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Follicular persistence, oestradiol level and conception on the first and second day of oestrus and influence of thermal stress in cross-bred dairy cows manifesting prolonged oestrus

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Abstract

Increasing incidence of prolonged oestrus reported in cross-bred dairy cows bred through artificial insemination (AI), is attributed to various causative factors and underlying mechanisms. The present study was to assess the pattern of follicular growth, oestrogen secretion, and conception on the first and second day of oestrus and to understand the influence of thermal stress as a causative factor for oestrus prolongation. The year-round study involved 52 cross-bred cows intensively managed under a hot humid climate, during their second and third months postpartum. Animals were examined for the status of ovarian follicles, oestrogen level, and conception to AI on Day 0 and Day 1 of oestrus confirmation and the findings were compared across seasons. While oestrus detection rate (ODR) and conception of AI were lowest during the summer season attributable to maximum TS, better ODR (P<0.05) and more prolonged oestrus (P<0.01) occurred during the seasons of favourable climate having least TS and better green fodder availability. Besides, the occurrence of larger follicles and almost similar oestradiol levels on Day 0 and Day 1 of oestrus, better conception rate for double AI (63.16 %) than total AI (48.48 %) indicated prolonged oestrus and involvement of delayed ovulation among the causation. Comparison of the seasonal pattern of follicular growth, oestrogen level, and conception to AI between Day 0 and Day 1 further clarifies the influence of TS and increased milk production as the potential contributors to the occurrence of prolonged oestrus in cross-bred cows under the hot humid tropical climate.

Keywords: *Crossbred cow, Oestrogen, Prolonged oestrus, Thermal stress*

An increasing incidence of prolonged oestrus has been reported in crossbred cows bred through artificial insemination (AI) (Kutty, 2020; Shakir, 2018) and various risk factors underlie its causation (Kutty, 2019; Singh *et al*., 2014). During the oestrous cycle of farm animals, oestrogen (E2) secreted mainly from granulosa cells of the dominant follicle produces various changes in ovaries like luteolysis and suppresses the growth of subordinate follicles (Schuller *et al*., 2017). In high-producing cows consumption of more feed causes a dramatic increase in liver perfusion and faster metabolism of ovarian steroids (Wiltbank *et al*., 2006), so a slow increase and inadequate peak of oestradiol leads to prolongation of the oestrus period, and the resultant poor quality of oocytes impairs fertilization and embryonic development (Sakatani *et al*. 2012a; Abdalla *et al.*, 2017). Alteration of follicular growth and secretory function has been reported in animals exposed to thermal stress (TS) (Wolfenson *et al*., 1995). Hence the present study investigated the

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pattern of follicular growth, level of oestrogen secretion, and conception to AI on the first and second day of oestrus and compared the findings across seasons to understand the influence of TS as a causative factor for the increasing incidence of oestrus prolongation in crossbred cows reared under the humid tropical climate.

Materials and methods

The study was carried out at Livestock Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University in India. The dairy farm of the station is located at an altitude of 60-70 meters above mean sea level, positioned at 11°21' N and 76°21' E latitude and longitude respectively, and having cross-bred cows intensively managed with feeding and breeding as per standard recommendations (ICAR-NIANP, 2013) formed the study setting. The yearlong study involved 52 cows belonging second and third months postpartum and at least eight cows were under observation every month.

Cows reported to have oestrus signs were verified based on history, clinico-gynaecological examination, and B mode ultrasonography of ovarian structures. Numbers of large follicles (8 mm or more) on both ovaries were recorded. Oestrus confirmed animals were inseminated with cryopreserved semen and blood samples were collected at the time of breeding (Day 0) and also 24 hours later (Day 1). The cows were re-examined on Day 1 for the persistence of internal changes of oestrus and ovarian follicles. Those having prominent tubular changes of oestrus persisting on Day 1 were re-inseminated and later verified for the return to oestrus and conception. Serum samples separated were stored frozen and subjected to oestrogen assay using ELISA Kit (Calbiotech inc®, El-Cajon Canada). Data were analyzed for the seasonal pattern of oestrogen level, follicle status and conception of Day 0 and Day 1, based on seasons prevailing in the region (Kutty, 2021) such as SON (Sep-Oct-Nov being Northeast Monsoon), DJF (Dec-Jan-Feb being Post monsoon), MAM (Mar-Apr-May being Summer) and JJA (Jun-Jul-Aug being Southwest monsoon) to assess the influence of TS on prolonged oestrus manifestations.

Results and discussion

The number of animals under the study, details of oestrus, and AI done during the four seasons are shown in Table 1. Classification of the seasons followed corresponding to four quarters (Kutty *et al*., 2019) such as SON (September October November representing Northeast monsoon), DJF (December January February – post-monsoon), MAM (March April May – Summer) and JJA (June July August – Southwest monsoon). A total of 46 oestrus periods were reported, 34 oestrus confirmed and 33 were inseminated (Day 0). Significantly high oestrus detection rate ($P<0.05$) as well as prolonged oestrus proportion ($P<0.01$) were observed during SON attributable to better expression of oestrus signs facilitated by the favourable climate of the region, after the seasons of extreme TS out of high ambient temperature during MAM and extreme humidity of JJA (Kutty *et al*., 2019) and also better availability of green fodder and increased consumption enhancing liver perfusion and faster metabolism of ovarian steroids prolonging expression of oestrus signs concurring the report of Wiltbank *et al*. (2006).

