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Seasonal influence on the luteal growth and regression characteristics of crossbred dairy cows of Kerala#

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Abstract

The study was aimed at investigating the effect of seasonal changes on luteal growth and regression characteristics in crossbred (CB) cows of Kerala. The seasons of the year were classified as rainy (June-September), post-monsoon (October-January) and summer (February-May). In each season, nine healthy regularly cycling CB lactating pluriparous cows were selected for the study. Luteal growth and regression pattern were studied at 24 h intervals from one ovulation to the next ovulation, by transrectal ultrasound (TRUS) examination of the ovaries. During the study period, parameters like the day of earliest detection of corpus hemorrhagicum (CH), size at detection, maximum size of corpus luteum (CL), the day of CL with maximum diameter, day of onset and completion of CL regression were recorded and compared between seasons. Corpus hemorrhagicum was detected one day after ovulation in 55.60, 66.70 and 80 per cent of animals during rainy, post-monsoon and summer seasons, respectively. There existed no statistically significant difference in the size of CL, either on the day of first detection or on the day of maximum size attainment. However, CL during the rainy season needed a significantly (P<0.05) longer period to attain maximum size (10.33 ± 0.58), compared to the post-monsoon (8.33 ± 0.62) and summer seasons (8.40 ± 0.56). The season did not affect the day of onset of luteal regression or its completion neither in animals with two-wave cycle nor with three- wave. The study concluded that the season had less or no impact on the growth and regression pattern of CL in CB cattle of Kerala.

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In tropical and subtropical areas, reproductive performance of cattle and buffaloes is considerably reduced during summer. Elevated ambient temperature (AbT) during the summer season results in a transient period of infertility in dairy cows, leading to longer inter-calving interval and consequent economic losses to the dairy producer.

In bovines elevated temperature and humidity during summer months result in reduced dry matter intake, poor expression of oestrus signs, altered endocrine mechanism due to the reduced steroidogenic capacity of theca and granulosa cells, impaired follicular activity including ovulation, decreased oocyte and embryo quality and embryonic loss due to alteration in the uterine environment (De Rensis and Scaramuzzi, 2003). Heat stress has a negative impact on bovine embryo by slowing down its developmental rates, raising the percentage of blastomeres that are apoptotic, and reducing the expression of crucial genes involved in its development. These changes are more pronounced in *Bos taurus* cattle. *Bos indicus* cattle, due to their superior adaptation to tropical and subtropical environments, typically outperform *Bos taurus* cows in terms of reproduction (Camargo *et al.*, 2007; Silva *et al*, 2013).

A functional CL is required for the maintenance of a healthy pregnancy. Researchers have hypothesized that elevated environmental temperature during the summer season may directly or indirectly impair luteal function. According to Howel *et al*. (1994), no seasonal variation in the length of the luteal phase and CL size was noticed in Holstein cows during spring and summer seasons. But the peak amplitude of progesterone and progesterone concentrations during the entire luteal phase tended to be lower in the summer. A similar observation was made in Jersey crossbred dairy cows by Satheshkumar *et al*. (2015) who reported that the size of CL increased to a mean maximum diameter of 22.10 to 22.60 mm on the mean Day of 9.00 to 9.60, remained variable around this size, and then began to regress continuously on the mean day of 15.90 to 16.80, with no appreciable changes in these parameters between the hot and cold seasons. But they observed that luteal function was greatly compromised in summer compared to the winter season, especially during mid-luteal phase.

Although there are reports of seasonal impact on follicular and endocrinological turnover in *B. taurus* and *B. indicus* cattle, studies related to growth and regression characteristics of corpus luteum were limited in Indian subcontinent. The objective of the present study is to compare the influence of season on luteal growth and regression characteristics in crossbred dairy cows of Kerala, by using transrectal real-time B-mode ultrasonography.

Materials and methods

The study was carried out at the University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Mannuthy, Thrissur district of Kerala from June 2021 to May 2022. The seasons in Kerala were classified as per Biya (2011), as rainy (June to September), post-monsoon (October to January), and summer (February to May). Twenty-seven healthy regularly cycling CB lactating pluriparous cows (nine cows in each season) were selected for the study. The body condition score (BCS) of all the selected animals was calculated as described by Edmonson *et al*. (1989). The cows having a body condition score of three or above were chosen for the study after an absolute body condition score was determined for each cow. Before conduct of the experiment, subclinical endometritis was ruled out by conducting a white-side test (Kumar *et al*., 2015). The data pertaining to age, breed, parity, nature of calving and postpartum complications like retention of fetal membranes, endometritis, metritis and pyometra were documented.

