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Ovarian follicular activity and hormonal profile during estrous cycle in cows: the development of 2 versus 3 waves

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Abstract

Most estrous cycles in cows consist of 2 or 3 waves of follicular activity. Waves of ovarian follicular development comprise the growth of dominant follicles some of which become ovulatory and the others are anovulatory. Ovarian follicular activity in cows during estrous cycle was studied with a special reference to follicular waves and the circulating concentrations of estradiol and progesterone. Transrectal ultrasound examination was carried out during 14 interovulatory intervals in 7 cows. Ovarian follicular activity was recorded together with assessment of serum estradiol and progesterone concentrations. Three-wave versus two-wave interovulatory intervals was observed in 71.4% of cows. The 3-wave interovulatory intervals differed from 2-wave intervals in: 1) earlier emergence of the dominant follicles, 2) longer in length, and 3) shorter interval from emergence to ovulation. There was a progressive increase in follicular size and estradiol production during growth phase of each wave. A drop in estradiol concentration was observed during the static phase of dominant anovulatory follicles. The size of the ovulatory follicle was always greater and produced higher estradiol compared with the anovulatory follicle. In conclusion, there was a predominance of 3-wave follicular activity that was associated with an increase in length of interovulatory intervals. A dominant anovulatory follicle during its static phase may initiate the emergence of a subsequent wave. Follicular size and estradiol concentration may have an important role in controlling follicular development and in determining whether an estrous cycle will have 2 or 3-waves.

Background

Ovarian follicular growth in cows occurs in waves. A wave of follicular growth involves the synchronous development of a group of follicles, one of which become dominant and achieves the greatest diameter suppressing the growth of the subordinate smaller follicles [1]. Transrectal ultrasonic imaging of ovarian follicles reveals that most estrous cycles in cows have two [2] or three [3] follicular waves. A retrospectively identified dominant follicle, its line of development was studied from the start of its growth till achieved the greatest diameter and was recognized as a dominant follicle, grew linearly for approximately 6 days (growing phase), remained approximately the same size for 6 days (static phase), and then began to regress (regressing phase), as indicated by decreasing in antral diameter. The follicular waves are first detectable as 4–5 mm follicles approximately days 0 and 10 for two-wave interovulatory intervals and on approximately days 0, 9 and 16 for three-wave interovulatory intervals [2]. It was reported that the bovine estrous cycle involves 2 waves [4] or 3 waves [5] of follicular activity. The purpose of this study was to monitor the ovarian follicular activity

Table 1: Follicular characteristics and hormonal concentrations during 2-wave and 3-wave interovulatory intervals

	No. of follicular waves during an interovulatory interval	
	2-wave	3-wave
Interovulatory Intervals		
No.	4	10
Length (days)	19.8 ± 0.6*	22.5 ± 0.8*
Length of days between emergence of waves:		
1st wave to 2nd	8.7 ± 0.3 [a]	7.2 ± 0.7 [a]
2nd wave to 3rd	10.8 ± 0.3 [b]	7.7 ± 0.4 [a]
3rd wave to ovulation		9.5 ± 0.4 [b]
Anovulatory follicles		
Maximum diameter (mm)	6.8 ± 0.3*	7.3 ± 0.7*
Ovulatory follicles		
Maximum diameter (mm)	10.9 ± 0.3	11.0 ± 0.9
Estradiol concentration (pg/ml)	5.40 ± 0.26	4.94 ± 0.15
Progesterone concentration (ng/ml)	0.96 ± 0.07*	1.43 ± 0.11*

Values are means ± SEM. Means with (*) in the same *row* are significantly different (P < 0.05)(LSD). Means with different letters (a,b) in the same *column* are significantly different (P < 0.05)(LSD).

in cows and to characterize the temporal association between follicular waves and the circulating concentrations of estradiol and progesterone during estrous cycle.

