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Ovarian Follicular Dynamics during the Estrous Cycle in Heifers Monitored by Real-Time Ultrasonography[1]

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ABSTRACT

It is not clear whether the turnover of ovarian follicles during the estrous cycle in cattle is continuous and independent of the phase of the cycle, or whether waves of follicular growth occur at specific times of the cycle. To clarify this controversy, the pattern of growth and regression of ovarian follicles was characterized during a complete estrous cycle in ten heifers by daily ultrasonographic examinations. Follicles ≥5 mm were measured and their relative locations within the ovary were determined in order to follow the sequential development of each individual follicle. Results indicated the presence of either two (n = 2 heifers), three (n = 7), or four (n = 1) waves of follicular growth per cycle. Each wave was characterized by the development of one large (dominant) follicle and a variable number of smaller (non-dominant) follicles. In the most common pattern observed (three waves/cycle), the first, second, and third waves started on Days 1.9 ± 0.3, 9.4 ± 0.5, and 16.1 ± 0.7 ($\bar{X}$ ± SEM), respectively. The dominant follicle in the third wave was the ovulatory follicle. The maximal size and the growth rate of the dominant follicle in the second wave were significantly lower than in the other waves, but no significant difference was observed between the first and third waves. For the two heifers that had two follicular waves/cycle, the waves started on Days 2 and 11, whereas in the remaining heifer (four waves/cycle), the waves began on Days 2, 8, 14, and 17, respectively. At 0, 1, 2, 3, and 4 days before estrus, the ovulatory follicle was the largest follicle in the ovaries in 100%, 95%, 74%, 35%, and 25% of follicular phases monitored, respectively. The relative size of the preovulatory follicle at the completion of luteolysis (progesterone<1 ng/ml) was negatively correlated (r = −0.90; p<0.0001) with the interval of time between the end of luteolysis and the luteinizing hormone surge, suggesting that the length of proestrus is determined by the size of the preovulatory follicle at the beginning of proestrus. In conclusion, this study shows that the development of ovarian follicles ≥5 mm in heifers occurs in waves and that the most common pattern is three waves per estrous cycle.

INTRODUCTION

Previous studies have generated conflicting hypotheses about the pattern of growth and regression of ovarian follicles during the estrous cycle in cattle (for recent reviews, see Spicer and Echternkamp, 1986; Ireland and Roche, 1987; Fortune et al., 1988). Initially, Rajakoski (1960) suggested that two waves of follicular growth occur during the cycle, the first wave starting a few days after estrus (estrus = Day 1) and the second follicular wave beginning around Days 12–14. The hypothesis of two waves per cycle was subsequently supported by Swanson et al. (1972), Mariana and Nguyen Huy (1973), and Pierson and Ginther (1984; 1986; 1987a,b). On the other hand, a number of other investigators concluded that follicular development is continuous and independent of the stage of the cycle (Choudray et al., 1968; Donaldson and Hansel, 1968; Marion et al., 1968; Dufour et al., 1972). On the basis of experiments that identified the presence of one dominant "estrogen-active" follicle (ovulatory or nonovulatory) during three different periods of the estrous cycle (Ireland and Roche, 1983a,b), and also increases in estradiol concentrations in the jugular and/or utero-ovarian veins during approximately the same three periods (Ireland et al., 1985; Fogwell et al., 1985), Ireland and Roche (1987) have recently

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hypothesized that three, rather than two, waves of follicular growth occur during an estrous cycle in cattle.

It is not surprising that several hypotheses about the pattern of follicular development in cattle were generated from the results of earlier studies because, at the time they were performed, no reliable technique was available to follow the development and regression of individual follicles during a complete estrous cycle. With the advent of real-time B-mode ultrasonography, and especially since the initial development of transducers for intrarectal use in horses (Palmer and Driancourt, 1980), it is now possible to visualize various reproductive events in large animal species. Palmer (1987) used this technique to monitor ovarian follicular dynamics during the estrous cycle in mares. In cattle, Pierson and Ginther (1984; 1986; 1987a,b) measured and counted by ultrasonography follicles ≥2 mm during a normal estrous cycle and early pregnancy. In another study, Quirk et al. (1986) monitored the growth and ovulation or regression of individual follicles ≥5 mm during the late luteal and the follicular phase of the estrous cycle in heifers undergoing spontaneous or prostaglandin (PG)$F_{2\alpha}$-induced luteolysis.

The main objective of this study was to follow and characterize the development of individual follicles during an entire estrous cycle and, consequently, to determine whether the turnover of ovarian follicles in heifers is continuous and independent of the phase of the cycle, or whether waves of follicular growth occur at specific times of the cycle.

