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FOLLICULAR DYNAMICS OF ESTROUS CYCLE IN POSTPUBERTAL AND POSTPARTUM CROSSBRED (HOLSTEIN FRIESIAN X KANKREJ) CATTLE

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ABSTRACT
The use of ultrasonography is gradually increasing in dairy animals for improving their reproductive efficiency through monitoring follicular dynamics and taking appropriate measures, including diagnosis of early pregnancy and pathology, if any. In this study, the follicular dynamics of an estrous cycle was studied daily between two consecutive estruses in 6 postpubertal crossbred (HF x K) heifers and 6 postpartum cows using 7.5 MHz trans-rectal linear array transducer. The crossbred heifers and cows showed identical follicular growth pattern, *i.e.* presence of either 2-waves (66.66%, n=4) or 3-waves (33.33%, n=2) of follicles in an estrous cycle. In two-wave cycle, the first and second wave began at day 0.50 ±0.29 and 9.50 ±0.50, respectively, and in three-wave cycle, the first, second and third wave began at day 0.50 ±0.50, 8.50 ±0.50 and 14.50 ±0.50, respectively. In heifers, the duration (12.00 ±0.70 vs 6.00±0.00 days) and end day (21.50 ±0.28 vs 14.50 ±0.50) of second dominant follicle were significantly ($p<0.05$) higher with early emergence (9.50 ±0.50 vs 14.50±0.50 day) and longer persistence (12.00 ±0.70 vs 7.00 ±0.00 days) of ovulatory follicle in 2-wave as compared to 3-wave cycles. The second wave appeared little earlier in the estrous cycle with 3-wave than with 2-wave (8.50 ±0.50 vs 9.50 ±0.50 days). The diameter of ovulatory follicle of 2-wave cycle was significantly ($p<0.05$) larger than 3-wave cycle (17.22±0.79 vs 14.65 ±0.45 mm). In crossbred cows, the ovulatory follicle of 2-wave cycle appeared significantly ($p<0.01$) earlier than 3-wave cycle (days 9.00±0.71 vs 14.50 ±0.50). The persistence of ovulatory follicle was significantly longer ($P< 0.01$) in 2-wave as compared to 3-wave cycle (12.00 ±0.70 vs 7.50 ±0.50 days). The linear growth rate (mm/day) of ovulatory follicle was significantly lower ($p<0.05$) in 2-wave than 3-wave cycle (0.90 ±0.07 vs 1.31 ±0.15). The other parameters were almost similar for 2- and 3-waves cycles of crossbred cows. The 2-waves cycles were found predominant in crossbred (HF x K) cattle without affecting the length of estrous cycle or inter-ovulatory intervals in 2- and 3-wave cycles.

KEY WORDS: Crossbred (HF x K) cattle, Follicular dynamics, Estrous cycle, Dominant follicle, Growth phase, Regression phase.

INTRODUCTION

Ovary is one of the central organs of reproductive system, its normal functioning is pivotal to the cow's breeding soundness and consequently profitability. Use of ultrasound technology in animal reproduction has an important role to study ovarian follicular dynamics (Ginther *et al*., 1996). During normal estrous cycle in bovine either two or three follicular waves are common (Savio *et al*., 1988; Ginther *et al*., 1989[b]; Satheshkumar *et al*., 2011). In each wave of follicular growth, one dominant follicle develops and suppresses other follicles. The first dominant follicle (DF) grows and reaches maximum diameter in the middle of the estrous cycle: when there is high level of progesterone, there is no ovulation; regression starts allowing a new wave growth to occur. The DF that develops during the last wave of follicular growth in each estrous cycle is the ovulatory follicle (OF). The mechanism that controls the follicular dynamics during estrous cycles needs to be understood to optimize the reproductive efficiency especially in crossbred cattle. The Holstein Friesian crosses of Kankej (HF x K) cattle are well known for their milk production and resistance to Indian environment. Study of follicular dynamics in crossbred cattle helps to decide the time/day of estrous cycle for onset of various controlled breeding techniques to achieve maximum fertility. No information is available in the literature on these aspects in HF x K crossbred cattle. Hence, this study was aimed to understand the follicular dynamics in postpubertal heifers and postpartum HF x K crossbred cyclic cows.

