Ovarian Follicular Dynamics Monitored by Real-Time Ultrasonography during Oestrous Cycle in Buffalo (*Bubalus bubalis*)

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ABSTRACT : Application of transrectal ultrasonography to buffaloes (n=6) to follow the growth of large antral follicles individually, on each day of one interovulatory interval revealed that follicular turnover during oestrous cycle occured in waves. There was a predominance of a two-wave pattern (5/6 animals) compared to that of a three-wave pattern (1/6 animals). For two-wave pattern, the first wave emerged at Day 0.20 ± 0.19 (Day 0 = day of ovulation) and was marked by development of a dominant anovulatory follicle which grew in size from 5.40 ± 0.24 mm at the day of detection to a maximum diameter of 12.40 ± 0.81 mm on Day 8.60 ± 1.57, with a growth rate of 0.88 ± 0.17 mm/day and then regressed, with a mean persistence of 19.40 ± 1.54 days. The second wave emerged at Day 9.20 ± 1.06 and was marked by development of a dominant ovulatory follicle which grew in size from 4.20 ± 0.37 mm at the day of detection to a maximum diameter of 13.80 ± 0.37 mm on Day 21.00 ± 1.38, with a growth rate of 0.66 ± 0.12 mm/day and then ovulated on Day 21.60 ± 1.25, with a mean persistence of 11.80 ± 1.39 days. The maximum diameters attained and the growth rates of dominant anovulatory and dominant ovulatory follicles, and the mean number of follicles ≥ 3 mm diameter detected at the time of emergence of first and second waves (11.80 ± 1.74 and 9.00 ± 2.81, respectively) were not significantly different. In the animal which showed a three-wave pattern, the first, second and third waves emerged on Days 1, 10 and 19, respectively. All animals, except one had at least one subordinate follicle in the first or second or both waves. The subordinate follicles increased in diameter over a few days and then regressed. The results indicate that in buffaloes, the follicular turnover during oestrous cycle occurs predominantly in a two-wave pattern.
(Key Words: Buffalo, Follicle, Oestrous Cycle, Ultrasonography)

INTRODUCTION

In studies using daily ultrasound monitoring of individual follicles, it has been established that in cattle, follicular development occurs in waves. Each wave of follicular growth is characterized by the synchronous development of a group of follicles followed by selection of a dominant follicle and subsequent regression of subordinate follicles (Savio et al., 1988; Sirois and Fortune 1988; Ginther et al., 1989a). Some reports indicate predominance of three waves (Sirois and Fortune, 1988; Savio et al., 1988; Fortune et al., 1991) whereas others suggest predominance of two waves of follicular growth during oestrous cycle in cattle (Pierson and Ginther 1988; Ginther et al., 1989b; Knopf et al., 1989). The first wave begins at the start of the oestrous cycle and is marked by development of a dominant anovulatory follicle which appears before midcycle and regresses during last half of the cycle. The second wave begins at midcycle and, in an oestrous cycle having two waves, culminates in the development of a dominant ovulatory follicle. In an oestrous cycle with three waves, the dominant follicle of the second wave also undergoes atresia, and the dominant follicle of the third wave which appears during the last half of the cycle becomes the ovulatory follicle (Savio et al., 1988; Sirois and Fortune 1988; Ginther et al., 1989a,b; Knopf et al., 1989; Fortune et al., 1991).

Buffalo is the principal dairy animal and the mainstay of dairy industry in Asia. Although ultrasonography has been used for studying ovarian follicular dynamics during superovulation in this species (Manik et al., 1994; Taneja et al., 1995a,b), detailed information on follicular dynamics in buffaloes during unstimulated oestrous cycle, which can be very useful in improving superovulation results is confined to only one recent report (Baruselli et

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al., 1997). The objective of the present study was to apply transrectal ultrasonography to buffalo to follow and characterize the growth and development of large antral follicles individually, on each day of the interovulatory interval to determine whether follicular growth during oestrous cycle occurs in waves in this species.