MATERIALS AND METHODS

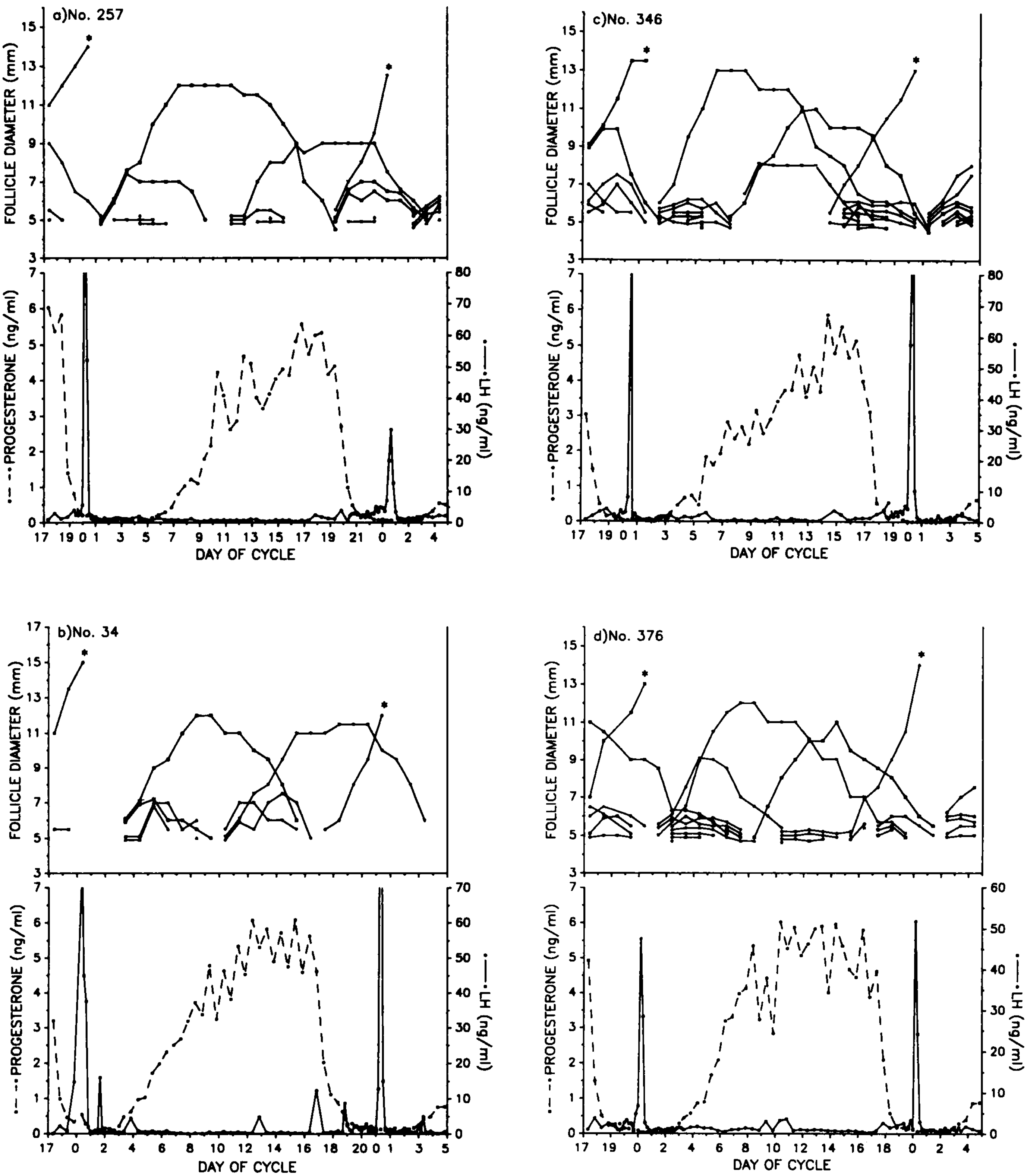
Animals

Ten Holstein heifers with normal estrous cycles, weighing 325–445 kg and between 2 and 3 yr old, were selected from our experimental herd. They had not received any luteolytic agent or other drugs during the 3 mo preceding this study. The experiment was carried out from February to July 1987. Animals were fed hay, corn silage, and mineral supplement, and were in good condition when the study was performed. The animals were kept in stanchions throughout the study except for an approximately 2-h period each day when they went outside to be checked for signs of estrus. A heifer was said to be in estrus (day of estrus = Day 0 of the estrous cycle) when she remained immobile while mounted by another female.

