

# Characterization of Postpartum Ovarian Follicular Development Pattern in Crossbred Cows

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# **ABSTRACT**

**Background:** The postpartum period is crucial for cattle reproduction. The length of postpartum anestrus significantly impacts reproductive efficiency. Apparently enhanced energy attribution to milk production may lead to anestrus by delaying the onset of follicular activity. The absence of an ovulation stimulus, rather than insufficient follicle growth, determines the limited duration until ovulation. The current study was aimed to document the postpartum ovarian dynamics in crossbred Jersey cows.

**Methods:** The study was conducted in ten postpartum crossbred Jersey cows. Transrectal ultrasonography was performed to record the follicular development pattern from parturition till two ovulation or 90 days postpartum on alternate days. The data was analyzed for follicular wave emergence, no of follicular waves, size of the dominant follicle and ovulatory status.

**Result:** Fourty per cent of crossbred Jersey cows showed cyclicity and rest 60 per cent of cows are in anestrum. The mean diameter size of the dominant follicle in ovulated cows is 12.2±1.2 mm and in anestrum cows is 10.6±0.4 mm. The first wave emergence is 5.7±0.3 and 10.8±5.9 day in OV and NOV group respectively. In conclusion significant difference could be observed in emergence of first, second, third, fourth and fifth follicular wave emergence, growth phase duration, rate, size of the dominant follicle and duration of wave between postpartum ovulated and anestrum cows.

Key words: Anestrum, Crossbred jersey cow, Follicular pattern, Ovulation, Postpartum.

#### INTRODUCTION

Ovarian rebound is a key determinant factor of the postpartum fertility in a dairy cow. The initial occurrence of ovulation after parturition indicates the restart and fulfillment of the preovulatory process of ovarian follicular development, as well as the restoration from the hormonal state of late pregnancy. Butler (2003) stated that insufficient energy availability during the postpartum period not only inhibits the pulsatile secretion of luteinizing hormone (LH) but also diminishes the ovarian response to LH stimulation. The researcher also stated that exposure to NEB during the early growth and development of ovarian follicles has a detrimental effect on subsequent progesterone secretion. Further, the incidence of anestrus in the postpartum period has a negative impact on fertility. Persistent anestrus refers to a prolonged period during which an animal fails to display estrous behavior or exhibit regular ovarian cyclicity without any estrous signs. Persistent anestrus can be caused by various factors, including suboptimal conditions or pathologic conditions like nutritional deficiencies and chronic debilitating diseases which could disrupt normal hormonal regulation of the estrous cycle (Crowe et al., 2014) and uterine and ovarian diseases which could compromise normal functioning of these reproductive organs. It could be hypothesized that the initiation of ovarian follicular growth, the subsequent development and fate of the dominant follicle (DF) in the postpartum period play a crucial role in reestablishing ovarian cyclicity. Sakaguchi et al. (2004),

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Rajmon *et al.* (2012) and Crowe *et al.* (2014) have documented the resumption of ovarian activity and follicular dynamics in postpartum *Bos taurus* cows. However, such thorough study is lacking in the crossbred cows of our nation. Hence the present study was designed to characterize the postpartum ovarian follicular development pattern in crossbred cows.

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## **MATERIALS AND METHODS**

The study was conducted at the Dairy Unit of Livestock Farm Complex, Veterinary College and Research Institute, Orathanadu, TANUVAS, Tamil Nadu India 10.6286°N, 79.2531°E. Pluriparous crossbred Jersey cows (n=10) in their postpartum period were subjected for the study during the period between April 2022 to March 2023. Body weight of all the cows were in range of 380-410 Kg; The cows were housed in a loose housing system and provided with a balanced diet as per standards consisting of concentrates, chopped green fodder, mineral mixture and ad-libidum drinking water. All the postpartum cows were subjected to transrectal ultrasonographic examination using real time ultrasound scanner with 7.5 MHz transducer (Sonoscape S2V, Italy) on alternate days from the day of parturition (Day 0) till two subsequent ovulations or 90 days postpartum period, whichever was earlier. The ovaries were scanned in the multiple planes to ensure thorough and precise examination of antral follicles and corpus luteum (CL) (Ginther, 1993). The images were frozen and internal ultrasound caliper was employed to measure the length and width of these structures. The diameters (mm) of these structures were calculated by averaging their length and width (Satheshkumar et al., 2012). Sequential analysis of daily data was followed to arrive at the follicular development pattern. The follicular population was classified according to their diameter into different categories: Class I (less than 5 mm), Class II (between 5 and 9 mm), and Class III (9 mm or larger) (Burns et al., 2005; Satheshkumar et al., 2012). The follicular inventories were closely followed and the animals were classified into two groups based on the ovulatory status of the DF during the study period. Group I (OV): The animals which had ovulated a DF during the study period and Group II (NOV): The animals which did not have any ovulations during the study period. Ovulation was confirmed by the sudden disappearance of DF after attaining the maximum diameter and subsequent verification of luteal tissue and vascular perfusion in the same location (Kim et al., 2007). The obtained data were statistically analysed and the level of significance was arrived by employing the t-Test as per snedecor and Cochran (1994).