Transrectal ultrasonography (TRUS) study

Routine TRUS was performed at 24 h intervals in all the animals to record ovarian status (follicular dynamics and luteal characteristics). The reproductive ultrasonography was performed using a real-time Ultrasound scanner (Mylab™ Gamma, Esaote SpA, Italy) outfitted with a linear array, trans-rectal transducer (5-10 MHz frequency) (SV3513, Esaote Europe B.V, Netherland).

The animals were adequately restrained and the scanner was placed at a sensible distance from the animals and transrectal ultrasonography was performed as per the standard procedure. In each season, TRUS of luteal growth and regression characteristics were recorded at 24 h intervals in all the animals (n=9) for one complete oestrous cycle, starting on the day of ovulation till the next ovulation. During the study period, diameter of the ovulatory follicle, the day of earliest detection of corpus hemorrhagicum (CH), size at detection, maximum size of CL, the day at which CL attains its maximum diameter, day of onset and completion of CL regression were recorded and compared between the seasons.

Results and discussion

In animals with two wave cycles, maximum diameter (mm) of ovulatory follicle (Mean ± SE) prior to ovulation during rainy, post-monsoon and summer was 14.40 ± 1.03, 12.85 ± 0.25 and 13.84 ± 0.53, respectively. Respective values in animals with three-wave cycle were 13.53 ± 0.20, 12.88 ± 0.25 and 14.77 ± 0.44mm. In animals with four-wave cycle, the size of the ovulatory follicle was 14.80 ± 0.25mm (Table 1). Irrespective of the nature of the wave pattern, there existed no significant difference in the mean maximum size of the ovulatory follicle between seasons. Contradictory to the present finding, Satheshkumar *et al.* (2015) recorded a significantly higher mean diameter of the ovulatory follicle during the summer than cold season (12.80 vs 11.20 mm). They reported that the increased diameter of ovulatory follicle could be due to alterations in steroidogenesis within the follicular microenvironment during summer season (Shimizu *et al*., 2005). Also, an imbalance in energy metabolism that happened to keep the body temperature at normal levels throughout the hot season might have impaired the aromatase activity in granulosa cells, delaying the functionality of the dominant follicle. This resulted in the prolongation of growth phase of the preovulatory follicle for the attainment of maximum size prior to ovulation in the summer season (Al- Katanani *et al*., 2002).

Even though, in the present investigation, the mean maximum diameter of three wave cycle is more in summer (14.77 ± 0.44mm) compared to rainy (13.53 ± 0.20mm) and post-monsoon (12.88 ± 0.25mm) seasons, the difference was non-significant, which might be due to the smaller sample size.

Sonographically, CH contrasted with the ovarian stroma by appearing as a more hypoechoic region. In the present study, CH was detected one day after ovulation in a majority of the animals (55.6 % in rainy, 66.7 % in post-monsoon and 80.0 % in summer season) and the data are depicted in Table 2. Niyas *et al*. (2018) also reported that, using TRUS, CH could be detected as early as one day after the end of oestrus.

In the present study, detection of CL by TRUS in all the animals was recorded by day three after ovulation in rainy and post-monsoon seasons. Niyas *et al.* (2019) also reported that CL can be identified by TRUS on the third day of ovulation as a poorly defined, uneven, greyish-black structure in the ovary. During the summer season, CL could be detected by day 2 itself. Similar to this finding, Pierson and Ginther (1984) reported that, with the exception of day 0–2 post-ovulation, the CL was discernible throughout the inter-ovulatory period. There existed no significant difference in the size of CL between seasons on the day of its first detection.

The mean maximum diameter (Mean ±SE) of CL observed were 22.71 ± 0.49, 21.92 ± 0.39 and 22.17 ± 0.32 mm during rainy, post-monsoon and summer seasons, respectively (Table 3). There existed no significant difference in the mean maximum diameter of CL between seasons. This was in agreement with the reports of Howel *et al*. (1994) who observed no seasonal variation in CL size in Holstein cows. Satheshkumar *et al*. (2015) also reported a similar mean maximum CL diameter ranging from 22.10 to 22.60 mm that in crossbred Jersey dairy cows, without any seasonal difference.