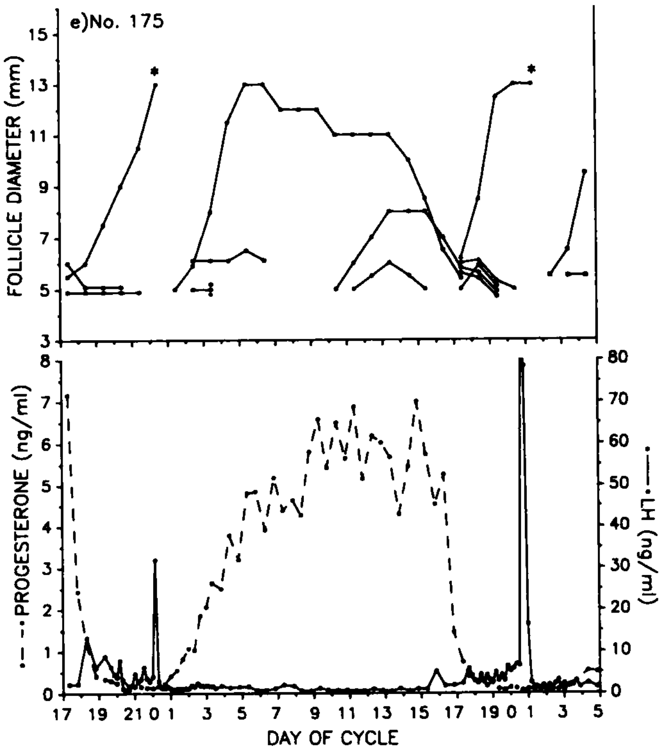
Ultrasonographic Examinations

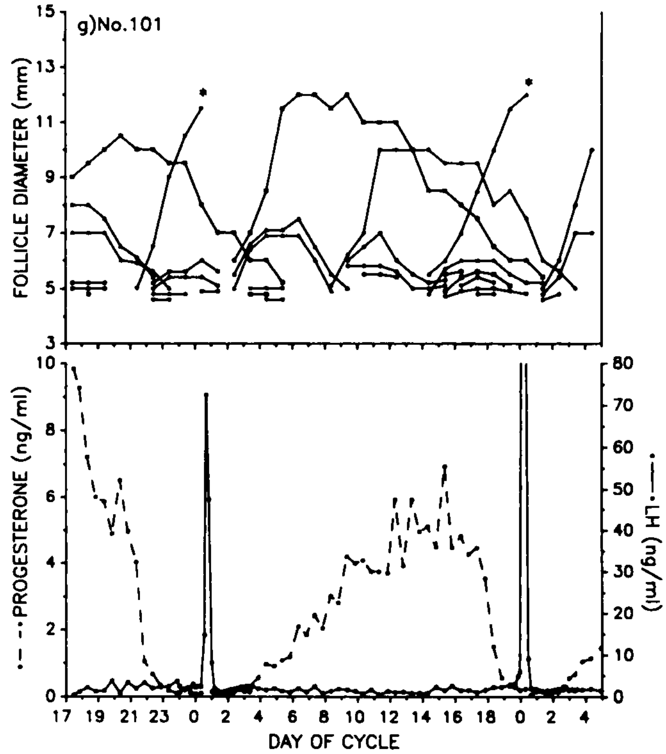
Ovarian follicular dynamics were monitored with a real-time B-mode linear array ultrasound scanner equipped with a 7.5 MHz intraractal probe (Technicare 210DX, Pitman-Moore, Washington Crossing, NJ). Ultrasonographic examinations were performed daily during one complete natural estrous cycle, starting on Day 17 of the previous cycle and ending on Day 4 of the subsequent cycle. No physical or chemical methods were used to restrain the animal during the procedure. Each examination was performed while the animal was in its stanchion. The reproductive tract was not palpated prior to ultrasonography. Our methods for examining the ovaries by ultrasonography and for following individual follicles from day to day have been described previously (Quirk et al., 1986). Briefly, the following routine was established for each examination: 1) feces were removed from the rectum; 2) the transducer was inserted into the rectum; 3) each ovary was scanned several times and in more than one plane, scanning first in a lateral to medial direction and then in a medial to lateral direction. Ovaries were scanned in a second direction: dorso-ventral, intermediate oblique or, in some cases, cranio-caudal. Each ultrasonography was recorded on videotape (VHS Video Cassette, Eastman Kodak Co., Rochester, NY). The tape was subsequently reviewed on the screen of the scanner and a diagram depicting the relative location of follicles ≥5 mm and the corpus luteum was made for each ovary. Each follicle ≥5 mm was located in relation to the lateromedial, dorso-ventral, and cranio-caudal aspect of the ovary, as well as in relation to other structures present within the ovary (other follicles or the corpus luteum). Follicles were measured with a ruler calibrated against the scale provided by the ultrasound unit. This procedure allowed us to follow the pattern of growth and regression of individual follicles ≥5 mm during the entire estrous cycle. Although follicles <5 mm could be detected, they were not included in the study since their individual development could not be accurately followed over time.

All ultrasonographic examinations and review of videotapes were performed by an operator who has used ultrasound scanners extensively since 1984 (Sirois et al., 1987a,b) and who, in the current study,



a)No. 257
c)No. 346
b)No. 34
d)No. 376
FOLLICLE DIAMETER (mm)
PROGESTERONE (ng/ml)
LH (ng/ml)
DAY OF CYCLE