# RESULTS AND DISCUSSION

Analysis of follicular dynamics in postpartum crossbred cows revealed that, four (40.0%) out of 10 animals had first postpartum ovulation of DF within the study period of 90 days (OV group), while the rest of the animals (60.0%) didn't ovulate in the same postpartum period (NOV group) and were considered to be acyclic. In the OV group, the mean day of first ovulation was 41.8 days (22-66) days). This initial ovulation is often silent as described by Sukareksi *et al.* (2019) and is followed by a short estrous cycle, commonly characterized by a single follicle wave. The short duration of this first luteal phase is attributed to the premature release of prostaglandin  $F_2$ á, which is believed to be triggered by increased levels of estradiol produced from the development

of the postovulatory DF between days 5 and 8 of the cycle. This process induces premature expression of estradiol and oxytocin receptors (Wiltbank *et al.*, 2018). As a result, the corpus luteum (CL) secretes lower amounts of progesterone and regresses prematurely around days 8 to 10 of the cycle. The second ovulation, typically occurring around days 9 to 11 after the first ovulation, is associated with the expression of estrous behaviour and is followed by a normal-duration luteal phase that produces normal concentrations of progesterone (Crowe *et al.*, 2014). In the current study, the average time between ovulations, known as the interovulatory period, was found to be 16.0±3.3 days in crossbred cows. All the animal in the OV group had a silent estrum in the first ovulation and behavioral signs are exhibited in the subsequent ovulation.

An average of 3.0 follicular waves (1-5) occurred before the first ovulation in the OV group, whereas in NOV group, an average of 4.7 follicular waves (3-5) were recorded without any ovulation in the 90 days postpartum period. The pattern of follicular wave development was generally consistent among all the animals. Therefore, in line with the perspective of Velazquez *et al* (2008), it is evident that the extension of the non-ovulatory postpartum period is not due to the absence of DFs (Rajmon *et al.*, 2012).

In the OV group, one animal (25.0%) exhibited the first ovulation in the first follicular wave (1 FW), two animals (50.0%) in the third follicular wave (3 FW) and one animal (25.0%) in fifth follicular wave (5 FW). The quantitative parameters of the follicular wave patterns in OV group are presented in Table 1 and Fig 1. Perusal of the data in the OV group revealed that there were no significant variations in the various follicular development patterns among the animals. However, the diameter of the DF of ovulatory wave was non-significantly larger than anovulatory waves. Even though, it was not statistically comparable, the day of first ovulation was much earlier in 1FW (Day 22), followed by 3 FW (Day 39.5) and 5 FW (Day 66) animals. Regarding dairy cows, the ovulation of the first DF after calving typically transpires in 30-80 per cent of cows, while it experiences atresia in 15-60 per cent of cows, or develops into a cyst in 1-5 per cent of cows (Sakaguchi et al., 2004). An earlier occurrence of the first ovulation was associated with the earlier onset of follicular wave growth. Moreover, the ultimate reproductive performance of cows was notably influenced by the outcome of the first dominant follicle. Cows that experienced early ovulation, specifically within 20 days postpartum, exhibited significantly higher fertility rates compared to those that ovulated later in lactation (Rajmon et al., 2012); which is similar to our results that the animals which got ovulated 50% (2/4) earlier got conceived in the second ovulation with proper behavioral estrum in OV group.

The quantitative parameters of follicular waves in NOV group were presented in Table 2 and Fig 2. On analysis it was found that the growth phase of the first follicular wave in NOV group was significantly (P<0.05) lengthier when compared to subsequent waves. The maximum diameter of

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the latter waves seems to be non-significantly increasing, however there were no oestrus signs or ovulation. The maximum diameter of the anovulatory and ovulatory follicle was 11.0±0.71 and 10.25±1.26 mm diameter respectively in Holstein Friesian crossbred cyclical cows (Satheshkumar et al., 2008) and 10.3±1.09 and 11.66±0.60 mm in 2-wave estrous cycle of Sahiwal Cattle (Dodiyar et al., 2022).