During rainy season, mean number of days required for the CL to attain its maximum size (10.33 ± 0.58) was significantly greater ($p<0.05$) than post-monsoon (8.33 ± 0.62) and

Table 1. Size of ovulatory follicle in CB dairy cattle with different follicular wave pattern, during different seasons prevailing in Kerala

Nature of wave pattern of follicular growth	Maximum diameter (mean ± SE) of ovulatory follicle (mm) during different seasons			F-value (p-value)
	Rainy (n=9)	Post-monsoon (n=9)	Summer (n=9)	
Two wave-cycle	14.40 ± 1.03 (n=3)	12.85 ± 0.25 (n=2)	13.84 ± 0.53 (n=6)	0.826[ns] (0.476)
Three wave-cycle	13.53 ± 0.20 (n=6)	12.88 ± 0.25 (n=5)	14.77 ± 0.44 (n=3)	9.999[ns] (0.003)
Four wave- cycle	Nil	14.80 ± 0.25 (n=2)	Nil	
Overall	13.82 ± 0.38 (n=9)	13.44 ± 0.22 (n=9)	13.78 ± 0.32 (n=9)	0.446[ns] (0.643)

ns non-significant

Table 2. Earliest day of detection of Corpus Hemorrhagicum in crossbred dairy cattle during different seasons prevailing in Kerala

Day of detection of CH	Number and per cent of animals in which CH was detected					
	Rainy (n=9)		Post monsoon (n=9)		Summer (n=9)	
	Progressive No.	Progressive per cent	Progressive No.	Progressive per cent	Progressive No.	Progressive per cent
1	5	55.60	6	66.70	8	80.00
2	7	77.80	7	77.80	9	100.00
3	9	100.00	9	100.00	-	-

Table 3. Diameter of corpus luteum at first detection, day of attainment of maximum diameter and maximum diameter attained in crossbred dairy cattle during different seasons prevailing in Kerala, assessed by trans-rectal ultrasonography

Variables	Rainy (n=9)	Post monsoon (n=9)	Summer (n=9)	F-value (P-value)
Size of CL at 1st detection (mm)	13.02 ± 0.53	13.02 ± 0.73	12.85 ± 0.75	0.022[ns] (0.978)
Day of attainment of maximum CL diameter	10.33 ± 0.58[a]	8.33 ± 0.62[b]	8.40 ± 0.56[b]	3.669* (0.040)
Maximum CL Diameter attained (mm)	22.71 ± 0.49	21.92 ± 0.39	22.17 ± 0.32	0.986[ns] (0.387)

**Significant at 0.05 level; ns non-significant*

Means having different letters as superscripts differ significantly between columns

summer seasons (8.40 ± 0.56) (Table 3).

Growth and regression pattern of CL in animals with two waves per cycle

Growth and regression pattern of CL in animals with two waves per cycle during different seasons are presented in Table 4 and 5. The CL grew to a mean maximum diameter of 23.05 ± 0.44, 23.18 ± 0.53 and 22.18 ± 0.54mm on 11.33 ± 2.03, 9.00± 0.00 and 7.67 ± 0.80 days, respectively, during rainy, post- monsoon and summer seasons. The CL remained at this size before it started regressing constantly on day 16.33 ± 0.67, 16.00 ± 0.00 and 16.00 ± 0.44 and the regression completed on days 19.33 ± 0.88, 19.00 ± 0.00 and 20.14 ± 0.55 during rainy, post monsoon and summer seasons, respectively. There existed no significant difference between seasons in any of these parameters. Non-significant differences recorded in the present study could be due to the small sample size utilised for the study.