MATERIALS AND METHODS

Animals

Nonlactating, sexually mature, multiparous buffaloes (n = 6), between 6 and 7 years of age, which weighed 450 to 550 kg and which had shown at least one normal oestrous cycle previously were used for the study. The animals were maintained under general herd managemental conditions in the National Dairy Research Institute animal herd. The study was completed in the winter season (January to February).

Ultrasonography

The animals were examined by transrectal ultrasonographic imaging with a real-time B-mode instrument with a 5.0 MHz linear array transducer (Tokyo Keiki LS-1000). Ultrasonographic examinations were performed daily between 10:00 and 12:00 hrs starting on the day of natural oestrus and ending on the day of ovulation at the end of the oestrous cycle. The animals were restrained by making them stand in an animal crate. No chemical methods were used to restrain the animals during ultrasonography. The transducer was inserted after evacuating the rectum and ultrasonography was performed as described earlier (Manik et al., 1994). The antra of follicles ≥ 10 mm were measured with the built-in callipers after freezing the ultrasound image, whereas the diameter of smaller follicles was measured against the in built centimeter scale displayed on the screen alongside the ultrasound image. This was done to minimize the errors during freezing of image. The diameter of nonspherical follicles was calculated by taking the average of the longest and widest measured points of the follicle. The follicles were characterized as small (3-5 mm diameter), medium (6-9 diameter) and large (≥ 10 mm diameter). Although follicles < 3 mm could be detected, these were not included in the study due to difficulty in following their individual development.

The day of ovulation was regarded as the day on which a large follicle which had previously been followed for many days was no longer seen. The day of ovulation at the beginning of the interovulatory interval was designated as Day 0. In buffaloes, ovulation has been reported to occur 30 hrs (range 18 to 45 hrs) following the onset of oestrus (Jainudeen, 1986). The data were analysed for one interovulatory interval. All the large follicles in individual animals were traced retrospectively to the day on which they were first detected. This was considered as the day of emergence of the follicle. Only follicles that grew to a diameter of ≥ 7 mm or which could be identified for at least 3 days, with a change in diameter by at least 1 mm were used for analysis. The criteria used for defining different components and events of follicular dynamics in the present study were based on those given by Ginther et al. (1989a). Briefly, the dominant follicle of the wave was defined as the one that grew to at least 10 mm and exceeded the diameter of all other follicles in the wave. The duration of growth of a dominant follicle was the interval of time (days) taken by that follicle to grow from the size at the time of its first detection to its maximum diameter. The day on which a dominant follicle grew to its maximum diameter was considered the end of growth phase. The days of first detection of a follicle that was subsequently identified as a dominant follicle was taken as the first day of the wave. A wave of follicular activity was identified by the presence of a dominant follicle irrespective of the presence or absence of subordinate follicles. A subordinate follicle was defined as one which originated from the same follicular pool as the dominant follicle of that wave by its first detection within 2 days of the first detection of the dominant follicle. Additioinally, it exhibited an increase in diameter during the initial period of growth of dominant follicle but it did not exceed 10 mm in diameter.

Statistical analyses

Since only one animal showed a three-wave pattern of follicular growth, data were analysed only for animals which had a two-wave pattern. The persistence, maximum diameters and growth rates of dominant anovulatory and ovulatory follicles were compared by paired 't' test. Difference was considered significant at $p < 0.05$. The rate of growth of each dominant follicle was calculated by regression analysis (slope = growth rate) as described by Ginther et al. (1989a) and Knopf et al. (1989).