Resumption of follicular activity in the postpartum period determines the fertility status of the dairy animal. Hence, the first follicular wave parameters in both the OV and NOV groups were compared and presented in Table 3. It was found that, the mean day of emergence of first follicular wave in the postpartum period was significantly (P<0.01) earlier in OV group (Day 5.7±0.3) than the NOV group (Day 10.8±5.9). In OV group, the DF of the first follicular wave attained the maximum diameter significantly (P<0.01) earlier (Day 19.3±2.6) within a significantly (P<0.05) shorter duration of growth phase (14.7±2.9 days) than the NOV group (Day 28.8±7.08 and 19.2±3.3 days respectively). Thus, early emergence of first follicular wave and early attainment of DF dominance seems to be the positive factors in determining the earlier ovulation during the postpartum period. A significantly (P< 0.05) larger diameter of DF was recorded in the first wave of OV group than the NOV group. The outcome of the first follicular wave and the fate of its DF depends on the concurrent frequency of LH pulses, which, in turn, relies on the follicle's ability to secrete adequate levels of estradiol to trigger a gonadotropin surge. The follicle's capacity to secrete estradiol is influenced by various factors, including the LH pulse frequency during the dominant phase of the follicle wave, the size of the dominant follicle, and the availability of IGF-I (Austin *et al.*, 2001; Canty *et al.*, 2006). Therefore, the primary determinant of DF ovulation during the postpartum period is the frequency of GnRH/LH pulses.

Following parturition, progesterone and estradiol concentrations decrease to baseline levels. The act of giving birth eliminates the inhibitory effects of elevated estradiol, allowing the synthesis of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) to resume. Synthesized FSH is released into the peripheral circulation, as indicated by the rapid occurrence of transient increases in blood FSH concentrations (within 3 to 5 days after parturition), which subsequently recur at 7- to 10-day intervals (Crowe et al., 1998); which is in accordance to the present findings in both the groups.

The DF size in both ovulatory and anestrous cows is nearly identical, with the distinction lying in the emergence

Table 1: Mean±SE of quantitative follicular wave parameters in Ovulatory group of post-partum crossbred cows.

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	5 FW (n=1)		3 FW (n = 2)		1 FW (n=1)	Overall an-	Ovulatory
	An- ovulatory	Ovula- tory	An- ovulatory	Ovula- tory-	Ovula- tory-	ovulatory waves	overall wave
	waves	wave	waves	wave	wave		
Growth phase duration (days)	10.0±1.4	24.0	14.3±2.7	13.5±2.5	19.0	12.1±1.6	17.3±2.9
Maximum diameter of DF (mm)	$9.9 \pm 0.3$	13.2	12.1±0.8	13.7±0.3	13.7	11.0±0.6	13.6±0.2
Growth phase rate (mm/day)	1.0±0.1	0.6	$0.9 \pm 0.2$	1.1±0.2	0.8	1.0±0.1	$0.9 \pm 0.2$
Static phase duration (day)	1.0±0.0	-	3.0±0.6	-		2.0±0.5	-
Regression phase duration (days)	10.3±2.3	-	7.3±1.5	-		8.8±1.4	-
Regression phase rate (mm/day)	1.1±0.2	-	1.9±0.4	-		1.5±0.3	-
Duration of the wave (days)	21.3±1.8	-	24.5±2.9	-		22.9±1.7	-
Average day of first ovulation		66.0		39.5	22.0		41.8

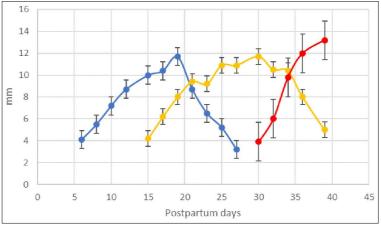


Fig 1: Follicular wave pattern in a postpartum ovulatory crossbred Jersey cow.

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of the follicular wave. The determining factor for ovulation or atresia, which subsequently impacts fertility, is the fate of the dominant follicle

Our emphasis should be on enhancing animal health and fertility through effective management practices rather than relying heavily on the widespread administration of external hormones. Achieving high reproductive efficiency during the postpartum period necessitates preventing metabolic diseases in the periparturient period and ensuring sufficient dry-matter intake after calving to meet the demands of milk production. Further research is warranted, particularly in the field of postpartum reproduction, to delve into the molecular pathology underlying anovulation and its association with anestrus (Peter *et al.*, 2009).

