

# ULTRASONOGRAPHIC CHARACTERIZATION OF OVARIAN FOLLICULAR DYNAMICS IN UMBLACHERY COWS

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## ABSTRACT

*Ultrasonographic characterization of ovarian follicular dynamics was carried out in two subsequent oestrous cycles in Umblachery cows (n =10) from the day of observed oestrus (Day 0) to next oestrus. Two subsequent oestrous cycles were studied in all the animals. Out of 20 cycles studied, seven (35.00%), twelve (60.00%) and one (5.00%) cycle(s) had two, three and four waves respectively. The day at which the Wave-1 dominant follicle (DF) attained the maximum diameter varied widely from Day 5 to Day 11. It was also found that the day of Wave-2 emergence varied widely from Day 7 to Day 13 among the individual animals. The average diameters of anovulatory wave DFs ranged from 9.07 to 9.86 and that of ovulatory wave DFs averaged from 9.20 to 9.67 mm. The mean inter-oestrus intervals were significantly ( $P < 0.01$ ) longer in cycles with three-follicular waves ( $22.17 \pm 0.27$  days) than two-follicular waves ( $19.43 \pm 0.28$  days). Even though follicular wave patterns were comparable with other crossbred and *B.indicus* cattle, smaller size of the DF and wide variation in the days to attaining maximum diameter and emergence of Wave-2 were found to be characteristic features of Umblachery cows.*

**Key words:** Umblachery cows, Follicular dynamics, Ultrasonography

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## INTRODUCTION

Umblachery is a recognized draught cattle breed reared in Thanjavur, Thiruvarur and Nagapattinam districts of Tamil Nadu. The population of these agro-friendly breed is reported to decline due to mechanization of

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agricultural activities (Thangaraju *et al.*, 2001). In 2007 its conservation status was reported by the FAO as "endangered" (Rischkowsky and Pilling, 2007). Hence, steps are being taken to conserve the breed by increasing their numbers in the breeding tract.

Application of assisted reproductive techniques, especially *in-vivo* and *in-vitro* production of embryos, is a probable mode of preserving and propagating the germ plasm. However, understanding the basic reproductive physiological parameters is of prime importance for application of these advanced techniques.

Follicular dynamics during an oestrous cycle was extensively studied and documented in exotic *Bos taurus* cattle (Ginther *et al.*, 1989; Pursley *et al.*, 1993) and *B. taurus* x *B. indicus* crossbred cattle (Satheshkumar *et al.*, 2008, 2012; Satheshkumar, 2015). Among the Indian *B. indicus* cattle, follicular turnover during oestrous cycle was documented in Gir (Vianna *et al.*, 1998), Rathi (Gaur and Purohit, 2007) and Sahiwal (Hassan *et al.*, 2021) breeds. Eventhough some basic reproductive characters of Umblachery cattle (oestrous cycle length, pubertal age, calving interval etc.) were reported earlier (Layeek *et al.*, 2011; Kannadhasan *et al.*, 2016), there were no extensive studies on follicular dynamics and their impact on the nature of the oestrous cycle. Hence the present study was aimed at ultrasonographic characterization ovarian follicular dynamics during normal oestrous cycles of Umblachery cows.

## MATERIALS AND METHODS

Umblachery cows maintained at District Livestock Farm (DLF), Animal Husbandry Department, Government of Tamil Nadu located in Korukkai, Thiruvavur district of Tamil Nadu were utilized for the study. Healthy and regularly cyclic Umblachery cows (n = 10) in their second to fifth parity were selected randomly. All the cows were maintained under identical semi-intensive conditions throughout the study. They were monitored regularly for cyclicity and the oestrus was confirmed by gynaecological examination and ultrasonographic investigation. They were subjected for ultrasonographic follicular dynamics study throughout the oestrous cycle, on alternate days, from the day of observed oestrus (Day 0) to next oestrus. Two subsequent oestrous cycles were studied in all the animals. Thus a total of 20 untreated oestrous cycles were completely investigated to characterize the follicular turnover during the oestrous cycles.