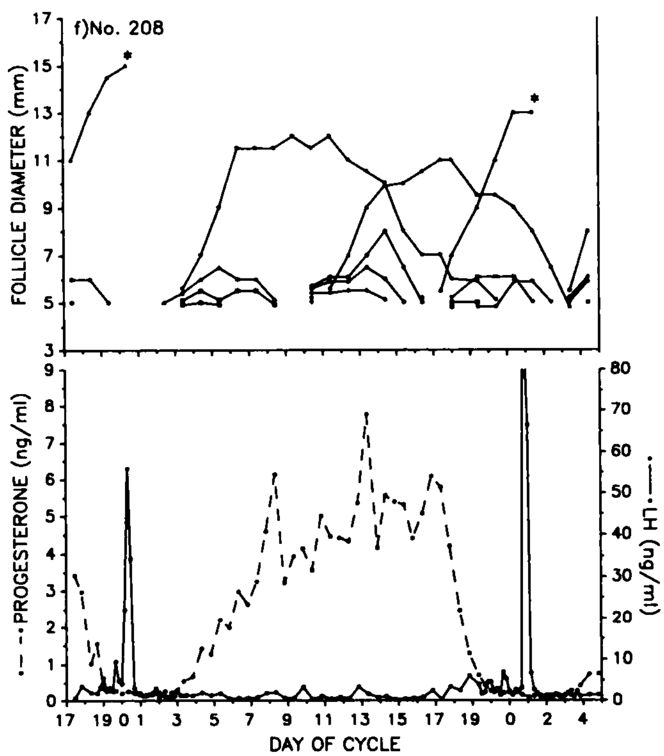


FIG. 1. Panels *a)* through *g)* show patterns of development and regression of individual follicles (*upper graph* in each *panel*, *solid lines* represent individual follicles on both ovaries) during a complete estrous cycle in seven heifers that had three follicular waves per cycle. The *asterisk* indicates the last day on which the ovulatory follicle was observed. The *lower graph* in each *panel* depicts progesterone and luteinizing hormone (LH) concentrations in plasma from the same heifer. Follicular diameter represents only the size of the antrum.

used the same scanner and the same methodology for ultrasonographic measurements of follicles that have been previously validated by our laboratory (Quirk et al., 1986). In our previous study we observed that diameters of dissected follicles were directly correlated (r = 0.98) with diameters observed by ultrasonography, but were consistently 2–3 mm larger than measurements determined from an ultrasonogram. This difference is to be expected, since the dissected follicles include the width of the granulosa, theca interna, theca externa, and a small amount of stroma (1–1.5 mm/side), whereas only the diameter of the antrum is measured by ultrasonography. Therefore, follicular diameters described in this paper represent only the size of the antrum.

Blood Sampling

Heifers were bled twice a day (approximately every 12 h) throughout the study. During a period starting with the regression of the corpus luteum (monitored by ultrasonography) and ending at noon on Day 3 of the subsequent cycle, a more intensive schedule of six samples a day (every 4 h) was conducted in order to detect changes in plasma luteinizing hormone (LH) around the time of estrus. Blood samples (10 ml) were collected from a jugular vein into evacuated tubes (Vacutainers; Becton-Dickinson, Rutherford, NJ) containing 200 µl of a saturated solution of sodium citrate as anticoagulant. The frequent samples (every 4 h) were taken by syringe through an indwelling catheter (Abbocath, 16-G × 14.0 cm, Abbott Labs, North Chicago, IL) in the jugular vein and then transferred into citrated tubes. The catheter was kept patent by flushing with a heparinized saline (200 IU/ml; Invenex, heparin sodium, Lyphomed, Inc., Rosemont, IL). Blood samples were immediately centrifuged at 5°C for 15 min, and the extracted plasma was stored at −20°C until measured for progesterone and LH by radioimmunoassay.

Radioimmunoassays (RIAs)

For progesterone determinations, 50 µl plasma aliquots were extracted with petroleum ether (recovery rate 77.4 ± 1.4%, X ± SEM) and then assayed according to a procedure previously described (Beal et al., 1980). The progesterone antiserum used cross-reacts 13.6% with 5β-dihydroprogesterone, <6% with other progestins tested, and <0.01% with the androgens and estrogens tested. The sensitivity of the assay is 12 pg/assay tube, and the intra- and interassay coefficients of variation were 8.6% and 8.5%, respectively.

For LH determinations, non-extracted plasma aliquots of 200 µl were assayed by specific RIA (Niswender et al., 1969; Quirk et al., 1986). Reagents included 1) anti-ovine LH antiserum (GDN No. 15), supplied by Dr. G. D. Niswender; 2) highly purified ovine LH (LER-1056-C2) for radioiodination, donated by L. E. Reichert, Jr.; 3) bovine LH (NIH-LH-B9) as standard; 4) goat anti-rabbit immunoglobulin (Miles Laboratories, Elkhart, IN) as second antibody. The sensitivity of the assay was 0.1 ng/assay tube. The intra- and interassay coefficients of variation were 10.7% and 11.7%, respectively.

Statistical Analysis

In this study, the persistence of a dominant follicle within the ovary was defined as the interval of time (days) elapsed between its appearance and disappearance as a follicle ≥5 mm. The growth rate (mm/day) of each dominant follicle was calculated by a linear regression (slope = growth rate) of the follicular diameters over the days of the cycle, including data from the first day the follicle was observed to the day on which the maximal size was reached. The relative diameter of the ovulatory follicle when progesterone first became <1 ng/ml was obtained by the ratio of its diameter at that time (at progesterone <1 ng/ml) to its maximal diameter at the last observation before ovulation. One-way analysis of variance was used for comparison between means; when a significant difference was found, a Duncan's multiple range test was used to determine which means were significantly different.

RESULTS

Follicular turnover was characterized by the presence of waves of follicular growth beginning at different times during the estrous cycle (Figs. 1–3, upper graphs). In terms of the number of waves per cycle, three different patterns of follicular development were observed in the ten heifers monitored. These patterns included either three (n = 7 heifers), two (n = 2), or four (n = 1) follicular waves per cycle. Each wave was characterized by the development of one large (dominant) follicle and a variable number of smaller (non-dominant) follicles.