Methods

Seven postpartum dairy cows (Native breed), 4–5 years of age, were used during September-December. Ultrasound examinations of the ovaries were carried out once every other day. The ultrasound scanner was a real-time, B mode, instrument equipped with a 5 MHz, linear array, intrarectal transducer. Cows were examined during 2 successive estrous cycles. 14 interovulatory intervals were used to assess the incidence of 2 versus 3 follicular waves. The day of ovulation at the beginning of an interovulatory interval was designated as Day 0. During each examination the diameter of identified individual follicles with antral diameter of ≥ 3 mm were recorded. A follicular wave was identified by the largest follicle (≥ 6 mm) attaining a diameter greater than the diameter of its subordinate follicles. The day of first detection of a ≥ 3 mm follicle that was retrospectively identified as a dominant follicle was taken as the first day of a wave. The interovulatory intervals were classified into: 1) 2-wave interovulatory intervals (1st wave with a dominant anovulatory follicle and 2nd wave with a dominant ovulatory follicle). 2) 3-wave interovulatory intervals (1st and 2nd waves with a dominant anovulatory follicle and 3rd wave with a dominant ovulatory follicle). Each dominant anovulatory follicle was partitioned into growing, static and regressing phases. The growing phase extended from the 1st day of a wave to the day that the follicle appeared to cease its increase in diameter. The static phase extended from the last day of growing phase to the 1st day that the follicle appeared to decrease in diameter. The regressing phase extended from the last day of static phase to the day the follicle was no longer detectable. During each ultrasound examination a single blood sample was drawn by jugular venepuncture from all cows. Serum was obtained from all samples and was analyzed by RIA for estradiol and progesterone using coat-A-count kits for each (Diagnostic Products Cooperation, USA). Statistical analysis for follicular dynamics and hormonal concentration were carried out using ANOVA [6].

Results

Of the studied 14 interovulatory intervals 5 out of 7 cows (71.4%) had 3 waves of follicular development. The mean length of the 2-wave interovulatory intervals (19.8 ± 0.6 days) was significantly (P < 0.05) shorter than that of 3-wave interval (22.5 ± 0.8 days). The mean length of days between emergences of waves (Table 1) did not differ significantly between the 1st and 2nd wave during 3-wave interovulatory intervals. While, there was a significant (P < 0.05) increase in length of days in the ovulatory wave of 2-wave interovulatory intervals (2nd wave) and of 3-wave interovulatory intervals (3rd wave). A significant (P < 0.05) increase in the maximum diameter of anovulatory follicle was observed during 3-wave interovulatory intervals (Table 1). Wave 1 was first detected on Day 0, while wave 2 was detected, on average, on Day 8.7 & 7.2 in 2-wave & 3-wave interovulatory intervals, respectively. Wave 3 was detected, on average, on Day 15 in 3-wave interovulatory intervals. The ovulatory follicle showed a significant (P < 0.05) increase in diameter compared with the anovulatory dominant follicle (Table 2, Fig. 1), in the 2-wave and 3-wave interovulatory intervals. A concomitant significant