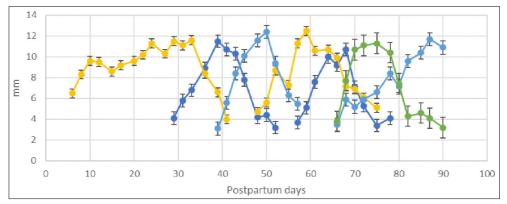


Fig 2: Follicular wave pattern in a postpartum anestrum crossbred Jersey cow.

Table 2: Mean±SE of quantitative follicular wave parameters in Anovulatory group of post-partum crossbred cows.

Wave parameters	W1	W2	W3	W4	W5	Significance
Day of wave emergence (day)	10.8±5.9	22.7±5.6	32.2±7.0	40.4±4.8	50.0±4.5	
Day of maximum diameter (day)	28.8±7.1	35.8±7.0	45.7±2.0	55.6±6.7	62.4±5.7	
Growth phase duration (days)	19.2±3.3 <sup>b</sup>	14.2±1.8 <sup>a</sup>	14.5±1.2a	16.2±2.7 <sup>b</sup>	13.4±2.8a	*
Maximum diameter of DF (mm)	10.6±0.4	10.6±0.4	11.0±0.6	12.1±0.3	12.0±0.4	NS
Growth phase rate (mm/day)	0.6±0.1	0.8±0.1	0.8±0.1	0.8±0.1	1.0±0.2	NS
Static phase (days)	4.0±0.8	2.8±0.7	3.3±1.0	4.4±1.2	4.0±1.4	NS
Day of regression (day)	32.8±6.9	38.7±7.5	49.0±6.2	60.0±6.9	66.4±4.9	
Regression phase (days)	8.8±2.4	6.8±1.0	8.8±1.5	8.4±2.0	18.8±3.6	NS
Regression phase rate (mm/day)	2.0±0.7	1.8±0.3	1.5±0.3	1.8±0.3	0.7±0.1	NS
Duration of the wave (days)	30.8±0.5 b	22.8±2.4a	25.7±1.5a	28±3.2 <sup>b</sup>	35.2±0.6 <sup>b</sup>	*

a,b - Values with different superscripts within a row vary significantly (P<0.05).

Table 3: Comparative analysis of first follicular wave parameters in Ovulatory and Anovulatory group of post-partum crossbred cows.

Wave parameters	OV	NOV	Significance	
Day of wave emergence (day)	5.7±0.3ª	10.8±5.9 <sup>b</sup>	**	
Day of Maximum diameter (day)	19.3±2.6 <sup>a</sup>	28.8±7.08 <sup>b</sup>	**	
Growth phase duration (days)	14.7±2.9 <sup>a</sup>	19.2±3.3 <sup>b</sup>	*	
Maximum diameter of DF (mm)	12.2±1.2a	10.6±0.4b	*	
Growth phase rate (mm/day)	0.9±0.1	0.6±0.1	NS	
Static phase duration (day)	2.3±0.9 <sup>a</sup>	4.0±0.8 <sup>b</sup>	*	
Day of regression (day)	21.7± 3.5°	32.8±6.9b	**	
Regression phase duration (days)	6.7±1.3	8.8±2.4	NS	
Day of complete regression (day)	28.3±3.8 <sup>a</sup>	41.7±6.5 <sup>b</sup>	**	
Regression phase rate (mm/day)	2.0±0.5	2.0±0.7	NS	
Duration of the wave (Days)	23.7±4.2 <sup>a</sup>	31.9±2.4 <sup>b</sup>	**	

a, b-Values with different superscripts within a row vary significantly (P<0.05).

<sup>\*</sup>Significant (P<0.05); NS: Not significant.

<sup>\*</sup>Significant (P<0.05); NS: Not significant.

## CONCLUSION

The present study has explored the postpartum ovarian dynamics in crossbred Jersey cows focusing on resumption of ovarian cyclicity; nevertheless, significant differences could be observed in follicular wave emergence and its duration; Perhaps the size of the DF is  $12.2\pm1.2$  mm and  $10.6\pm0.4$  mm in OV and NOV cows, the fate of cyclical resumption in terms of ovulation is decided by various factors. The lesser percentage of ovulation in the present study signals that the management care in terms of transition cow placing emphasis on postpartum partum cyclicity and fertility needs to be addressed.

#### Disclosure statement

Authors declare no conflict of interest.

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