Real-time B-mode ultrasound scanner (Aeroscan CD10 PRO, Japan) equipped with a rectal probe (7.5 MHz frequency) was employed to study the follicular dynamics. Each ovary was scanned in various planes to identify all the follicular structures (Ginther, 1993). The images of follicles were frozen and the length and width of these structures were measured using the in-built ultrasound calliper to arrive at the diameter (mm) as described by Zeitoun *et al.* (1996). Characteristics of dominant follicles (DF) and follicular wave patterns during the oestrous cycles were documented.

The Completely Randomized Design and independent 'T' - test were followed for the experiment and the data collected were analyzed using SPSS® 20.0 software package.

## RESULTS AND DISCUSSION

Out of 20 oestrous cycles studied in the Umblachery cows, seven (35.00 %), twelve (60.00 %) and one (5.00 %) cycle(s) had two-, three- and four-follicular waves respectively. Similarly, Dodiya *et al.* (2022) recorded that majority (57.10%) of non-lactating pluriparous Sahiwal cows exhibited three-wave follicular patterns than two-wave patterns during normal oestrous cycle. However, Figueiredo *et al.* (1997), Gaur and Purohit (2007) and Hassan *et al.* (2021) observed higher incidence of two-follicular waves in Nellore (83.30 %), Rathi (78.57 %) and Sahiwal (100.00 %) cows respectively. Satheshkumar *et al.* (2011) stated that Jersey crossbred cows exhibited more of the three-wave pattern (75.00 %) similar to our observations. In the present study, only one cycle (5.00%) had four-follicular waves. In contrary, Alvarez *et al.* (2000) documented higher incidence of four-wave cycles in *B. indicus* cows and attributed the higher incidence of four-wave cycles in Brahman cattle to elevated IGF-I and lower FSH concentrations, which would have resulted in less persistent DFs and more follicular wave development during an oestrous cycle. The varied reports on follicular wave patterns among breeds by various researchers could be attributed for diverse climatic conditions, seasonality associated with decreased pre-ovulatory LH surge and physiological

differences between these breeds of cattle (Hassan *et al.*, 2021).

Among the 10 Umblachery cows studied, five (50.00 %) cows exhibited same follicular wave patterns consistently for two consecutive cycles, but the remaining cows varied in their follicular wave patterns between the cycles. Figueiredo *et al.* (1997) also recorded that 30.00 per cent of Nellore cattle had different follicular wave patterns of development as that of the previous cycle.

The characteristics of the DF during normal oestrous cycle with three- and two-follicular waves in Umblachery cows are presented in Table 1. In three-follicular wave oestrous cycles the first (Wave-1), second (Wave-2) and third (Wave-3) waves emerged on  $2.08 \pm 0.18$ ,  $9.91 \pm 0.56$  and  $16.58 \pm 0.57$  days respectively, while in two-follicular wave oestrous cycles the Wave-1 and Wave-2 waves emerged on the mean days of  $1.86 \pm 0.13$  and  $10.86 \pm 0.62$  respectively. Similar period of Wave-1 emergence was recorded by Gaur and Purohit (2007) and Hassan *et al.* (2021) in Rathi and Sahiwal cows respectively.

Martinez *et al.* (2003) stated that the day of emergence of the second follicular wave tended to decrease as the number of waves per cycle increased. Similarly, in the present study, the emergence of Wave-2 occurred non-significantly earlier in oestrous cycles with three-follicular waves than two-follicular waves. It was also found that the day of Wave-2 emergence varied widely from Day 7 to Day 13 among the individual animals as against