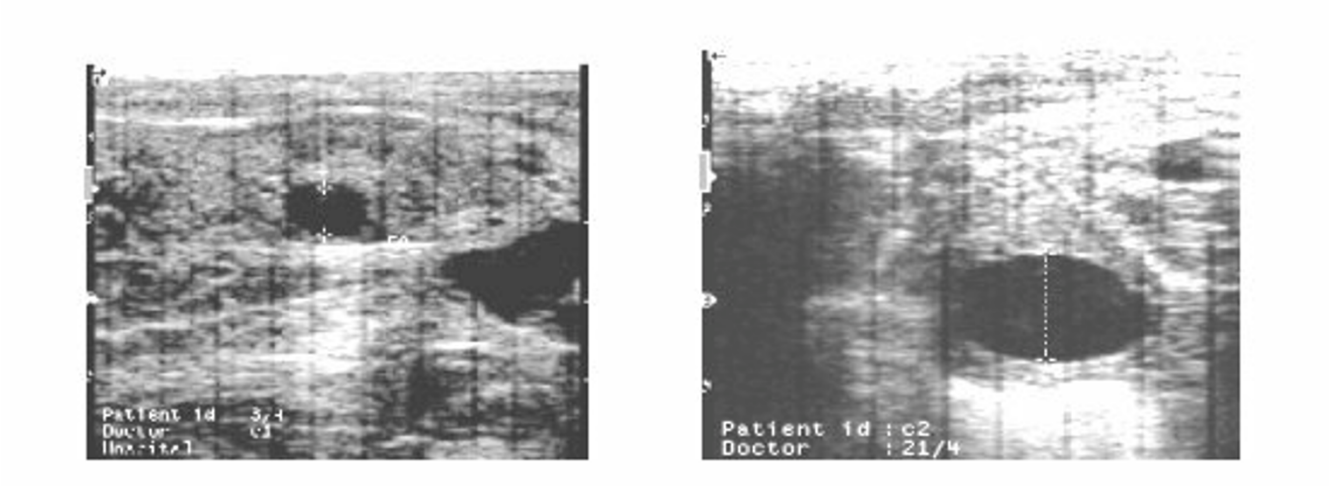

Figure 1
Ultrasonograms of ovarian follicles, showing a dominant anovulatory follicle (6 mm) in its static phase (on the left) and an ovulatory follicle (12 mm) a day before ovulation (on the right).

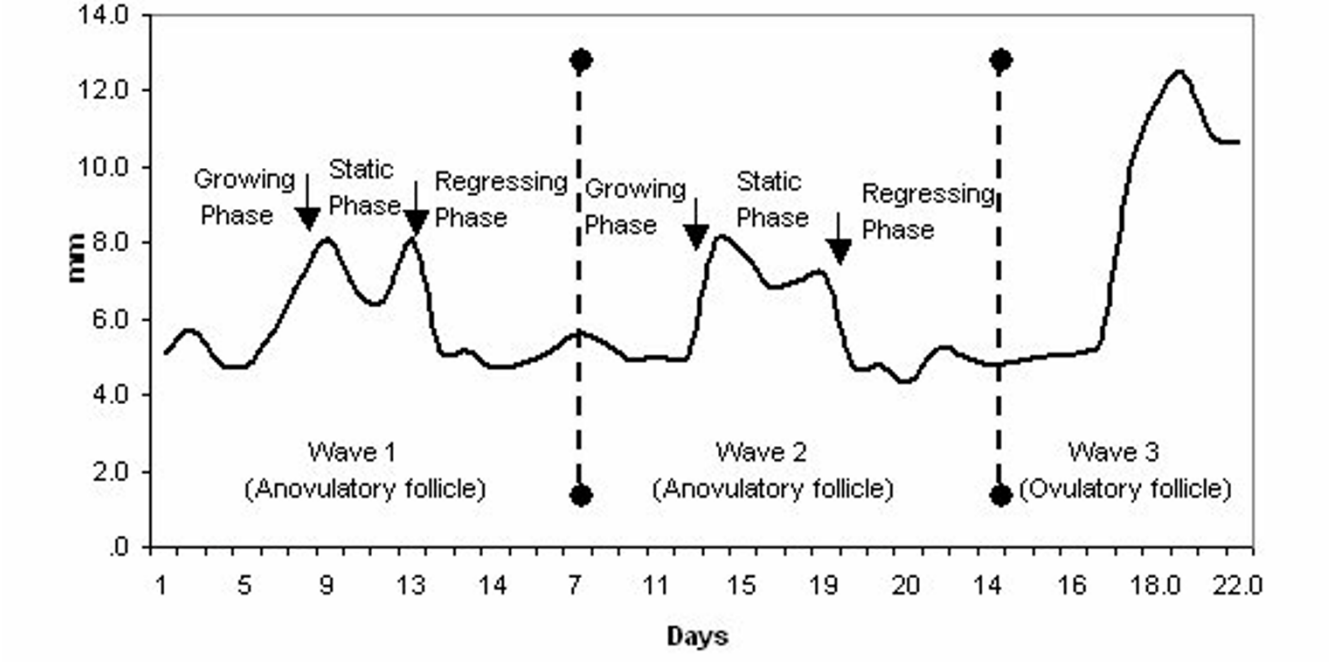

Figure 2
Profile of the dominant follicles during 3-wave interovulatory intervals.

