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# Influence of Interval From Calving and Season on Follicular Development, Onset of Oestrus, Conception and Embryonic Mortality During Early Post Partum Period of Cross Bred Cows Under Humid Tropical Climate

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## ABSTRACT

Dairy cows suffer extreme physiological stress during the early stages of lactation depending on the milk yield and climatic adversities, delaying post-partum (PP) fertility. The present study intended to assess the influence of interval from calving and seasons on the resumption of PP fertility. Reproductive ultrasonography of eight early PP cows each was performed every week and continued year-round replacing four cows every month. Details of ovarian structures, oestrus detected, AI done, conception, and embryonic loss were analyzed for variations between weeks of early PP and across seasons. A total of 381 ultrasonography (USG) checks revealed functionally active ovaries throughout the period of study having tiny follicles, prominent antral follicles, and evidence of recent ovulation in 99.23%, 94.17%, and 64.56% respectively. Functionally active follicles were highest during summer and lowest in post-summer ($P<0.05$) attributable respectively to the direct and delayed effect of thermal stress (TS). Oestrus and conceptions were almost uniform across the weekly intervals, while AI done and conception were lowest (28.57%) during summer. Overall conception of AI and embryonic loss were 48.71 % and 21.05 % respectively and all the embryonic losses occurred before 35 days PP. In spite of the highest conception rate (75.00 %), the embryonic loss was maximum (40.00 %) during post-summer, being the delayed impact of TS. To conclude, even though ovaries are functionally active throughout the early PP period, seasons of direct and delayed effect of TS seriously affect the fertility of cross-bred dairy cows under the humid tropical climate.

***Keywords****:* Conception, Dairy Cow, Embryonic Loss, Ovarian Follicle, Thermal Stress

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# INTRODUCTION

Fertility of dairy cows has declined continuously in the past 30 years simultaneous with the increase of milk production (Butler, 2000; Lopez-Gatius, 2003) and thermal stress (TS) caused by climatic adversities (Hansen, 2009), reducing the fertilization rate of artificial insemination (AI) in the species even to the extent of 80 per cent (Saacke *et al*., 2000). Lactating cows suffer extreme physiological stress during the transition period, which continues during the early part of lactation depending upon the milk yield (Sachin *et al*., 2024) so that regaining of body condition and resumption of post partum (PP) fertility gets delayed beyond the stage of peak yield period and onset of regaining positive energy balance (Gomez *et al*., 2018).

Cross bred cow herd under the study were found to suffer TS throughout the year indicated by the prevalence of high temperature humidity index, accompanied by elevated levels of HSP 70 in the serum (Kutty, 2021), delaying the first PP oestrus beyond 72 days (Kutty *et al.,* 2019). Negative influence of high milk yield on PP fertility gets corrected as the milk yield starts to decline after the stage of peak productivity (Krishnan *et al*., 2017). Whereas the influence of adverse climate continues to affect the reproductive processes, which can be understood by comparing the fertility trends across predominant seasons of the locality (Lopez-Gatius, 2003) and is very essential, in the context of increasing climatic adversities, to initiate necessary interventions for enhancement of PP fertility. Hence, present study was carried out to describe the ovarian activity, occurrence of oestrus, conception to AI and embryonic loss during the early PP period of cross bred dairy cows and to understand the seasonal pattern of fertility parameters under the humid tropical climate of the region.

# MATERIALS AND METHODS

The study was carried out at Livestock Research Station, Thiruvazhamkunnu under Kerala Veterinary and Animal Sciences University in India. Dairy farm of the station 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. Out of the cows calved each month and uneventfully completed the initial fortnight of PP period, belonging second to fifth parity, optimum body condition score and history of regular milk yield in the previous lactation, four cows were randomly selected every month. Selected cows were subjected to the study from day 28 to 91 PP, so that there were only eight cows under the study each month and continued year round from August to July.

Cows being studied were routinely observed for oestrus signs from Day 15 PP and checked by ultrasonography (USG) at weekly intervals from Day 28 onwards for resumption of cyclical activity. Both the ovaries were scanned for antral follicles and luteal structures. Follicles were categorized based on the size and structural features as extra-large (15-20 mm), large (9-14 mm), medium (6-8 mm), small (3-5 mm) and tiny (less than 3 mm) as suggested by Sartorelli *et al.* (2005) and Ginther (2016). Follicles of more than 20 mm size, associated with characteristic clinical manifestations were considered as cysts. Tiny follicles recruited to grow further formed the prominent category comprised of large, medium and small follicles. Luteal structures detected were categorized based on the size, blood flow characteristics and associated tubular manifestations into functional corpus luteum (FCL) and regressing corpus luteum (RCL) (Morris *et al*., 2011).