In the most common pattern observed (3 waves/cycle), the first, second, and third waves started on Days 1.9 ± 0.3, 9.4 ± 0.5, and 16.1 ± 0.7, respectively (X ± SEM; Fig. 1; Table 1). The dominant follicle in

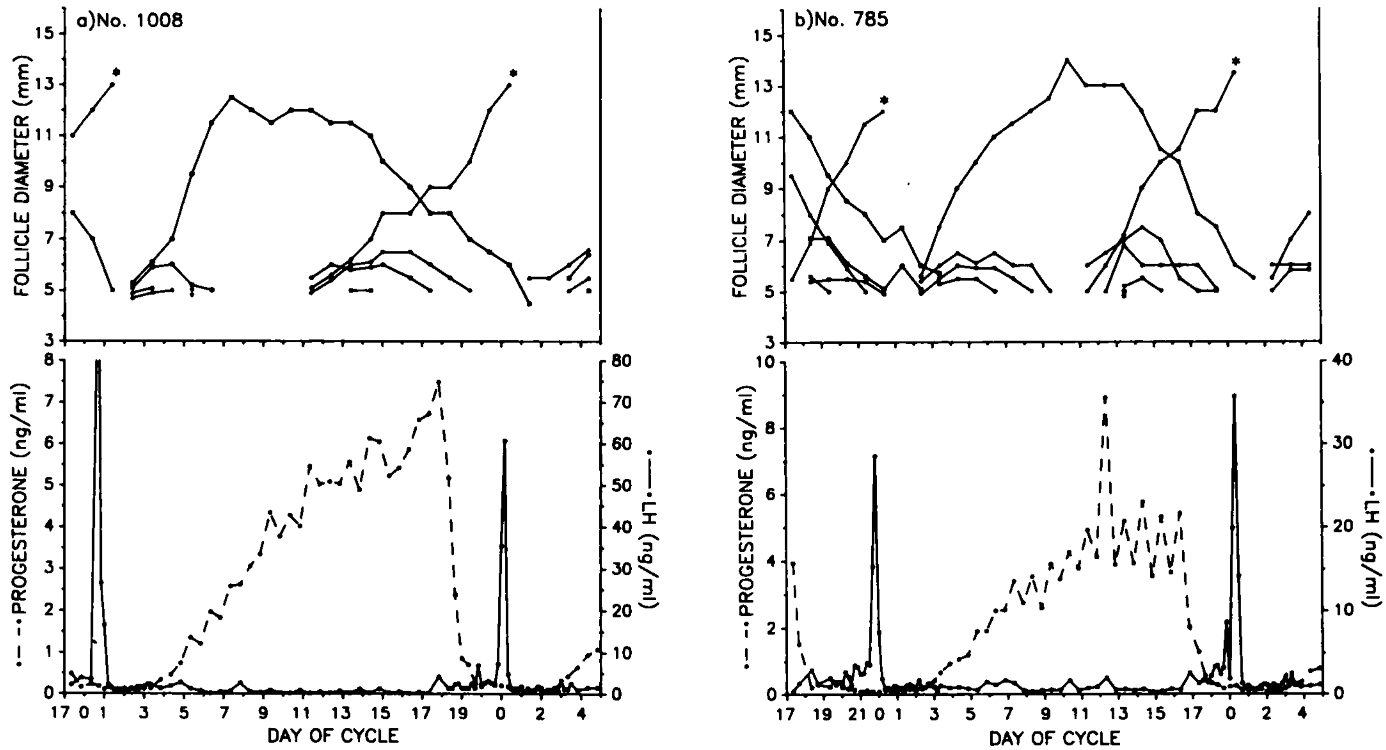


FIG. 2. Panels *a)* and *b)* show patterns of development and regression of individual follicles (*upper graph* in each *panel*, *solid lines* represent individual follicles on both ovaries) during a complete estrous cycle in two heifers that had two follicular waves per cycle, with corresponding plasma progesterone and luteinizing hormone (LH) concentrations (*lower graph*). The *asterisk* (*upper graph*) indicates the last day on which the ovulatory follicle was observed. Follicular diameter represents only the size of the antrum.

the third wave was the ovulatory follicle and appeared 5.9 ± 0.3 days before ovulation (cycle length: 20.7 ± 0.4 days). The maximal size reached by the dominant follicle in the first, second, and third waves was 12.3 ± 0.2, 10.2 ± 0.5, and 12.8 ± 0.3 mm, respectively. The dominant follicle in the second wave was significantly smaller ($p<0.01$) than dominant follicles in the two other waves. Also, the growth rate of the dominant follicle was slower during the second wave than during the first and third waves (1.1 ± 0.1 vs. 1.6 ± 0.2 and 1.7 ± 0.2 mm/day, $p<0.05$). The persistence of the dominant non-ovulatory follicles in the first two waves differed significantly (16.9 ± 0.9 vs. 13.1 ± 0.8 days). The mean number of follicles ≥5 mm per wave were, in order, 5.3 ± 0.7, 3.9 ± 0.7, and 4.7 ± 0.8, with no significant differences between the waves. On average, non-dominant follicles could be observed for 6 days during each of the three waves.