**Table 1. Mean  $\pm$  SE of dominant follicle characteristics during normal oestrous cycle in Umblachery cows**

Parameters	Three follicular waves			Two follicular waves		Significance	F value
	Wave-1	Wave-2	Wave-3	Wave-1	Wave-2		
Day of wave emergence (Day)	2.08 $\pm$ 0.18 <sup>a</sup> (0 - 3)	9.91 $\pm$ 0.56 <sup>b</sup> (7 - 13)	16.58 $\pm$ 0.57 <sup>c</sup> (13 - 20)	1.86 $\pm$ 0.13 <sup>a</sup> (1 - 2)	10.86 $\pm$ 0.62 <sup>b</sup> (9 - 13)	**	<b>215.6038</b>
Day of maximum diameter (Day)	7.92 $\pm$ 0.46 <sup>a</sup> (5 - 11)	15.58 $\pm$ 0.59 <sup>b</sup> (12 - 20)	22.17 $\pm$ 0.33 <sup>c</sup> (21 - 25)	8.57 $\pm$ 0.57 <sup>a</sup> (7 - 11)	19.43 $\pm$ 0.28 <sup>b</sup> (19 - 21)	**	<b>206.5838</b>
Maximum diameter of DF (mm)	9.07 $\pm$ 0.23	9.08 $\pm$ 0.65	9.20 $\pm$ 0.14	9.86 $\pm$ 0.28	9.67 $\pm$ 0.30	N.S	<b>0.02903</b>
Growth phase (days)	6.83 $\pm$ 0.45	6.67 $\pm$ 0.41	6.58 $\pm$ 0.46	7.71 $\pm$ 0.52	9.57 $\pm$ 0.75	N.S	<b>0.07527</b>
Growth rate (mm/day)	1.39 $\pm$ 0.09	1.44 $\pm$ 0.14	1.47 $\pm$ 0.09	1.31 $\pm$ 0.07	1.06 $\pm$ 0.10	N.S	<b>0.0876</b>
Static phase (days)	1.42 $\pm$ 0.14	1.33 $\pm$ 0.14	@	1.43 $\pm$ 0.19	@	N.S	<b>0.1642</b>
Day of initiation of regression (Day)	10.17 $\pm$ 0.37 <sup>a</sup> (9 - 12)	17.58 $\pm$ 0.52 <sup>b</sup> (14 - 21)	@	11.14 $\pm$ 0.55 <sup>a</sup> (9 - 13)	@	**	<b>123.9416</b>
Regression phase (days)	5.67 $\pm$ 0.62	4.25 $\pm$ 0.44	@	4.71 $\pm$ 0.48	@	N.S	<b>3.195</b>
Regression rate (mm/day)	1.80 $\pm$ 0.17	2.40 $\pm$ 0.31	@	2.29 $\pm$ 0.28	@	N.S	<b>2.6016</b>
Duration of wave (days)	12.58 $\pm$ 0.67 <sup>a</sup>	11.25 $\pm$ 0.59 <sup>ab</sup>	7.17 $\pm$ 1.24 <sup>c</sup>	14.43 $\pm$ 0.40 <sup>a</sup>	9.43 $\pm$ 0.80 <sup>b</sup>	**	<b>9.4204</b>
Duration of oestrous cycle (days)		22.17 $\pm$ 0.33 (21 - 25)		19.43 $\pm$ 0.28 (19-21)			

(Range with parenthesis)

Values within the row with different superscripts differ significantly \*\* (P < 0.01)

NS – Not significant (P > 0.05)

@ Non-determinable in ovulatory wave of the cycle

Day 8 – 11 in crossbred cows (Satheshkumar *et al.*, 2012)

The ovulatory wave DF emerged significantly ( $P < 0.01$ ) earlier in two-follicular wave cycles than three-follicular wave cycles in Umblachery cows, which was in concurrence with the observations of Ginther *et al.* (1989), Satheshkumar (2015) and Dodiya *et al.* (2022) in exotic, crossbred and indigenous cows respectively.

The day at which the Wave-1 DF attained the maximum diameter varied widely from Day 5 to Day 11 of oestrous cycle, with an average of Day 7.92 and 8.57 in three- and two-follicular waves respectively, in Umblachery cows. However, Satheshkumar *et al.* (2012) reported that Wave-1 DF attained maximum diameter much earlier (Day 6.70 - 7.30) than Umblachery cows. In Umblachery cows, the duration of anovulatory waves ranged between 11.25 and 12.58 days in three-follicular wave cycles and 14.43 days in two-follicular wave cycles. The mean duration of ovulatory waves in three- and two-follicular wave cycles were  $7.17 \pm 1.24$  and  $9.43 \pm 0.80$  days respectively. Similar wave durations were recorded by Gaur and Purohit (2007) in Rathi cows.