MATERIALS & METHODS

The present study was carried out at Livestock Research Station of the College in Anand. The ultrasound examination were performed in postpubertal heifers (>2.0 years old) and postpartum cyclic crossbred (HF x K) cows using a real-time B-mode ultrasound scanner (M-5 Vet, Mindray, China) equipped with a 5.0 to 8.0 MHz linear array transducer designed for intra-rectal placement. By placing the lubricated gloved hand in the rectum, the evacuation of feces was accomplished and one of the ovaries was grasped from its base. After liberally applying the ultrasound transmission gel, the transducer was placed over the ovary through rectal wall and scanning was accomplished in several planes using 7.5 MHz frequency to identify all the follicles > 4 mm in diameter for both the ovaries. Desired images were frozen on the screen and the measurements were taken using a built in caliper system.

Hard copies (sonograms) were taken using videographic thermal printer (Brother, India).

The dominant follicle (DF) was characterized as the one, which grew at least 10 mm and exceeded the diameter of other follicles. Subordinate follicles (follicular cohort) were defined as those that appeared to originate from the same follicular pool as the dominant follicle (Ginther, 1995). A dominant follicle and its cohort were defined as a wave (Knopf *et al.*, 1989). The day of emergence of follicular wave was defined by first follicle to grow above 4 mm (Ginther, 1995). For individual follicle, the time from the first measurable diameter by ultrasonography (>4 mm) and the last day that the maximum diameter recorded was defined as the growth period; the time from decline in maximum diameter until the measurable size was defined as the atresia period. Growth rate was calculated by extracting the minimum diameter (> 4mm) from the maximum diameter and dividing by growth period. The ovulation was recorded as described by Nasser *et al.* (1993). The data were analyzed statistically using't' test (Snedecor and Cochran, 1994).

RESULTS & DISCUSSION

The findings on follicular dynamics observed in postpubertal heifers and postpartum cyclic crossbred cows throughout an estrous cycle are presented in Tables 1 to 3 and illustrated in Figures 1 to 4. Some of the sonograms of ovarian structures are also depicted in plates 1-4. The results indicated that there were presence of two patterns of ovarian follicular development per cycle, viz., 2-waves (66.66%, n=4) and 3-waves (33.33%, n=2) in both heifers and cows as noted by Patel *et al*. (2006), Satheshkumar *et al*. (2011), Ramana *et al.* (2013) and Hadiya *et al*. (2016) in HF, crossbred, Ongole and Gir cattle, respectively.

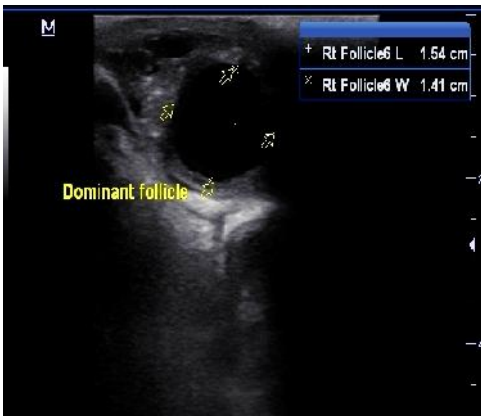

PLATE 1: Graafian follicle

PLATE 2: Mature corpus luteum

PLATE 3: Dominant follicle

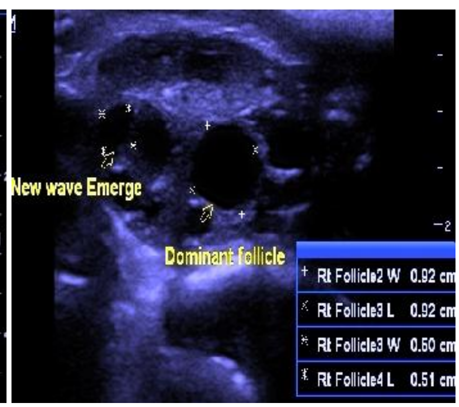

PLATE 4: New wave emergence

Follicular Dynamics in Crossbred Heifers

In two-wave cycle of crossbred heifers, the first and second wave began at day 0.50±0.29 and 9.50±0.50, respectively**,** while in three-wave cycle, the first, second and third wave began at day 0.50±0.50, 8.50±0.50 and 14.50±0.50, respectively (Table 1; Figs. 1-2). There was no significant difference between 2- and 3-wave cycles with regard to the day of the emergence of the first wave and second wave. The end day, duration, linear growth rate and regression rate of first dominant follicle also did not differ significantly between 2- and 3-wave cycles. However, duration and end day of second dominant follicle were significantly higher in 2-wave as compared to 3-wave cycle (12.00±0.70 vs 6.00±0.00, 21.50±0.28 vs 14.50±0.50; Table 1; Figs. 1-2).