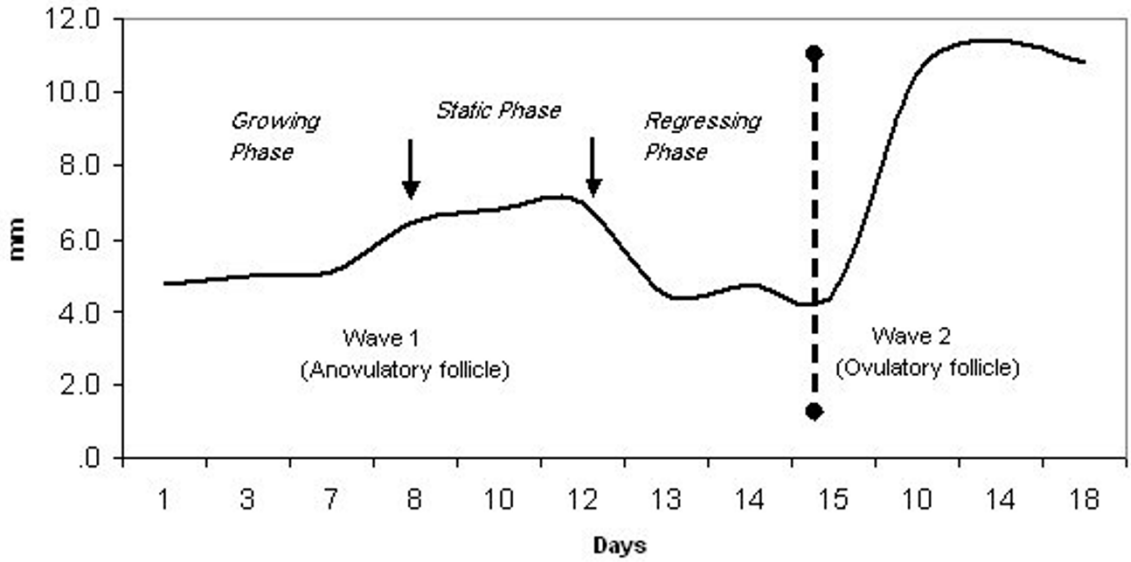

Figure 3
Profile of the dominant follicles during 2-wave interovulatory intervals.

Table 2: Phases of follicular development during 2-wave and 3-wave interovulatory intervals

	2-wave interovulatory interval		3-wave interovulatory interval	
	Maximum follicular diameter (mm)	Estradiol concentration (Range pg/ml)	Maximum follicular diameter (mm)	Estradiol concentration (Range pg/ml)
Anovulatory follicles				
Growth phase	6.8 ± 0.2	3.54 – 5.66	7.3 ± 0.2	3.93 – 5.68
Static phase	6.8 ± 0.1	5.66 – 4.14	7.3 ± 0.1	5.68 – 4.14
Regression phase	6.1 ± 0.2	4.14 – 5.70	6.3 ± 0.1	4.14 – 5.25
Ovulatory follicles				
Interval from detection to ovulation	10.9 ± 0.3	5.46 – 6.57	11.0 ± 0.9	5.35 – 6.94

Values are means ± SEM.

increase in estradiol concentration (Fig. 4), and decrease in progesterone concentration accompanied this increase in the diameter of the ovulatory follicle.

The development of dominant anovulatory follicles comprised three phases: growing, static, and regressing phases. A progressive increase in the size of dominant anovulatory follicle and estradiol concentration was observed (Table 2) during growth phase, and progressive decrease in estradiol concentration during static phase with more or less constant size of the follicles. While, during regressing phase there was a constant decrease in follicular size and increase in estradiol production. The 2-wave and 3-wave periods showed a positive correlation between follicular size and estradiol concentration and a negative correlation between the two variables and progesterone concentration (Table 3).

Profiles of follicular dynamics during 3-wave and 2-wave interovulatory intervals were represented in Figures 2 &3.