For the two heifers that exhibited two waves/cycle, the first and second waves started on Days 2 and 11 respectively (cycle length: 20 ± 1.0 days; Fig. 2). The dominant (largest) follicle in the second wave was the ovulatory follicle and was observed 10 ± 1.0 days before ovulation. The mean maximal diameter reached by the dominant follicles was 13.3 mm during both waves, and their growth rates were 1.3 ± 0.3 and 0.9 ± 0.1 mm/day for the dominant follicle in the first and second wave, respectively. A mean number of 4.0 and 5.0 follicles ≥ 5 mm was present during the first and second wave.

In the remaining heifer (4 waves/cycle), the waves began on Days 2, 8, 14 and 17, respectively (cycle length: 23 days; Fig. 3). The ovulatory follicle (fourth wave) appeared 7 days before ovulation and had a growth rate of 2.3 mm/day. The sizes reached by the dominant follicle in these four waves were, in chronological order, 13.5, 12, 9.5, and 17 mm.

The mean maximal diameter (± SEM) reached by the ovulatory follicles monitored was 13.4 ± 0.3 mm (n = 20; 10 ovulatory follicles in the complete cycle studied and 10 ovulatory follicles in the preceeding cycle). On the day of estrus, the ovulatory follicle was always the largest follicle present in either ovary (20/20 estrous periods monitored). However, on 1, 2,

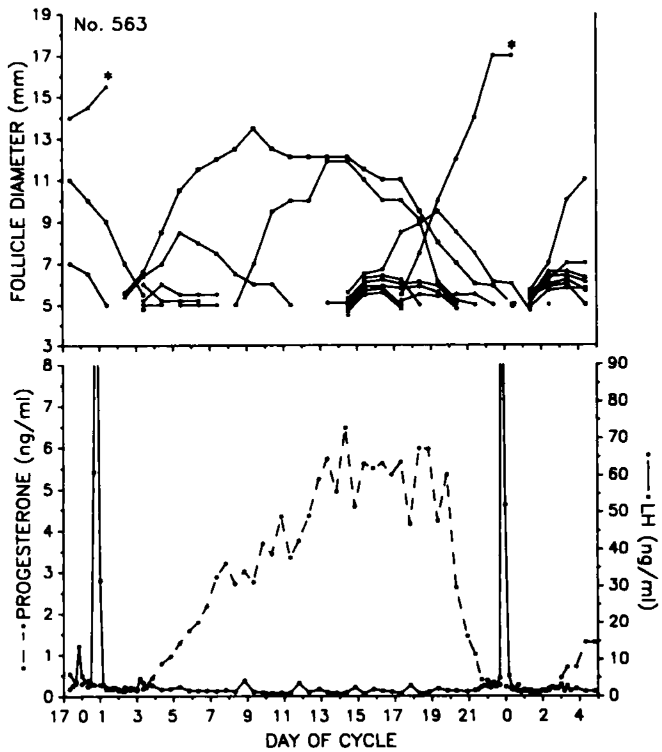


FIG. 3. Pattern of development and regression of individual follicles (*upper graph* in each *panel, solid lines* represent individual follicles on both ovaries) in one heifer that had four follicular waves per cycle. The *asterisk* indicates the last day on which the ovulatory follicle was observed. The *lower graph* depicts progesterone and luteinizing hormone (LH) concentrations in the plasma. Follicular diameter represents only the size of the antrum.

3, and 4 days before estrus, it was the largest follicle in only 19/20, 14/19, 6/17, and 3/12 cases, respectively. The gradual decrease, from 20 to 12, in the number of estrous periods monitored occurred because each experiment was initiated near the end of an estrous cycle (Day 17). Therefore, the ovulatory follicle of that cycle could be monitored only for a limited (and variable) length of time (see Figs 1–3). The sequential location (right vs. left ovary) of the dominant follicles did not follow any specific pattern (Table 2). However, even though the corpus luteum did not alternate from one ovary to the other during two consecutive cycles, it was not observed on the same ovary during three consecutive estrous cycles (Table 2). Overall, the dominant follicle was on the left ovary in 27/47 waves, and on the right ovary in 20/47 waves. There was no significant difference in the growth rate and the maximal size reached by the dominant follicle whether it was ipsilateral to the ovary bearing the corpus luteum or contralateral (growth rate = 1.3 ± 0.1 vs. 1.5 ± 0.1 mm/day, respectively; maximal size = 12.2 ± 0.5 vs. 12.7 ± 0.3 mm, respectively).

In all heifers, a preovulatory surge of LH was observed around the time of estrus (Figs. 1, 2, and 3, lower graphs). The relative diameter of the preovulatory follicle (diameter when progesterone first declined below 1 ng/ml: maximal diameter) was negatively correlated ($r = -0.90$; $p<0.0001$) with the interval of time between progesterone <1 ng/ml and the LH peak (Fig. 4).

DISCUSSION

To our knowledge, this is the first complete description of ovarian follicular dynamics during the bovine estrous cycle, based on ultrasonographic analyses of development and regression of individual follicles. This study indicates that it is feasible and

TABLE 1. Characteristics of follicular development during normal estrous cycles in seven heifers with three follicular waves per cycle.