TABLE 1: Characteristics (Mean ± SE) of dominant/ovulatory follicle of each wave in two- (22.00±0.21 d) and three-waves (22.00±0.00 d) follicular cycle in crossbred heifers

Characteristics	First DF	Second DF	Third DF

	2-waves (n=4) 66.7%	3-waves	2-waves (OF)	3-waves (n=2) 33.3%	2-waves	3-waves (OF)
Growth Phase						
• beginning day	0.50±0.29	0.50 ±0.50	9.50±0.50^{c}	8.50±0.50	-	14.50±0.50^{d}
• end day	10.50±0.64	10.50±0.50	21.50±0.28^{a}	14.50±0.50^{b}	-	21.50±0.50
• duration (days)	10.00±0.40	10.00±0.00	12.00±0.70^{ac}	6.00±0.00^{b}	-	7.00±0.00^{d}
• linear growth rate (mm/day)	0.81±0.08	0.79±0.10	1.04±0.06	1.19±0.20	-	1.27±0.14
Static Phase						
• duration (days)	4.75±0.25	4.50±0.50	-	3.00±0.00	-	-
• maximum diameter (mm)	12.10±0.45	11.85±0.55	-	11.40±0.20	-	-
Regression phase						
• beginning day	15.25±0.47	15.00±0.00	-	17.50±0.50	-	-
• end day	21.50±0.29	20.00±0.00	-	20.50±0.00	-	-
• duration (days)	6.25±0.47	5.00±0.00	-	3.00±0.00	-	-
• linear regression rate (mm/day)	-1.14±0.10	-1.26±0.11	-	-1.26±0.22	-	-

DF = Dominant follicle, OF = Ovulatory follicle.

a,b Values with different superscript differ significantly (P < 0.05) between columns within each dominant follicle.

c,d Values with different superscript differ highly significantly (P < 0.01) between ovulatory follicles.

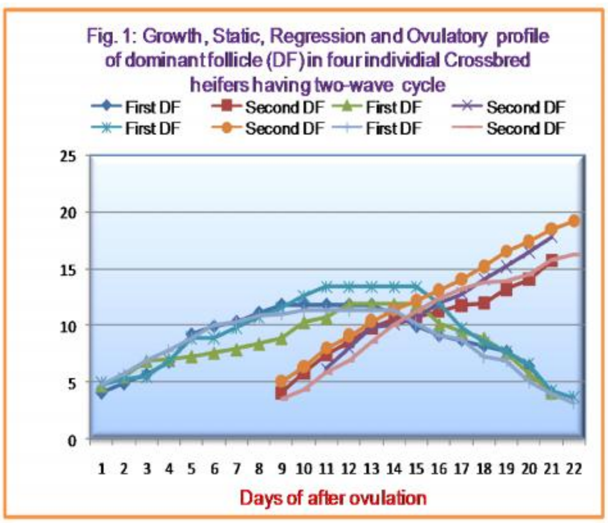

Fig. 1: Growth, Static, Regression and Ovulatory profile of dominant follicle (DF) in four individial Crossbred heifers having two-wave cycle

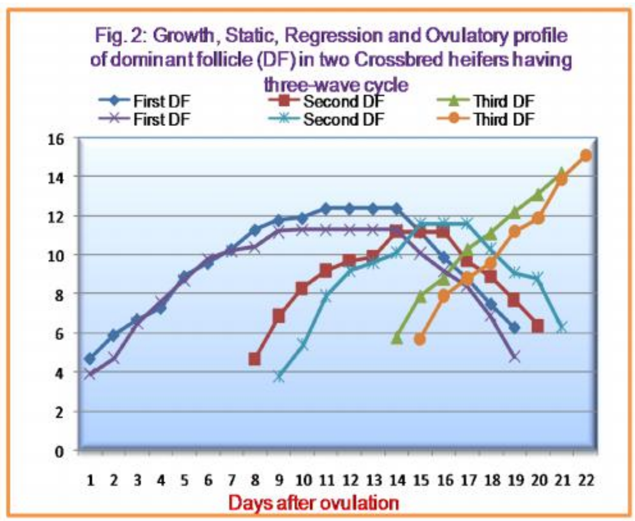

Fig. 2: Growth, Static, Regression and Ovulatory profile of dominant follicle (DF) in two Crossbred heifers having three-wave cycle