Table 3: Mean follicular diameter and hormonal concentrations within the waves of follicular development during 2-wave and 3-wave interovulatory intervals

	2-wave interovulatory intervals		3-wave interovulatory intervals		
	Wave 1	Wave 2	Wave 1	Wave 2	Wave 3
Length of days	8.7 ± 0.3	10.8 ± 0.3	7.2 ± 0.7	7.7 ± 0.4	9.5 ± 0.4
Maximum follicular diameter (mm)	6.65 ± 0.14 [a]	10.9 ± 0.90 [b]	7.0 ± 0.19 [b]	6.9 ± 0.18 [b]	11.0 ± 0.9 [a]
Estradiol concentration (pg/ml)	4.68 ± 0.18 [a]	5.95 ± 0.70 [b]	4.60 ± 0.13 [b]	4.81 ± 0.23 [b]	5.23 ± 0.29 [a]
Progesterone concentration (ng/ml)	1.17 ± 0.13 [a]	0.75 ± 0.27 [b]	1.21 ± 0.18 [b]	1.78 ± 0.15 [a]	1.03 ± 0.10 [b]

Values are means ± SEM. Means with different letters (a,b) in the same *row* and same *intervals* are significantly different (P < 0.05)(LSD).

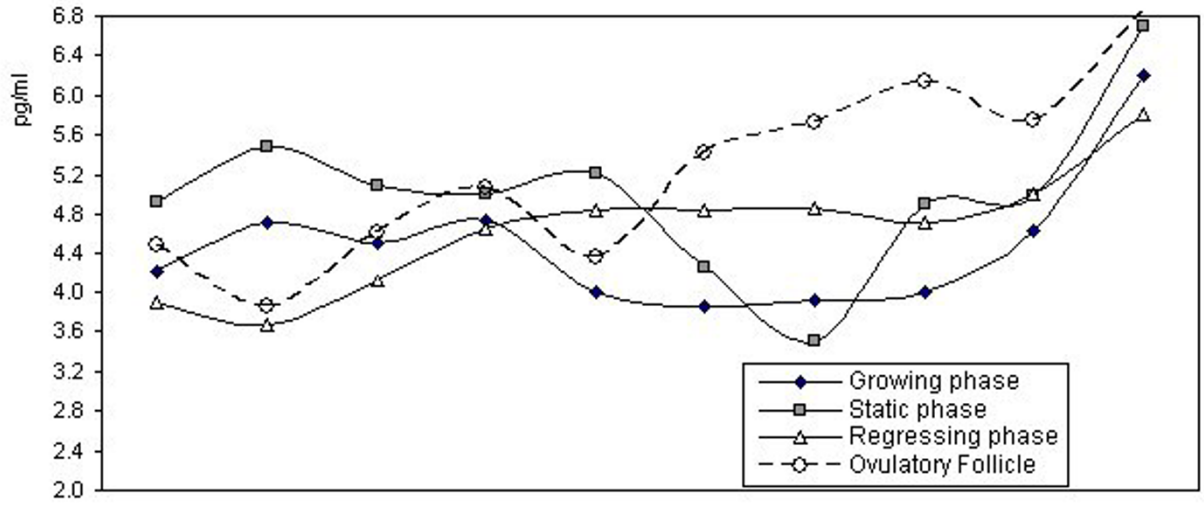

Figure 4
Profiles of estradiol concentration (pg/ml) during follicular development in 3-wave interovulatory intervals including growth, static, and regression phases of dominant anovulatory follicles (1st & 2nd wave) and growth of the ovulatory follicles (3rd wave).