Animals showing prominent oestrus signs within 40 days PP (voluntary waiting period) were not inseminated and those having even minor signs of oestrus thereafter were verified through clinico-gynecological examination. Cows in proper oestrus were inseminated using frozen-thawed semen once or twice depending upon the persistence of oestrus signs on the subsequent day. Inseminated animals were excluded from the study unless returned to oestrus and non-return animals were subjected to B mode USG for early indications of pregnancy at 25 days after AI. For those found pregnant, scanning was repeated at 35 and 45 days post AI to assess viability and growth of embryo, structural features of the CL and progression of the uterine changes. Features of early pregnancy such as presence of fluid *in utero,* foetal membranes, embryonic structures, blood flow characteristics and foetal heart beat were recorded. Further confirmation of pregnancy was performed at 60 and 90 days post-service in all the positive animals to catch any pregnancy loss until these stages.

Data collected on different types of follicles were compared between weekly intervals of scanning to assess the pattern of follicular activity as the days from calving advances. Similarly, follicular types, luteal structures, conception and embryonic loss details were compared across the four seasons prevailing in the locality such as SON (September October November – North east monsoon), DJF (December January February – Post monsoon), MAM (March April May – Summer) and JJA (June July August – South west monsoon / post summer) to assess the seasonal pattern of ovarian activity and fertility parameters during the early phase of PP period beyond the days of peak lactation.

## RESULTS AND DISCUSSION

Among 48 animals included in the study 381 USG checks were done distributed across the weekly intervals of PP period as shown in Figure 1. Number of USG at each PP interval were decreasing from day 40 onwards attributable to the exclusion of inseminated animals from scanning until confirmation of non-pregnancy either based on return to oestrus or USG of uterus at 25 days post AI.

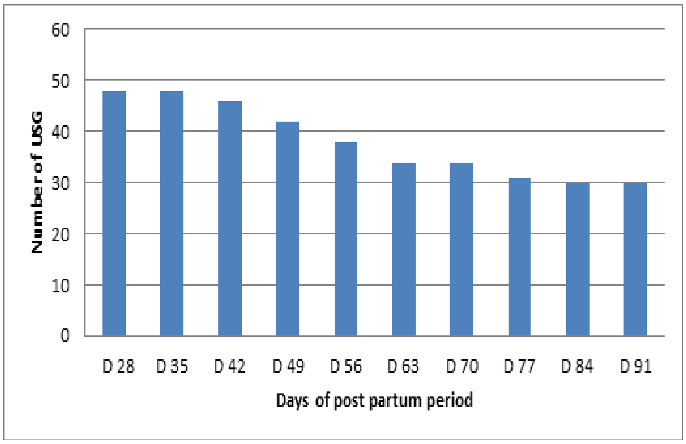


**Fig. 1:** Number of ultrasonography scanning at weekly intervals of post-partum period

Extra-large follicles and cysts detected were 7 and 9 respectively during the entire (63 days) periods of the study in 48 animals. Large follicles were detected in 15scanning including presence of double in two cases. Number of medium follicles in a single scanning ranged from 0 to 5 with 45.41 % having single and 20.21 % having no medium follicle on either of the ovaries. Similarly, detection of small follicles ranged from 0 to 10, having single in 28.87 % scanning and 39.37 % having nil. Tiny follicles were in plenty ranging from 1 (11.02 %) to 15 (0.26 %) distributed on one or both the ovaries reflecting the rhythmic occurrence of follicular waves (Ginther *et al.,* 2016). While, 45.67 % scanning detected 2 to 4 tiny follicles, 9.19 % had Nil. Overall, presences of prominent antral follicles were detected in 94.23 % of the scanning and there were only three scanning wherein none of the follicle types could be detected. Thus, ovaries are very active during the early PP period indicated by the follicular recruitment and growth in large numbers concurring the earlier reports that follicular recruitment and growth occurs irrespective of the season (Choudhary *et al.,* 2023), and the process is regulated by various factors acting at the intra-ovarian level (De-Rensis *et al.*, 2002).