The average diameters of anovulatory wave DFs ranged from 9.07 – 9.86 mm without any significant differences within the waves and between the wave patterns. Similar results were observed in Sahiwal cows (Dodiya *et al.*, 2022). However, the DF sizes in Umblachery cows were found to be smaller when compared to Nelore and Gir cows (Sartorelli *et al.*, 2005;

Viana *et al.*, 1998). The diameter of ovulatory wave DF in Umblachery cows averaged 9.20 - 9.67 mm, which was much smaller than preovulatory follicles of Holstein ( $14.6 \pm 0.5$  mm) and Jersey crossbred ( $12.83 \pm 1.37$  mm) cows (Taponen *et al.*, 2000; Satheshkumar *et al.*, 2008). Relatively larger ovulatory follicles were recorded in other *B. indicus* cattle when compared to Umblachery cows. Lima *et al.* (2020) documented that the diameter of ovulatory follicle in Nelore cows ranged from 13.4 - 14.6 mm. Similarly, in Sahiwal cows, a range of 11.66 - 14.71 mm was reported by Hassan *et al.* (2021) and Dodiya *et al.* (2022). Baldighi *et al.* (2022) reported  $13.1 \pm 0.75$  mm as the maximum diameter of ovulatory follicle in Gir heifers. On perusing these literatures, Umblachery cattle were found to be possessing considerably smaller follicles when compared among the Indian *B. indicus* and crossbred cattle.

In two-follicular wave cycles, the mean growth phase (GP) of ovulatory wave DFs were significantly ( $P < 0.01$ ) increased and their growth rate (GR) were significantly ( $P < 0.05$ ) slower than the ovulatory wave DFs in three-follicular waves. Knopf *et al.* (1989) suggested that lower plasma concentrations of progesterone ( $P_4$ ) during a phase of cycle might increase the GR of DF present at that time. The development period of third wave DF of three-follicular wave cycles usually coincides with the luteolytic phase of the cycle with decreasing  $P_4$  concentrations and thus would have contributed for increased GR before ovulation. In present study, the mean inter-oestrus intervals for three- and two-

follicular wave oestrous cycles were  $22.17 \pm 0.27$  and  $19.43 \pm 0.28$  days respectively. The three-follicular wave oestrous cycles were significantly ( $P < 0.01$ ) longer in duration than two-follicular wave cycles. The findings were in concurrence with those of Figueiredo *et al.* (1997), who recorded an increased duration of oestrous cycle length in Nellore cattle with three-follicular waves (22.0 days) than two-follicular waves (20.7 days). Similarly, Dodiya *et al.* (2022) found that the oestrous cycle length was shorter by 2.17 days in two-follicular cycles than the three-follicular wave cycles in Sahiwal cows.

On perusing the literatures, to the best of our knowledge, this is the first extensive report on the ultrasonographic characterization of *in-vivo* follicular turnover during the oestrous cycles of Umblachery cows. In conclusion, analysis of ovarian follicular dynamics in Umblachery cows revealed that they exhibited similar wave patterns as other Indian *B. indicus* and crossbred cattle, but the individual follicular parameters differ uniquely. Notably, the maximum diameters of the DFs were relatively smaller when compared to exotic, crossbred and other Indian *B. indicus* cattle. Further, the days of Wave-1 DF attaining the maximum diameter and days of Wave-2 emergence were highly variable between animals, which were considered to be the major concerning factors for application of programmed breeding and assisted reproductive techniques like multiple ovulation and embryo transfer technology. Further research needs to be carried out to explore the protocols on synchronizing the

follicular wave emergence in order to achieve more precise results in advanced reproduction techniques, towards the conservation of the breed.

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