Even though the persistence of large follicles on the second day of oestrus was detected in all the cows, only 19 were re-inseminated. Details of AI done, return to oestrus, and conception from double AI as well as total AI across the seasons are shown in Table 2. Even though the numbers of AI done in any of the seasons were not

Table 1. Season-wise distribution of oestrus detected, animals observed and proportion of oestrus persisting on the second day among the observed animals

Season	Oestrus detection			Oestrus Detection Rate (%)	Oestrus on the second day	Prolonged oestrus (%)
	Cows observed	Oestrus reported	Oestrus confirmed			
SON	12	11	9	75.00[a]	6	66.67[b]
DJF	15	16	10	66.67[b]	6	60.00[b]
MAM	12	9	7	58.33[b]	4	57.14[b]
JJA	13	10	8	61.54[b]	3	37.50[a]
Overall	52	46	34	65.38*	19	55.88**

** Significant at 1 %, * Significant at 5 %, Values with different superscripts varies significantly

Table 2. Details of AI done, return to oestrus, conception from double AI and Total AI done across four seasons

Season	AI done			Return to Oestrus		Conception (%)	
	Day 0	Day 0 & 1	Double AI %	Double AI	Total AI	Double AI	Total AI
SON	8	6	75.00	2	4	50.00	62.50
DJF	10	6	60.00	1	4	66.67	60.00
MAM	7	4	57.14	1	2	50.00	28.57
JJA	8	3	37.50	1	3	100.00	37.50
Overall	33	19	57.58	5	13	63.16	48.48

Table 3. Ultrasonographic findings of ovaries during Day 0 and Day1 of oestrus

Parameters	Day of oestrus	
	Day 0	Day 1
Total oestrus studied	34	34
Follicles of 8 mm or more	57	51
Single follicle of 8 mm or more	15	18
Size of the largest follicle (mm)	8 to 16	11 to 16
Size of the second largest follicle (mm)	8 to 14	8 to 12
Mean size of largest follicle (mm)	12.85± 0.36	13.56 ± 0.29
Mean size of the second largest (mm)	9.68 ± 0.36	9.43± 0.28
Size of largest follicle on right ovary	22	19
Size of largest follicle on left ovary	12	15
Total follicles on right ovary	35	29
Total follicles on left ovary	22	22

large enough to make valid conclusions, the double AI proportion was highest during SON. Despite the highest conception rate for total AI, conception out of double AI was less during SON, indicating reduced conception rate in prolonged oestrus cases (Shakir, 2018). However, conceptions from double AI as well as total AI were the lowest during MAM indicating the adverse influence of TS on conception concurring the report of Dash *et al*. (2016).

Details of follicles detected at scanning on both the ovaries during Day 0 and Day 1 of oestrus are compared in Table 3. The occurrence of larger follicles was slightly more during Day 0 (57 versus 51), while the mean size of the largest follicle in mm was more on Day 1 than Day 0 (13.56 versus 12.85). Size of the second largest follicle appeared to be slightly more on Day 0 and is in agreement with the earlier finding of sub-ordinate follicles growing more during the TS prone seasons as reported by Wolfenson *et al.* (1995). More often the right ovary was having the largest follicle irrespective of Day 0 or Day 1, so the total follicles detected on the right ovary were 35 and 29 respectively on Day 0 and Day 1, as against 22 each on the left ovary. This is in agreement with the functional dominance of right ovary already established in bovine species (Stevenson, 2019). Details of follicles detected on Day 0 and Day 1 of oestrus are compared between the four seasons in Table 4. There was no significant difference in size of the largest follicle of the oestrus across seasons.

Oestradiol level

Oestradiol level (pg/ mL) on Day 0 and Day 1 of oestrus during the four seasons and overall mean are compared in Table 5. There were significant differences in the E2 levels of day 0 ($P < 0.01$) and day 1 ($P < 0.05$) between the seasons, the highest being during JJA attributable to the favourable shift of climate following summer and lowest during SON because of the increased intake of feeding materials enhancing the rate of steroid degradation irrespective of the days of collection. Since E2 levels can be correlated with the intensity and duration of oestrus signs, elevated E2 levels of JJA together with almost equal levels on Day 0 and Day 1 can be attributed to the prolongation of the oestrus period, even though external signs were lacking. However, the number of larger follicles was low and the size of the dominant follicle was smaller during JJA than in other seasons (Table 4.)