RESULTS AND DISCUSSION

Follicular development was characterized by waves of follicular growth in all the animals. In conformity with the preplanned definition (Ginther et al., 1989a), 5/6 buffaloes had a two-wave and one buffalo had a three-wave pattern of follicular turnover in the interovulatory interval (figure 1). For two-wave pattern, the first and

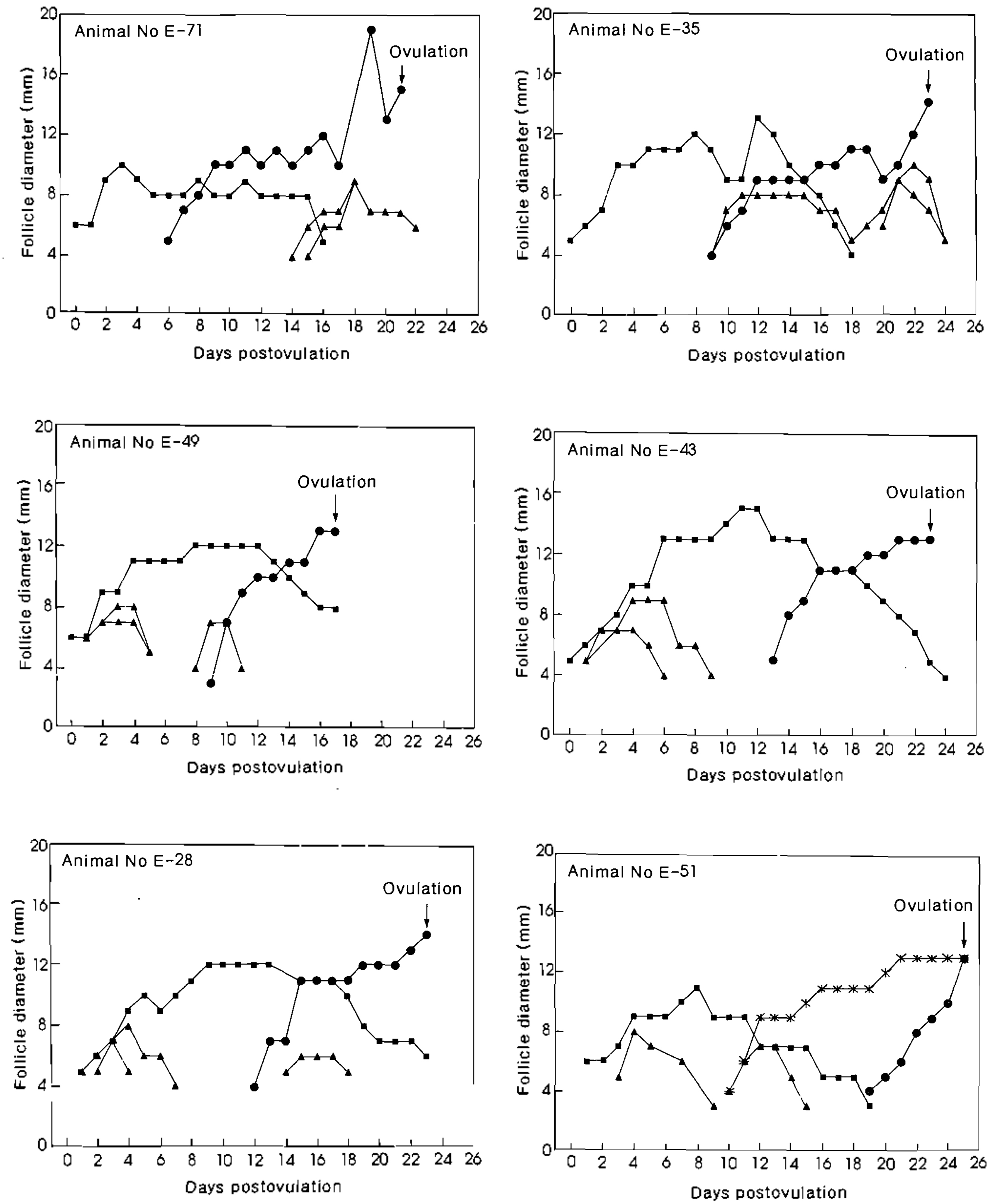

Figure 1. Patterns of growth and regression of individual dominant follicle of the first wave (square), second wave (circle) and third wave (asterisk), and subordinate follicles (triangle). In Animal E-51 which had a three-wave pattern of follicular turnover, the dominant follicle of the third wave was ovulatory, whereas in all other animals which had a two-wave pattern, the dominant follicle of the second wave was ovulatory.

second waves emerged at Days 0.20 ± 0.19 and 9.20 ± 1.06, respectively (Day 0 = day of ovulation) whereas in the animal which showed a three-wave pattern, the first, second and third waves emerged at Days 1, 10 and 19, respectively (table 1). Each wave was characterized by the presence of a variable number of small-sized follicles, one of which subsequently developed to a size of > 10 mm and emerged as the dominant follicle.