Table 4. Growth characteristics of CL in CB dairy cattle with different pattern of oestrous cycle, during different seasons prevailing in Kerala

Number of waves	Variables	Growth characteristics of CL in different seasons			F-value (P-value)
		Rainy	Post-monsoon	Summer	
Two wave	Day of maximum size	11.33 ± 2.03 (n=3)	9 .00 ± 0.00 (n=2)	7.67 ± 0.80 (n=6)	2.446^{ns} (0.148)
	Maximum diameter attained (mm)	23.05 ± 0.44 (n=3)	23.18 ± 0.53 (n=2)	22.18 ± 0.54 (n=6)	0.886^{ns} (0.449)
Three wave	Day of maximum size	10.67 ± 1.17 (n=6)	8.60 ± 1.08 (n=5)	9.00 ± 1.53 (n=3)	0.903^{ns} (0.433)
	Maximum diameter attained (mm)	22.53 ± 0.71 (n=6)	21.51 ± 0.56 (n=5)	22.25 ± 0.25 (n=3)	0.741^{ns} (0.499)
Four wave	Day of maximum size		10.50 ± 0.50 (n=2)		
	Maximum diameter attained (mm)		22.63 ± 1.08 (n=2)		

ns-non significant (P > 0.05)

Table 5. Comparison of the day of onset and completion of luteal regression in crossbred dairy cattle with different follicular wave pattern, between different seasons prevailing in Kerala

Number of wave	CL regression	Day of CL regression (Mean ± SE)			F-value (P-value)
		Rainy	Post monsoon	Summer	
Two wave	Onset	16.33 ± 0.67 (n=3)	16.00 ± 0.00 (n=2)	16.00 ± 0.44 (n=6)	0.105^{ns} (0.901)
	Completion	19.33 ± 0.88 (n=3)	19.00 ± 0.00 (n=2)	20.14 ± 0.55 (n=6)	0.700^{ns} (0.522)
Three wave	Onset	18.50 ± 0.76 (n=6)	16.60 ± 0.40 (n=5)	18.33 ± 0.33 (n=3)	2.829^{ns} (0.102)
	Completion	21.67 ± 0.42 (n=6)	20.60 ± 0.24 (n=5)	22.00 ± 0.58 (n=3)	3.017^{ns} (0.090)
Four wave	Onset		30.50 ± 3.50 (n=2)		
	Completion		34.00 ± 3.00 (n=2)		

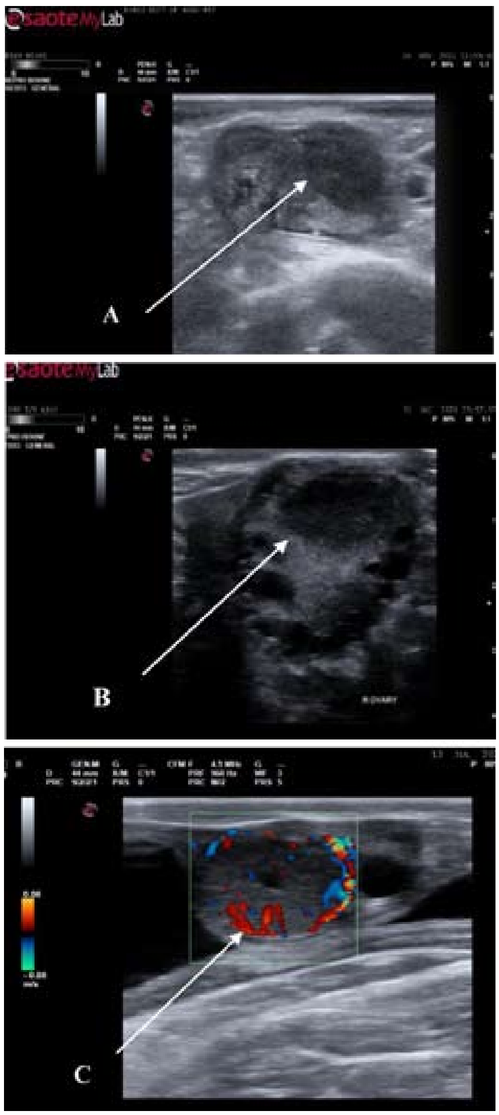
ns-non significant

Growth and regression pattern of CL in animals with three waves per cycle

The CL attained a mean maximum diameter of 22.53 ± 0.71, 21.51 ± 0.56 and 22.25 ± 0.25 mm on the days 10.67 ± 1.17, 8.60 ± 1.08 and 9 .00 ± 1.53 during rainy, post-monsoon and summer seasons, respectively. The CL remained at this size before it started regressing constantly on the mean days 18.50 ± 0.76, 16.60 ± 0.40 and 18.33 ± 0.33, respectively during rainy, post-monsoon and summer seasons. Luteal regression was completed on days 21.67 ± 0.42 (rainy), 20.60 ± 0.24 (post-monsoon) and 22.00 ± 0.58 (summer) (Table 4 and 5).