Table 5. Oestradiol levels (pg/mL) on Day 0 and Day 1 of oestrus across seasons

Seasons	E2 levels (pg/mL)	
	Day 0	Day 1
SON	31.9 ± 5.1^{a}	32.8 ± 6.0.^{a}
DJF	51.6 ± 4.6^{b}	46.5 ± 6.0^{ab}
MAM	52.8 ± 9.1^{b}	49.6 ± 3.8^{ab}
JJA	62.1 ± 5.6^{b}	61.9 ± 4.3^{b}
Overall	47.8 ± 3.31	45.7 ± 3.43
F-value (p-value)	4.89** (0.007)	3.34* (0.032)

*. Significant (P<0.05) **. Significant (P<0.01)

Values with different superscripts varied significantly within the columns

Table 4. Ultrasonographic findings on Day 0 and Day1 of oestrus across seasons

Parameters	Day of oestrus	Seasons				Overall
		SON	DJF	MAM	JJA	
Animals under study		9	10	7	8	34
Total follicles on ovaries	Day 0	19	18	10	10	57
Large follicles on the Right		6	9	4	3	22
Large follicles on the Left		4	4	1	3	12
Size of the largest (mm)		13.1 ± 0.69	12.4 ± 0.59	13.4 ± 0.40	13.0 ± 1.15	12.85 ± 0.34^{ns}
Total follicles on ovaries	Day 1	16	16	8	8	48
Large follicles on the Right		5	9	2	3	19
Large follicles on the Left		4	4	3	3	14
Size of the largest (mm)		12.9 ± 0.73	13.8 ± 0.38	13.8 ± 0.66	13.3 ± 0.61	13.45 ± 0.29^{ns}

ns – non-significant

Oestradiol levels across seasons were related to the growth pattern of follicles on the ovaries. The highest E2 level was during JJA when number of follicles was lowest. This can be attributed to the increased secretory activity of dominant follicles after the summer stress, inhibiting the growth of more follicles. Whereas TS causes an increase in size and number of larger follicles with lowered functional competence, especially during summer and subsequent months, concurring with the report of Wolfenson *et al.* (1995). Accordingly, lowering of AbT during JJA might have resulted in increased production of E2 by the dominant follicles. All types of follicles were maximum during SON and DJF, whereas the E2 level was significantly lowest during SON. Favourable weather and an increase in dry matter intake cause rapid metabolism of steroid hormones in the liver so that the E2 level remains low during the phases of increased milk production as reported by Abdalla *et al.* (2017).

Untimely detection of oestrus due to lack of behavioral signs can contribute to the variation of E2 level between Day 0 and Day 1. Comparatively low level of E2 (3.19 pg/mL) on Day 0 together with an elevated level on Day 1 (3.65 pg/mL), as in SON can be attributed to early detection of oestrus, so that E2 level continues to increase between Day 0 and Day 1. Reduction of E2 level from Day 0 to Day 1 can be due to late detection or early arrest of oestrus as seen in DJF (5.16 to 5.04 pg/ml) and MAM (5.28 to 4.96 pg/mL). Despite elevated oestradiol levels in JJA, the number of oestrus detected was not much increased and is attributable to the lack of behavioural signs already prevalent in the herd (Nasir and Kutty, 2005). Shifting of oestrus exhibition from daytime towards night hours as reported by Bolocan (2009) also might have caused lesser detection even though prolongation of E2 secretion was evident causing almost equal levels on Day 0 and Day 1.

Since none of the animals were showing prominent behavioural signs of oestrus, the accuracy of heat detection was low so that the actual onset of signs or even pro-oestrus become indistinguishable by human observation alone (Bolocan, 2009), unless more focused approaches like scoring for oestrus intensity could be adopted as a measure to improve heat detection rates (Kutty, 2023). Thus lack of behavioural signs and difficulty in distinguishing the actual onset and end of the oestrus period complicates the interpretation of E2 level on Day 0 and Day 1.

Since the study was restricted only to early PP cows, the total number of oestrus detected and AI done were less, and variations between seasons were non-significant. However, monthly averages of oestrus detected and AI done (Table 4.30) were higher in DJF and lowest in MAM being the periods respectively of minimum and maximum TS (Kutty *et al.,* 2019). Out of 33 inseminations, cows subjected to double AI during the oestrus were relatively more (57.58 %) than normally expected. Persistence of ovarian follicles in almost all the cows on the second day of oestrus, indicative of delayed ovulation, formed the major reason for a high proportion of DAI (Ghuman *et al.,* 2014). More non-returns and conception among cows subjected to double AI clarifies the possibility of, besides poor oestrus detection due to weakened behavioral signs, the occurrence of delayed ovulation in a high proportion of prolonged oestrus cows.

Conclusion

Persistence of almost similar numbers and sizes of the largest and second largest follicles on Day 0 and Day 1 and maintenance of almost the same level of oestradiol on Day 1 (45.7 ± 3.43) as that of Day 0 (47.8 ± 3.31) support the earlier finding of a very high proportion of prolonged oestrus in the herd. Achievement of better conception for double AI (63.16 %) as against total AI (48.48 %) further confirms the possibility of delayed ovulation being the underlying reason for prolonged oestrus complicated by faulty detection out of lack of prominent behavioural signs. Comparison of the seasonal pattern of follicular growth, oestrogen level, and conception to AI between Day 0 and Day 1 clarifies the influence of thermal stress and increased milk production as the potential contributors to the occurrence of prolonged oestrus in cross-bred cows under the hot humid tropical climate.

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Conflict of interest

The authors declare that they have no conflict of interest.

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