Regarding luteal activity, FCL could be detected in 154 (40.42 %) scanning, including detection of double FCL in seven cases of scanning. Similarly, RCL were detected in 93 (24.40 %) scanning including double in 10 of the cases. Detected luteal structures were on right ovary in 170 (64.39 %) scanning as against left ovary in 88 (33.34%) and both the ovaries in 6 (2.27 %) scanning concurring the established finding that right ovary is more functional in ruminants (Stevenson, 2018). Thus, evidence of recent ovulation in the form of FCL or RCL was detected in 246 (64.56 %) scanning. Further, in spite of active follicular activity in 94.23 % scanning, corresponding luteal structures were not detected, indicating anovulation / lack of luteinization attributable to the stress of peak lactation and/or adverse climate prevailing throughout the year (Kutty, 2021).

**Table 1.** Average numbers of different types of follicles and corpus luteum detected at each scanning during weekly intervals of the postpartum period

| Days Post Partum | No of Scans | Type of follicles | | | | | | Type of corpus luteum | |
|---|---|---|---|---|---|---|---|---|---|
| | | Large | Medium | Small | Prominent | Tiny | All | Functional | Regressing |
| 28 | 48 | 0.04 | 1.35 | 1.13 | 2.52 | 4.44 | 6.96 | 0.19 | 0.40 |
| 35 | 48 | 0.06 | 1.19 | 1.52 | 2.77 | 2.02 | 4.79 | 0.33 | 0.19 |
| 42 | 46 | 0.07 | 1.15 | 1.07 | 2.28 | 4.43 | 6.72 | 0.43 | 0.22 |
| 49 | 42 | 0.02 | 1.40 | 0.64 | 2.07 | 4.69 | 6.76 | 0.43 | 0.24 |
| 56 | 38 | 0.00 | 1.13 | 1.45 | 2.58 | 3.95 | 6.53 | 0.32 | 0.32 |
| 63 | 34 | 0.12 | 1.00 | 1.32 | 2.44 | 3.41 | 5.85 | 0.62 | 0.26 |
| 70 | 34 | 0.03 | 1.29 | 0.88 | 2.21 | 3.32 | 5.53 | 0.56 | 0.35 |
| 77 | 31 | 0.10 | 1.03 | 1.29 | 2.42 | 4.84 | 7.26 | 0.32 | 0.32 |
| 84 | 30 | 0.00 | 1.30 | 0.93 | 2.23 | 3.73 | 5.97 | 0.57 | 0.27 |
| 91 | 30 | 0.00 | 1.23 | 1.17 | 2.40 | 4.00 | 6.40 | 0.63 | 0.13 |
| Mean | 38.1 | 0.04 | 1.21 | 1.14 | 2.39 | 3.88** | 6.28** | 0.44 | 0.27 |

** Significant at 1% level

Details of the mean numbers of different types of follicles and corpus luteum detected at each scanning of weekly intervals during the PP period are shown in Table 1. Among different types of follicles, number of tiny follicles showed highly significant variation (P<0.01) between the scanning intervals, with the lowest at day 35 PP, attributable to being the period of peak lactation. All other periods had more or less similar number of tiny follicles ranging from 3.32 ± 0.49 to 4.84 ± 0.55 and the average for the entire study period was 3.88 ± 0.14 per scanning. Attributable to the variation of tiny follicles, total number of follicles also showed highly significant variation (P<0.01) between scanning periods with the lowest (4.79 ± 0.39) and highest per scanning (7.26 ± 0.53) respectively at 35- and 77-days PP with the mean of 6.28 ± 0.15. Thus, it is evident that different types of follicles as well as luteal structures occur more or less uniformly during the early PP period except a reduction around the stage of peak lactation as reported by Choudhary *et al.* (2023).

Mean number of different types of follicles detected at each scanning are compared across four seasons of the region in figure 2. Number of medium follicles showed highly significant variation (P<0.01) between seasons with the lowest mean per scanning (0.97 ± 0.10) during JJA and highest (1.40 ± 0.08) during MAM. Accordingly, the number of prominent follicles and total number of follicles also showed significant variation (P<0.05) with the same pattern across seasons as that of medium sized follicles. Increased growth of follicles during summer has been reported by Torres-Junior *et al.* (2008) as well and is attributed to an increase in the level of FSH and decreased inhibin because of TS, leading to enhancement of follicular growth and follicular co dominance in cows during summer (Roth *et al.*, 2000). Medium sized follicles being functionally more important, the significant variation between seasons can influence the PP fertility in a major way. Despite being the rainy season, reduced occurrence of functionally active follicles during JJA can be attributed to the delayed effect of TS from the preceding summer season, in agreement with the altered steroid profile consequent to TS reported by Pavani *et al.* (2015) and Abdalla *et al.* (2017).