There existed no significant difference between seasons on the day of attainment of the maximum size of CL, maximum diameter (mm) of CL, day of onset of regression or day of complete CL regression in animals with three wave patterns. Non-significant differences recorded in the present study could be due to the small sample size utilized for the study.

During the initial growth phase (Day 3), a significantly higher (P<0.05) mean diameter



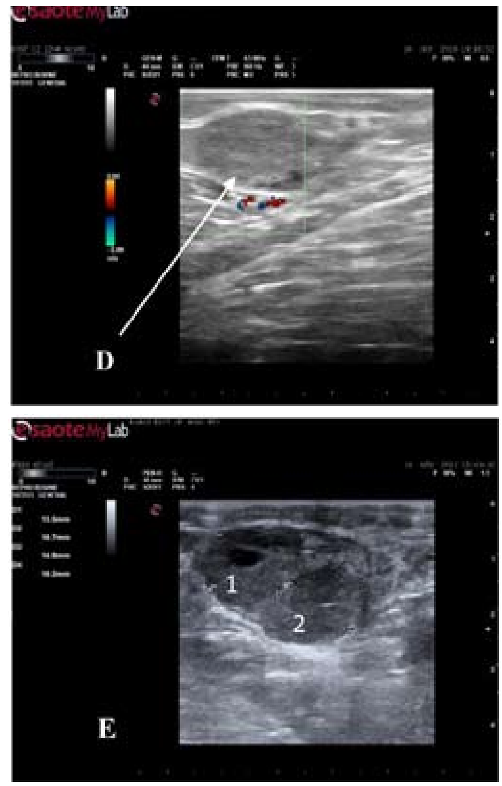


Fig. 1. Sonographic imaging of growth and regression characteristics of CL (A) Corpus hemorrhagicum (B) Developing CL on day 5 (C) Developed CL on day 10 with active blood supply (D) Regressing CL: Reduction in size and blood supply to CL (E) Ovary with double ovulation: Double CL.

for CL was observed during the summer **(**17.15 ± 0.52mm) compared to the rainy season (14.28 ± 0.8mm). In the summer season, ovulation occurred from the Graafian follicles of higher diameter (14.77 ± 0.44mm) in animals with three wave pattern when compared to rainy (13.53 ± 0.20mm) and post-monsoon seasons (12.88 ± 0.25mm) (Fig.4). This could be the reason for the increased size of CL at day 3 after ovulation during the summer season. Vasconcelos *et al*. (1999) reported that the reduction in the size of the ovulatory follicle resulted in the formation of CL with reduced size. Significantly lesser mean diameter ($p<0.05$) of midcycle (day 10) CL observed during the post-monsoon season can also be related to the lesser diameter of the ovulatory follicles recorded in the same season.

Early onset of luteal regression was recorded during the post-monsoon season indicated by a significant reduction ($P<0.01$) in the mean diameter (mm) of CL at day 16 (18.99 ± 0.46) when compared to rainy (21.28 ± 0.46) and summer (21.38 ± 0.38) seasons.

Two animals in post-monsoon season exhibited four-wave pattern of oestrous cycle. The growth and regression pattern of CL were recorded in both animals. Season did not affect the day of onset of luteal regression or its completion neither in animals with two-wave cycle nor three-wave cycle (Table 5). This was in accordance with the findings of Satheshkumar *et al.* (2015) who reported that seasonal changes did not influence the luteal regression characteristics in Jersey crossbred dairy cows.

Conclusion

The present study concluded that the increased growth phase of CL was observed during the rainy season but all other parameters like earliest detection of CH, size at detection, the maximum diameter of CL, and onset and completion of luteal regression did not vary significantly between the seasons. Thus, the season in Kerala had less or no impact on the growth and regression characteristics of CL in CB dairy cows.

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Conflict of interest

The authors declare that they have no conflict of interest**.**

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