In the animals which had a two-wave pattern, two dominant follicles could be identified. The first dominant follicle which was anovulatory was retrospectively traced back to Day 0.20 ± 0.19 with a mean diameter of 5.40 ± 0.24 mm. It continued to grow upto Day 8.80 ± 1.82, when it attained a maximum diameter of 12.40 ± 0.81 mm, following which it regressed and disappeared in the last half of the oestrous cycle. This follicle could be detected for 19.40 ± 1.54 days during the cycle. The second dominant follicle which was ovulatory was retrospectively traced back to Day 9.20 ± 1.06 with a mean diameter of 4.20 ± 0.37 mm. It continued to grow

Table 1. Characteristics of follicular turnover during oestrous cycles in buffaloes having two- or three-wave pattern (Values are mean ± SEM for two-wave pattern).

	Number of waves	
	2	3
Number of interovulatory intervals	5	1
Length of interovulatory intervals (days)	21.6 ± 1.25	25
Emergence of first wave (day)	0.2 ± 0.19	1
Emergence of second wave (day)	9.2 ± 1.06	10
Emergence of third wave (day)	–	19
Interval between emergence of waves (days)		
1st wave to 2nd wave	9.6 ± 1.16	9
2nd wave to 3rd wave	–	8
No. of follicles ≥ 3 mm at emergence of wave		
1st wave	11.8 ± 1.74	6
2nd wave	9.00 ± 2.81	4
3rd wave	–	12
First dominant anovulatory follicle		
Diameter at the time of emergence (mm)	5.40 ± 0.24	6
End of growth phase (day)	8.8 ± 1.82	8
Maximum diameter (mm)	12.40 ± 0.81	11
Maximum size reached on (day)	8.60 ± 1.57	8
Growth rate (mm/day)	0.88 ± 0.17	0.71
Persistence (days)	19.40 ± 1.54	24
Second dominant anovulatory follicle		
Diameter at the time of emergence (mm)	–	4
End of growth phase (day)	–	11
Maximum diameter (mm)	–	13
Maximum size reached on (day)	–	21
Growth rate (mm/day)	–	0.82
Persistence (days)	–	15
Ovulatory follicle		
Diameter at the time of emergence (mm)	4.20 ± 0.37	3
End of growth phase (day)	21.60 ± 1.25	25
Maximum diameter (mm)	13.80 ± 0.37	13
Maximum size reached on (day)	21.00 ± 1.38	25
Growth rate (mm/day)	0.66 ± 0.12	1.50
Persistence (days)	11.80 ± 1.39	6
Interval from detection to ovulation (days)	11.80 ± 1.39	6

upto Day 21.00 ± 1.38, by which time it had attained a maximum diameter of 13.80 ± 0.37 mm. It ovulated on Day 21.60 ± 1.25, with the interval from detection to ovulation being 11.80 ± 1.39 days. There was no significant difference in the miximum diameters attained and the growth rates (0.88 ± 0.17 and 0.66 ± 0.12, respectively) of dominant anovulatory and dominant ovulatory follicles. The mean number of follicles ≥ 3 mm diameter detected at the time of emergence of first and second waves (11.80 ± 1.74 and 9.00 ± 2.81, respectively) were not significantly different.

All the animals except E-71 had at least one subordinate follicle in either first or second or both waves. Mean number of subordinate follicles was 1.17 ± 0.40 and 0.67 ± 0.21 in the first and second waves, respectively. The largest subordinate follicles of the first and second waves which were 5.67 ± 0.66 and 4.20 ± 0.20 mm in diameter, respectively at the day of first detection had a growth phase characterized by an increase in diameters to 8.25 ± 0.25 and 7.25 ± 0.25 mm, respectively, following which they regressed and subsequently disappeared, with persistence of 5.75 ± 1.03 and 5.25 ± 0.31 days, respectively.