	X̄ ± SEM (range)		
	Wave 1	Wave 2	Wave 3[a]
No. of follicles ≥5 mm/wave	5.3 ± 0.7[b] (3–8)	3.9 ± 0.7[b] (2–7)	4.7 ± 0.8[b] (1–8)
Dominant ovulatory and non-ovulatory follicles			
Maximum size (mm)[g]	12.3 ± 0.2[b] (12–13)	10.2 ± 0.5[c] (8–11.5)	12.8 ± 0.3[b] (12–14)
Growth rate (mm/day)	1.6 ± 0.2[d] (1.2–2.1)	1.1 ± 0.1[e] (0.9–1.6)	1.7 ± 0.2[d] (1.2–2.5)
Appearance on day (day of cycle)	1.9 ± 0.3 (1–3)	9.4 ± 0.5 (8–11)	16.1 ± 0.7 (14–19)
Maximum size reached on day	6.4 ± 0.4 (5–8)	14.2 ± 1.0 (11–18)	21.0 ± 0.4 (12–14)
Persistence of dominant follicles (days)	16.9 ± 0.9[b] (12–19)	13.1 ± 0.8[c] (9–15)	5.9 ± 0.3[f] (5–7)

[a]The dominant follicle of this wave ovulated; this explains the difference in its persistence as compared with Waves 1 and 2.

[b,c,f]Data with different superscripts within the same row differ significantly ($p<0.01$).

[d,e]Data with different superscripts within the same row differ significantly ($p<0.05$).

[g]Diameter of the antrum only.

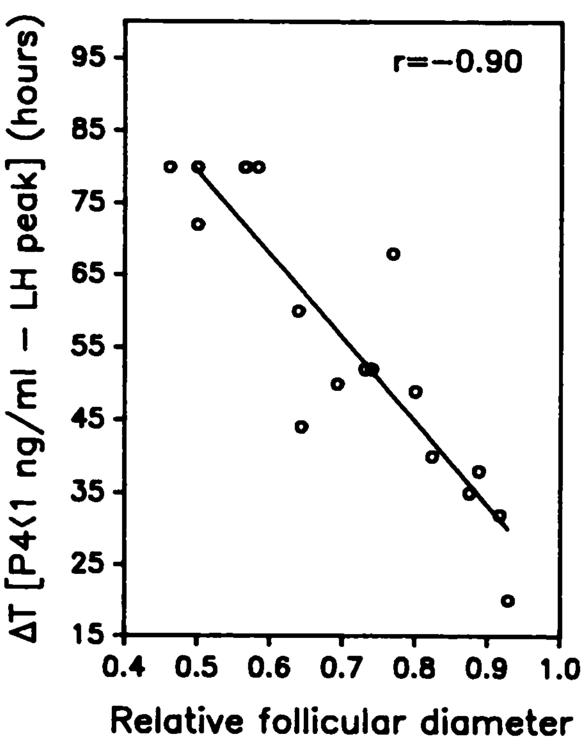


FIG. 4. Correlation between the relative diameter of preovulatory follicles at the completion of luteolysis (progesterone <1 ng/ml) and the interval of time between the first plasma progesterone <1 ng/ml and the luteinizing hormone (LH) peak (regression equation: $y = -1.15x + 137$ for $0.46<x<0.93$, $r = -0.90$). The relative diameter of the preovulatory follicle was obtained by the ratio of the follicular diameter when progesterone first became <1 ng/ml to its maximal diameter at the last observation before ovulation.

TABLE 2. Sequential location (L = left vs. R = right ovary) of the dominant follicle and the corpus luteum.

	Ovary bearing the dominant follicle (the corpus luteum) Follicular wave no.					
Heifer no.	−1[a]	1	2	3	4	+1[b]
785	L (R)	L (L)	R (L)	-	-	R (R)
1008	R (L)	R (R)	R (R)	-	-	L (R)
34	L (L)	R (L)	R (L)	R (L)	-	- (R)
346	L (R)	L (L)	L (L)	L (L)	-	L (L)
376	L (R)	L (L)	L (L)	R (L)	-	R (R)
257	L (L)	R (L)	L (L)	R (L)	-	- (R)
175	L (R)	L (L)	L (L)	R (L)	-	R (R)
208	R (L)	L (R)	L (R)	L (R)	-	L (L)
101	L (R)	L (L)	L (L)	L (L)	-	L (L)
563	L (L)	R (L)	R (L)	R (L)	R (L)	R (R)

[a]Last follicular wave of the preceding cycle.

[b]First follicular wave of the following cycle. In this wave, because the experiment ended on Day 4, the dominant follicle was designated the largest follicle on that day (Day 4), except with Heifers 34 and 257 where dominance could not be determined on Day 4.

practical to trace by ultrasonography the development of individual follicles ≥5 mm throughout the estrous cycle in heifers. Repeated examinations proved to be a safe procedure since no rectal injury was induced during the approximately three hundred examinations performed. Also, since normal estrous cycle length, normal progesterone levels, and typical preovulatory LH surges were observed, there is no evidence that repeated ultrasonographies modified the course of a normal estrous cycle.