The emergence of ovulatory follicle (OF) was significantly earlier in 2-wave as compared to 3-wave cycle (9.50 ±0.50 vs. 14.50 ±0.50 days). Similarly, the duration of ovulatory follicles differed significantly (P < 0.01) between 2- and 3-wave cycles (12.00 ±0.70 vs 7.00 ±0.00 days; Table 1; Figs. 1-2). In the static phase of first dominant follicle, the duration and maximum diameter were not different statistically between 2- and 3-waves. The second wave appeared little earlier in the estrous cycle with 3-wave than with 2-wave (8.50 ±0.50 vs 9.50 ±0.50 days) as reported by Baruselli *et al*. (1997). It has been hypothetized that the first dominant follicle of a 3-wave cycle become incapable of suppressing FSH and thereby allows FSH to surge permitting the early emergence of the next wave (Adams *et al.*, 1992; Adams *et al.*, 2008).

The persistence of the first dominant follicle was non-significantly longer in 2-wave than in 3-wave cycle (21.00 ±0.41 vs 19.50 ±0.50 days). The results of present study demonstrated that 2- and 3-wave cycles do not differ with respect to the maximum diameter of the first dominant follicle, however the diameter of ovulatory follicle of 2-wave cycle was significantly (P<0.05) higher than 3-wave cycle (17.22 ±0.79 vs 14.65 ±0.45 mm), and corroborated with the previous studies (Baruselli *et al.*, 1997; Satheshkumar *et al.*, 2011). The mean intervals between estruses (22 days) and between ovulations (23 days) were similar between 2- and 3-wave cycles. These findings are however in contrast to those of Figuelredo *et al*. (1997), Townson *et al.* (2002) and Patel *et al.* (2006). The linear growth rate of ovulatory follicle was non-significantly higher in 3-wave as compared to 2-wave cycle as observed by Ahmed *et al.* (1997) and Manik *et al.* (1998)

Follicular Dynamics in Crossbred Cows

In postpartum cyclic crossbred cows also, there were presence of either 2-waves (66.66%, n=4) or 3-waves (33.33%, n=2) of follicular growth pattern as documented by Baruselli *et al.* (1997) and Satheshkumar *et al.* (2011), but no single wave cycle as reported in buffaloes (Taneja *et al*., 1996; Awasthi *et al.*, 2009), or four-wave pattern as reported in cattle (Rhodes *et al.*, 1995) could be seen in crossbred cows under study.

There was no significant difference for the period of emergence of first as well as second dominant follicle between 2- and 3-wave cycles. However, the ovulatory follicle of 2-wave cycle appeared significantly (P<0.01) earlier than 3-wave cycle (days 9.00±0.71 vs 14.50±0.50) as has been documented earlier (Baruselli *et al.*, 1997). The end day, duration (days) and linear growth rate (mm/day) of first dominant follicle were almost similar between 2- and 3-wave cycles in crossbred cows (Table 2; Figs. 3-4).

TABLE 2: Characteristics (Mean ± SE) of dominant/ovulatory follicle of each wave in two- (21.50±0.29 d) and three-waves (22.00±0.00 d) follicular cycle in crossbred cows

Characteristics	First DF		Second DF		Third DF	
	2-waves (n=4) 66.7%	3-waves	2-waves (OF)	3-waves (n=2) 33.3%	2-waves	3-waves) (OF)
Growth Phase						
• beginning day	0.50±0.29	0.00 ±0.00	9.00±0.71[c]	8.00±0.00	-	14.50±0.50[d]
• end day	8.25±0.25	8.50±0.50	21.00±0.00[a]	15.00±0.00[b]	-	22.00±0.00
• duration (days)	7.75±0.48	8.50±0.50	12.00±0.70[ac]	7.00±0.00[b]	-	7.50±0.50[d]
• linear growth rate (mm/day)	1.21±0.12	1.22±0.17	0.90±0.07[ae]	1.62±0.14[b]	-	1.31±0.15[f]
Static Phase						
• duration (days)	5.75±0.25	4.50±0.50	-	4.00±0.00	-	-
• maximum diameter (mm)	14.40±0.24	14.65±1.05	-	13.80±0.10	-	-
Regression phase						
• beginning day	14.00±0.41	13.00±1.00	-	17.00±0.00	-	-
• end day	21.00±0.00	20.00±0.00	-	22.00±0.00	-	-
• duration (days)	7.00±0.40	7.00±1.00	-	3.00±0.00	-	-
• linear regression rate (mm/day)	-1.39±0.09	-1.33±0.14	-	-1.80±0.29	-	-

DF = Dominant follicle, OF = Ovulatory follicle.
a,b Values with different superscript differ significantly (P < 0.05) between columns within each dominant follicle.
c,d Values with different superscript differ highly significantly (P < 0.01) between ovulatory follicles.
e,f Values with different superscript differ significantly (P < 0.05) between ovulatory follicles.