Variation in the number and size of luteal structures are compared in table No 2. There was no significant variation for the type and size of CL across the seasons. However, there was a positive association (P<0.05) between the presence of FCL as well the size of RCL with the number of small follicles. Number of RCL was also found to have positive association (P<0.05) with the number of oestrus detected and AI done, as normally expected.

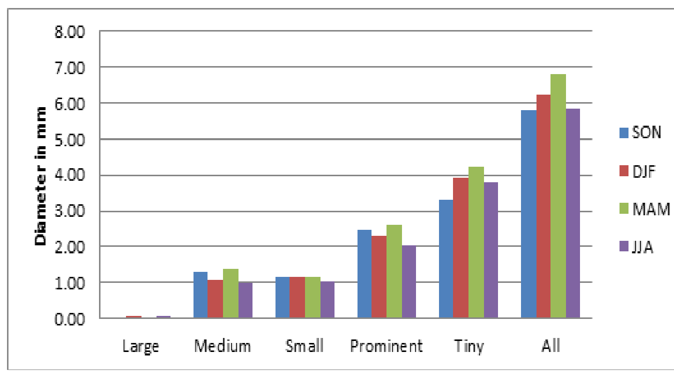


**Fig.2.** Different types of follicles at each scanning compared across the four seasons

**Table 2.** Mean ± SE of the numbers and size of different types of luteal structures across the seasons

| Parameter | Details of CL detected at USG checks across the season | | | | |
|---|---|---|---|---|---|
| | SON | DJF | MAM | JJA | Overall |
| Number of FCL | 12.33±1.76 | 17.33±1.33 | 18.00±2.52 | 17.00± 3.79 | 16.17 ± 1.27 |
| Size of FCL (mm) | 17.43±0.81 | 18.28 ± 0.69 | 17.98 ±0.41 | 17.57 ± 1.07 | 17.81± 0.35 |
| Number of RCL | 8.00±0.58 | 12.00± 1.00 | 10.33± 2.85 | 6.00± 1.73 | 9.18 ± 1.02 |
| Size of RCL (mm) | 10.16±0.52 | 12.34 ± 0.59 | 11.40 ± 0.51 | 12.04 ± 1.23 | 11.48 ± 0.41 |

NS- non-significant variation between the seasons (P>0.05)

## Occurrence of Oestrus

Out of the 48 animals in the study, eight cows exhibited oestrus during the VWP (upto 40 days PP), including 3 shown before 28 days and were not inseminated. Between 40 to 90 days, 41 oestruses were detected in 35 cows and 13 remained anoestrus. However, growth of antral follicles on one or both the ovaries was detected in 47 of these cows during USG at 28 days PP and was reduced 46 only at 35 days. As shown in table No 1, there were around 2.52 ± 0.17 prominent follicles at day 28 PP, which came down to 2.07 ± 0.18 at 49 days PP as against the annual mean number of 2.39 ±0.04 and is attributable to the peak lactation period affecting follicular growth (Sachin et al., 2024) Distribution of oestrus detected, AI done and details of conception during the weekly intervals of PP periods are shown in table 3. Oestrus and conception occurred more or less uniformly across the PP intervals studied in agreement with the report of Macias-Cruz *et al*. (2016). Out of the 39 inseminations carried out between day 46 to 91 PP, 6 returned to service before 25 days of AI. Among 33 cows subjected to USG at 25 days post AI, 11 were diagnosed non-pregnant at the first check itself and three animals needed a second check 2 days later to declare the non-pregnancy. Also among 19 (48.70 %) found pregnant at 25 days, two cows needed recheck after 2 days in order to make sure of the conception.

**Table 3.** Distribution of oestrus and conception during the post-partum period

| PP Period | Oestrus | AI done | Pregnant | Conception (%) |
|---|---|---|---|---|
| 41-48 | 6 | 6 | 2 | 33.33 |
| 49-55 | 5 | 5 | 3 | 60.00 |
| 56-62 | 7 | 6 | 4 | 66.67 |
| 63-69 | 5 | 5 | 2 | 40.00 |
| 70-76 | 8 | 8 | 4 | 50.00 |
| 77-83 | 6 | 5 | 2 | 40.00 |
| 84-91 | 4 | 4 | 2 | 50.00 |
| Total | 41 | 39 | 19 | 48.72 |

Conception to AI

A total of 64 USG checks were made in 33 animals for the detection of early pregnancy, which included 47 and 17 examinations respectively in 19 and 14 conceived and non-conceived cows. Distribution of AI done, details of conceptions and pregnancy loss during the four seasons are shown in Table 4.