Discussion

It has been postulated [7] that most estrous cycles in cows consist of 2 or 3 waves of follicular activity. In the present study, transrectal ultrasonographic monitoring of the ovarian follicular dynamics during estrous cycle of native cows has revealed predominance (71.4% of cows) of 3-wave follicular activity. Similar findings have been reported in two previous studies [3,8]. They demonstrated that only 20% of cows had two follicular waves during each estrous cycle, and the remainder had three waves. On the contrary, several studies shown the prevalence of 2-wave follicular activity during an estrous cycle in cattle [4,9,10]. This discrepancy, could be attributed to the high incidence of the 2 or 3 waves of follicular activity might be based on presence of 2 or 3 peaks of gonadotrophic hormones particularly FSH. The peaks of FSH were also related to lower estrogen concentration, which in turn depended on regression in follicular size. Genetic predisposition or uncontrolled environmental conditions may play an important role in regulation of incidence of the 2 or 3 follicular waves within one estrous cycle, through influences on follicular development and the level of their estrogen secretion. The mean length of the interovulatory intervals, in this study, was on average of 2.7 days longer in 3-wave than in 2-wave intervals. 2.4 days longer in length was reported [10] for 3-wave interovulatory intervals and this increase was attributed to a longer luteal phase. While, on another study [8] there was no significant difference in the mean length of estrous cycles between 2-wave and 3-wave patterns. In agreement with the results of this study, wave 2 of 3-wave estrous cycles in cows was detected earlier than in 2-wave cycles [11]. Also,

similar reported results [3] have shown intervals of 9 days between waves in 2-wave estrous cycles and approximately 7 days between waves in 3-wave cycles.

Within a wave of follicular development, the follicle that attains the largest diameter during its growth phase was defined as the morphologically dominant follicle (the largest follicle of the 1st and 2nd waves in cows with 3-wave cycles). While the morphologically and functionally dominant follicle (the largest follicle of the final wave, i.e. ovulatory follicle) appears to have 2 aspects: its ability to suppress the growth of other smaller subordinate follicles, and its ability to ovulate under appropriate hormonal conditions [12]. In the present study, the static phase of all dominant anovulatory follicles did not give way to the regressive phase until after emergence of the next dominant anovulatory or ovulatory follicle (depending on, whether it will have 2-wave or 3-wave interovulatory intervals). The dominant follicle exerts an inhibitory effect on its subordinate follicle and on the static dominant follicle of the preceding wave. In all studied waves, the dominant follicle was in the static phase when the next wave was first detected.

In this study, the increase in follicular size was associated with an increase in estradiol concentration and a decrease in progesterone concentration. It seems that ovulation occurred when the follicles attained their maximum diameter (10.9 mm in 2-wave and 11.0 mm in 3-wave periods), and when estradiol concentration attained its peak (5.95 ± 0.70 pg/ml in 2-wave periods and 5.23 ± 0.29 pg/ml in 3-wave periods), and meanwhile, when progesterone attained its minimum concentration. Hence, it might be concluded that the ovulatory follicle could produce estradiol in a concentration necessary for LH stimulation and induction of ovulation. This concentration of estradiol was only possible when the ovulatory follicle attained its maximum size. Therefore, when the follicle reaches its maximum size (> 10 mm) during a wave ovulation occurred and the cycle become 2-wave cycle and if does not attain this size another wave starts during which it attains this size (> 10 mm) and the cycle become 3-wave cycle. Along with follicular growth, estradiol concentrations associated with ovulation were > 5.0 pg/ml, while concentrations < 5.0 pg/ml were not enough to induce ovulation. A peak of serum concentration of estradiol was associated with the end of the growth phase of the dominant follicle [13]. Serum FSH concentration reached a peak values on days that approximated to follicle wave emergence [13]. It seems that the decrease in estradiol concentration, in this study, during the static phase of anovulatory follicles may have a role in initiating the growth of the dominant follicle of the subsequent wave. This effect of estradiol might be through its positive influence on FSH secretion from the pituitary. The ovulatory dominant follicle might suppress FSH secretion by increasing the concentration of estradiol [14].

Further studies could be conducted to test whether environmental factors have an effect on the development of 2 versus 3 follicular waves. Although, from the author point of view, nutrition did not play a significant role, as all studied animals received the same level of balanced ration.

Conclusions

In conclusion, there was a predominance of 3-wave follicular activity that was associated with an increase in length of interovulatory intervals compared with 2-wave follicular activity. Incidence of 2 or 3-wave cycles depends mainly on follicular size (>10 mm) and estradiol concentration (>5.0 pg/ml), When both are attained after emergence of the 2nd wave, the cycle will be 2-wave cycle, and when not attained, the cycle continues to be 3-wave cycle.

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