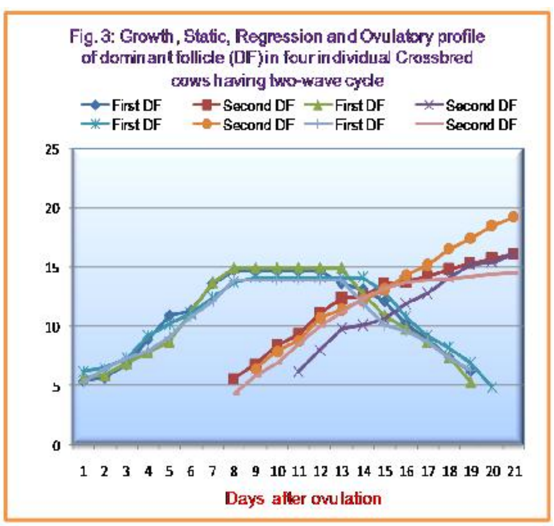

Fig. 3: Growth, Static, Regression and Ovulatory profile of dominant follicle (DF) in four individual Crossbred cows having two-wave cycle

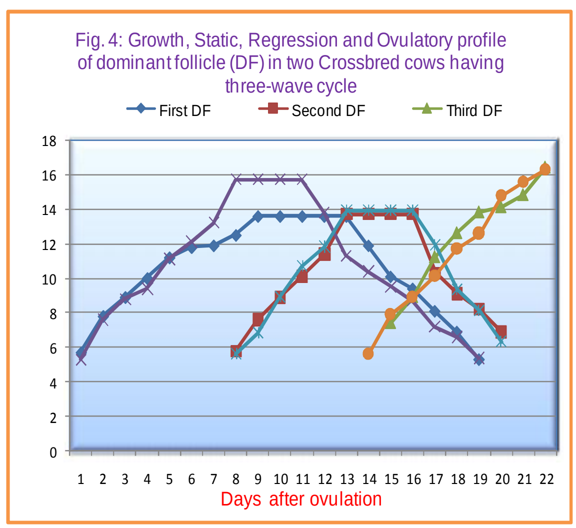

Fig. 4: Growth, Static, Regression and Ovulatory profile of dominant follicle (DF) in two Crossbred cows having three-wave cycle

The differences in persistence and maximum diameter of the first dominant follicle were statistically non-significant between 2-wave and 3-wave cycles. Similarly, no significant differences were observed in the duration of the static and regression phase of the first dominant follicle between 2- and 3-wave cycles (Table 1; Figs. 3-4). Similar trend was observed in the regression phase for beginning day, end day and linear regression rate of first dominant follicle, where the differences were not significant between 2- and 3-wave cycles (Table 2; Figs. 3-4).

The inter-ovulatory intervals (22-23 days) and duration of estrous cycle (21-22 days) were also statistically not different between 2- and 3-wave cycles. These finding were in contrast to the observations of Patel *et al.* (2006), who observed significantly longer inter-ovulatory intervals for 3-wave than 2-wave cycles in HF cows. The persistence of ovulatory follicle was significantly longer (P< 0.01) in 2-wave as compared to 3-wave cycle (12.00 ±0.70 vs 7.50 ±0.50 days) as reported earlier by Figuelredo *et al*. (1997). The linear growth rate (mm/day) of ovulatory follicle was significantly lower (P<0.05) in 2-wave than 3-wave cycle (0.90 ±0.07 vs 1.31 ±0.15) as documented in others study (Ahmed *et al.,* 1997).The growth profile of the solitary dominant follicle was atypical in two- and three-waves pattern of crossbred cattle. This will help to understand the development pattern of dominant follicles of various waves and time of ovulation, which improve the results of various controlled breeding protocols in crossbred cattle.

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