an increase in E_2 production which resulted in expression of oestrus signs during mid gestation. A similar hormonal environment (low P_4 and high E_2) was recorded in the present study during the mid-luteal phase of MCO group animals. Considering these endocrine facts, the theory of gestational oestrus can be extrapolated to the occurrence of MCO too. The increased E_2 concentration which was secreted by the DFs of first or second follicular wave (Satheshkumar *et al.*, 2012b) would have overrode the low P_4 and induced

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the animal to exhibit oestrus like signs during the mid cycle. This luteal deficiency during the mid-luteal phase might compromise the embryonic sustenance (Satheshkumar *et al.*, 2015) leading to repeat breeding condition in these animals. It corroborated with the retrospective analysis which recorded 68.3 per cent repeat breeders among the animals that exhibited MCO.

Blood supply to individual follicle ensures adequate supply of hormones, oxygen, growth factors etc. and is critical for the attainment of dominance and ovulatory capacity (Acosta, 2007). Satheshkumar *et al.* (2012a) had opined that the lower DPD (indicative of rapid Doppler pulse frequency) and lower PI (indicative of lesser resistance) values of the peri-follicular blood flow ensures better vascular perfusion of ovulatory follicle aiding in ovulation process. On the contrary, high DPD and PI values of the peri-follicular blood flow were recorded during MCO in the present study. Inspite of low P_4 concentration during MCO, its suprabasal level (>1 ng/ml) was sufficient enough to prevent the ovulation by inhibiting the E_2 induced LH surge (Duchens *et al.*, 1994). Thus, the deficient blood flow parameters and suprabasal P_4 levels confirmed the anovulatory status of DF in MCO group. Hence insemination during MCO will not lead to conception.

Conclusion

From the fore going findings it could be concluded that luteal deficiency is the major factor contributing to the occurrence of MCO and hence remedial measures should be aimed at increasing the circulating P_4 levels during the mid luteal phase of the oestrous cycle. Satheshkumar *et al.* (2012b) confirmed that the endogenous production of P_4 could be substantially increased by administration of GnRH analogue on Day 6 post insemination which induces accessory CL formation in crossbred cattle. At the management level, steps should be taken to distinguish between OES and MCO before attempting the AI which will definitely reduce the number of inseminations per conception and improve the fertility rate in crossbred cattle.

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