Our results show that the growth of ovarian follicles ≥5 mm in heifers occurs in waves. They strongly support the model hypothesized by Ireland and Roche (1987) regarding the development of dominant follicles during the bovine estrous cycle. This model is similar to the model hypothesized for the primate menstrual cycle, which holds that during the follicular phase, the dominant follicle goes through three consecutive phases: the selection, the dominance, and the ovulation phase (diZerega et al., 1980; Hodgen et al., 1985). Patterns of follicular development depicted in Figures 1 through 3 indicate that the bovine pattern varies from that observed in primates in that waves of follicular development occur throughout the cycle. During each wave in heifers, one follicle is selected, becomes larger and "dominates" the other follicles, possibly by inhibiting their growth. After the dominance phase, this follicle will either ovulate (dominant ovulatory follicle) or will become atretic (dominant non-ovulatory follicle), depending on whether the phase of dominance is associated with luteolysis or not.

In the most common pattern observed (three waves/cycle, 7/10 heifers), waves occurred at an average interval of 7 days, the first, second, and third waves starting, on average, on Day 2, 9, and 16 respectively (Fig. 1; Table 1). Our results do not support the hypothesis of Rajakoski (1960), who proposed that only two waves of follicular growth occur during the bovine estrous cycle, one beginning between Days 3 and 4 of the cycle and the second starting between Days 12 and 14 and producing the ovulatory follicle (estrus = Day 1 in their study). Support for the two-wave model was recently inferred by Pierson and Ginther (1984, 1986, 1987a,b), who measured and counted follicles ≥2 mm by ultrasonography during a complete estrous cycle. However, it should be emphasized that their methodology and endpoints were different from ours, since they measured and counted follicles each day of the cycle without following individual follicles from day to day. On the other hand, we were interested in following individual follicles over time, and the videotaped record of each ultrasound exam ensured accuracy in tracking individual follicles ≥5 mm.

Our results also do not support the concept that the turnover of ovarian follicles ≥5 mm is continous and independent of the phase of the cycle (Choudray et al., 1968; Donaldson and Hansel, 1968; Marion et al., 1968; Dufour et al., 1972). They also do not support the model recently proposed by Spicer and Echternkamp (1986), which incorporates the hypothesis of Matton et al. (1981) that growth and replacement of large follicles is more rapid at the end of the estrous cycle. In heifers with three follicular waves per cycle, the growth rate of the dominant non-ovulatory follicle in the first wave was not different from the growth rate of the dominant ovulatory follicle in the third wave (Table 1). The slower growth rate and smaller maximum size reached by the dominant non-ovulatory follicle of the second wave might be explained by a negative influence of constant high level of progesterone during its entire development. Our results are consistent with studies by Staigmiller and England (1982) and Fortin and Seguin (1984), who suggested that a minimum interval of 4–5 days is required by a growing follicle of approximately 5 mm to reach an ovulatory size. The mean interval between the appearance of the ovulatory follicle and the time of ovulation was 5.9 and 10 days for heifers with three and two follicular waves per cycle, respectively. Contrary to the previous report of Matton et al. (1981), but consistent with that of Pierson and Ginther (1987b), the size of the dominant follicle was not different when its location was ipsilateral or contralateral to the corpus luteum (12.2 vs. 12.7 mm, respectively).

Three of the ten heifers monitored exhibited different patterns of follicular development, two had two waves, whereas one heifer had four waves of follicular growth (Figs. 2 and 3, respectively). So far, it is not possible to identify the factors that generate this variation among animals, but the presence of more than one pattern might explain differences in the results among studies. However, in a recent preliminary report of a study in which ovarian follicular development was monitored by daily ultrasonography in thirteen heifers, Savio et al. (1987) observed the growth of three dominant follicles during 21/26 estrous cycles studied. These results, like ours, suggest that the most common pattern of follicular development in heifers is three waves per estrous cycle. In the present study, cycles with two waves were characterized by a second wave that started about 2 days later than the average for animals with three waves, and also by early regression of the corpus luteum or slow growth rate of the dominant ovulatory follicle (Fig. 2). The number of animals is too small for definitive conclusions, but perhaps cycles with two waves occur when luteal regression is relatively early and the dominant follicle in the second wave is still healthy at that time.

The results confirmed our previous report that the preovulatory follicle is not the largest follicle until the day of estrus (Quirk et al., 1986) since in the 20 peri-ovulatory periods monitored, the ovulatory follicle was consistently the largest follicle in the ovaries only on the day of estrus. Many of the ovulatory follicles were also the largest follicles one and two days before estrus (19/20 and 14/19, respectively), whereas before that time the proportion was lower (6/17 and 3/12 at 3 and 4 days before estrus, respectively).

The results presented in Figure 4 clearly suggest that the length of proestrus is influenced by the size of the preovulatory follicle at the beginning of proestrus. Heifers with a small preovulatory follicle at the beginning of proestrus had a longer interval [progesterone <1 ng/ml − LH peak] than heifers with a large preovulatory follicle ($r = -0.90$, Fig. 4). This finding supports the suggestion of Scaramuzzi et al. (1980) and Macmillan and Henderson (1984) that the size or maturity of the largest non-atretic follicle present at the time of a $PGF_{2\alpha}$-induced luteolysis could influence the interval from the $PGF_{2\alpha}$ injection to estrus.

In conclusion, our results suggest that in heifers the development of follicles ≥5 mm occurs in waves, with the most common pattern being three waves per cycle, and that during each wave, a single follicle becomes dominant whereas other follicles in the same wave regress. These results also show that tracing the development and regression of individual follicles by ultrasonography is a powerful tool for studying ovarian follicular dynamics in cattle.

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