The results of the present study indicate that in buffaloes, the follicular turnover during oestrous cycle occurs in a distinct wave-like pattern, with predominance of a two-wave pattern over a three-wave pattern. This is in agreement with the results of earlier studies in cattle (Pierson and Ginther 1988; Ginther et al., 1989a,b; Knopf et al., 1989) and a recent report in buffalo (Baruselli et al., 1997). In cattle, however, a number of reports indicate a predominance of a three-wave pattern (Savio et al., 1988; Sirois and Fortune, 1988; Fortune et al., 1991). The factors responsible for this diversity, which have led to different proportions or two- and three-wave patterns in different studies are not understood.

There is a similarity between the results of the present study and those reported earlier in buffalo (Baruselli et al., 1997) and in cattle in terms of the time of emergence of first and second waves (Ginther et al., 1989a; Knopf et al., 1989; Savio et al., 1990; Adams et al., 1994; Bo et al., 1995), persistence of anovulatory follicle and time of end of growth phase of anovulatory follicle, day on which anovulatory follicle attained maximum size and length of interovulatory interval (Knopf et al., 1989; Savio et al., 1990). Our results of presence of at least one subordinate follicle in the first and second waves in 5/6 animals, and the average number of subordinate follicles observed in the present study are in agreement with those reported in cattle (Knopf et al., 1989).

The mean maximum diameters of dominant ovulatory and dominant anovulatory follicles reported in this study are similar to those observed in some studies in cattle (Fortune et al., 1988; Bo et al., 1995) but smaller than those reported in buffalo (Baruselli et al., 1997) and some other reports in cattle (Ginther et al., 1989a; Savio et al., 1990). In the present study, the differences in the maximum diameters and growth rates of dominant anovulatory and dominant ovulatory follicles were not found to be significant within the two-wave cycles, as also reported in an earlier study in buffalo (Baruselli et al., 1997). Others have observed that although the growth rate of dominant ovulatory follicle was significantly lower than that of dominant anovulatory follicle, the differences between the mean maximum diameters of these follicles were not significant (Ginther et al., 1989a; Knopf et al., 1989).

In the present study no differences were observed in the number of follicles ≥ 3 mm in diameter present at the time of emergence of the first and second wave. In earlier studies on follicular populations during oestrous cycle in cattle no differences have been found in the total number of follicles ≥ 2 mm in diameter, or in the number of follicles in the 2-3 mm, 4-6 mm and 7-10 mm categories (Ginther et al., 1989a; Adams et al., 1994). The two waves may, therefore, be similar in their follicular population and composition at the time of emergence of the waves and any compositional changes may occur only during the progress of growth of the cohort of follicles. There were no significant diameter differences among follicles that later became dominant or subordinate. The dominant follicle, however, grew faster and was larger than the largest subordinate follicle at all times.

Based on extensive work carried out in cattle, the current hypothesis is that the emergence of each follicular wave is preceded by a surge in circulatory FSH levels 1-2 days earlier and that this surge is responsible for triggering the emergence of follicular waves. The post surge decline in FSH is an integral component of the mechanism of selection of dominant follicle (Adams et al., 1992a,b). It has been shown that exogenous FSH treatment initiated 9 days after oestrus i.e. around the expected time of the endogenous wave-eliciting FSH surge results in a higher superovulatory response, compared to treatments initiated early in cycle (Goulding et al., 1990). A higher superovulatory response has been reported to be elicited when treatments were initiated on the day of, or the day before wave emergence compared to that with later treatments (Nasser et al., 1993). Others have, however, found that superovulatory treatments can be initiated at the time of emergence of wave 1 (Adams et al., 1994). Based on the results of the present study it can be suggested that the day of oestrus and days 8-9 after oestrus may be more suitable for initiation of superovulation in buffalo than other days of the oestrous cycle.

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