**Table 4**. Number of inseminations, conception, embryonic loss and pregnancies confirmed during the four seasons

| Season | AI done | Conception | | Embryonic loss | | | | Pregnancy confirmed | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | % | 25-35 d | 35-45 d | Total | % | Number | % |
| SON | 11 | 5 | 45.45 | 2 | 0 | 2 | 40.00 | 3 | 27.27 |
| DJF | 13 | 6 | 46.15 | 1 | 0 | 1 | 16.67 | 5 | 38.46 |
| MAM | 7 | 2 | 28.57 | 0 | 0 | 0 | 0.00 | 2 | 28.57 |
| JJA | 8 | 6 | 75.00 | 1 | 0 | 1 | 16.67 | 5 | 62.50 |
| Total | 39 | 19 | 48.71 $^{ns}$ | 4 | 0 | 4 | 21.05 | 15 | 38.46 |

Chi square = 2.641 $^{ns}$, p value = 0.450, Ns – Non-significant

Out of 39 AI, 19 conceived giving a conception rate of 48.71 per cent, which is higher than the average reported for the same herd in an earlier study (Kutty *et al.*, 2019) and is attributed to better attention under the study. The conception rate was lowest in summer (28.57 %), attributable to extreme TS during the season and highest conception rate was obtained during JJA (75.00 %) due to the onset of rainy season and better environment (Kutty, 2021), even though the variations were non-significant between seasons because of the small number of inseminations during each of the seasons.

Conception rates of SON and DJF are almost similar and nearer to the annual mean figure. Despite better conception rate, number of AI was very low during JJA, and is attributable to the extended time required for recovery from the exhaustion of summer stress, in agreement with the report of Torres-Junior *et al.* (2008) that TS exerted a delayed deleterious effect that lasted up to 105 days on ovarian follicular growth, hormone concentrations and oocyte competence. Even though follicular activity was more during summer, there were only very few oestrus with obvious external manifestations and the conception rate was also poor because of the prevalence of high ambient temperature during the period concurring the report of Choudhary *et al.* (2023). Heat stress has been reported to lower the oestrus manifestation and conception altering the steroid profile and oocyte quality during summer (Pavani *et al.,* 2015; Abdalla *et al.,* 2017).

## Embryonic Loss

Out of 19 conceptions, four embryos were lost between days 25 and 35 of pregnancy and no loss was detected thereafter, and in agreement with the earlier report that embryonic mortality is more likely to occur within 35 days of conception in cattle (Das *et al.,* 2016). Non detection of pregnancy loss from 35 to 90 days can also be attributed to the smaller number of pregnancies that could be followed up in the season to make valid conclusions. Death of many fertilized oocytes is usual occurrence before 16 days, but goes unnoticed because of non-alteration of the cycle length. However, embryonic loss between 16 to 24 days gets manifested as delayed return to service, while easy detection is not possible otherwise. However, embryos could be easily observed using USG at 25 days and loss thereafter could be detected by repeated USG examination.

Four out of 19 formed a higher rate of pregnancy loss and was distributed in three seasons with more numbers in SON and equal numbers in DJF and JJA. Non detection of any embryonic loss during MAM can be attributed to very few non return animals available for checking. Detected embryonic loss was more during SON (40%), even though the weather and management conditions were better during the period and is attributable to more conceptions occurred during JJA immediately following the summer and delayed effect of TS as reported by Krishnan *et al.* (2017) that deleterions effect of TS may persist upto 40 days after the service. Cows inseminated at a THI of >75 has been reported to have high risk of EED, abortion and still birth compared to the low risk for AI at THI <65 (El-Tarabany and El-Tarabany, 2015; Abdalla *et al.,* 2017). Overall EED rate was 21.05 per cent in the study and concurs the earlier studies that reported high variability in the proportions of EED ranging from 7.2to 29 per cent (Silke *et al.,* 2002; Santos *et al.,* 2004; Abdalla *et al.,* 2017).

# CONCLUSION

Even though, ovaries were highly active throughout the early PP period, functionally active follicles were significantly low during south-west monsoon attributable to the delayed effect of TS during summer. Likewise, despite almost uniform distribution of oestrus and conception across the PP intervals, AI done and conception rate was lowest during summer – the season of extreme TS, and embryonic loss was highest during south-west monsoon - the season subsequent to summer. Thus, seasons of direct and delayed effect of TS appear to be highly influential in deciding the fertility performance of cross bred cows during the early PP period.

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# CONFLICT OF INTEREST

The author has no conflict of interest to declare.

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