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Follicle Selection in Cattle: Follicle Deviation and Codominance Within Sequential Waves[1]

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ABSTRACT

Follicle deviation during bovine follicular waves is characterized by continued growth of a developing dominant follicle and reduction or cessation of growth of subordinate follicles. Characteristics of follicle deviation for waves with a single dominant follicle were compared between wave 1 (begins near ovulation; n = 15) and wave 2 (n = 15). Follicles were defined as F1 (largest), F2, and F3, according to maximum diameter. No mean differences were found between waves for follicle diameters at expected deviation (F1, ≥8.5 mm; Hour 0) or observed deviation or in the interval from follicle emergence at 4.0 mm to deviation. For both waves, circulating FSH continued to decrease ($P < 0.05$) after Hour 0, estradiol began to increase ($P < 0.05$) at Hour 0, and immunoreactive inhibin began to decrease ($P < 0.05$) before Hour 0. A transient elevation in circulating LH reached maximum concentration at Hour 0 ($P < 0.01$) in both waves and was more prominent ($P < 0.0001$) for wave 1. Waves with codominant follicles (both follicles >10 mm) were more common ($P < 0.02$) for wave 1 (35%) than for wave 2 (4%). Codominants (n = 6) were associated with more ($P < 0.05$) follicles ≥4 mm and a greater concentration ($P < 0.04$) of circulating estradiol at Hours −48 to −8 than were single dominant follicles (n = 15). A mean transient increase in FSH and LH occurred in the codominant group at Hour −24 and may have interfered with deviation of F2. In codominant waves, deviation of F3 occurred near Hour 0 (F1, approximately 8.5 mm). A second deviation involving F2 occurred in four of six waves a mean of 50 h after the F3 deviation and may have resulted from a greater suppression ($P < 0.05$) of FSH in the codominant group after Hour 0. In conclusion, follicle or hormone differences were similar for waves 1 and 2, indicating that the deviation mechanisms were the same for both waves. Waves that developed codominant follicles differed in hormone as well as follicle dynamics.

estradiol, follicle, follicle-stimulating hormone, luteinizing hormone, ovary

INTRODUCTION

The two or three waves of follicular activity that develop during the bovine estrous cycle emerge (follicles first detected at 4 mm) approximately 0, 9, and 16 days after ovulation and are termed waves 1, 2, and 3, respectively [1]. The follicles of a wave undergo a common-growth phase for 2 or 3 days after emergence at 4.0 mm [2]. At the end of the common-growth phase, deviation begins and becomes established in <8 h [3]. Deviation is characterized by continued growth of the largest follicle to become the dominant follicle and a reduction or cessation of growth of the smaller follicles to become subordinate follicles [4]. Deviation has been proposed to be the eminent event in follicle selection in monovular species [5]. Mean diameter of the largest follicle at the beginning of deviation was 8.5 mm [4, 6].

Each follicular wave is stimulated by a surge of FSH that reaches a peak when the emerging follicles are about 4 mm [7]. The FSH concentrations decline during the common-growth phase. Several recent studies using blood sampling every 1–4 h have found that the nadir is reached 10–24 h after the beginning of deviation [3, 8–10]. According to an FSH/follicle coupling hypothesis, the growing follicles cause the FSH decline from the peak of the wave-stimulating FSH surge until deviation, even though the follicles continue to require FSH [9, 10]. At the beginning of deviation, only the more developed largest follicle is able to utilize the low FSH concentrations and becomes the only follicle involved in FSH/follicle coupling. The smaller follicles have not reached a similar developmental stage at the beginning of deviation, but because of their continued and close temporal dependency on FSH, they eventually become susceptible to the low concentrations. On average, a small transient elevation in LH begins before deviation and decreases after deviation [10, 11]. Gene expression for LH receptors increases in the granulosa cells of the future dominant follicle about 8 h before the beginning of deviation [12]. The LH stimulates the production of estradiol and insulin-like growth factor 1 (IGF-1) [11], based on a decrease in these factors in follicular fluid when LH is experimentally reduced. These intrafollicular factors and perhaps others may account for the responsiveness of the largest follicle to the low concentrations of FSH.

Periodic follicular waves occur not only during the estrous cycle but also during pregnancy [13, 14], the postpartum period [14], the prepubertal period [15, 16], and prolonged progesterone administration [17]. The occurrence of waves under many diverse hormonal conditions attests to the robustness of the mechanisms that underlie follicle deviation and is a consideration in the development of hypotheses concerning the controlling mechanisms. However, most of the research on follicular waves has been limited to wave 1 of the estrous cycle, with only limited comparisons to wave 2. In this regard, the maximum diameter of the dominant follicle of wave 1 and the interval between emergence of waves 1 and 2 were similar for two-wave and three-wave interovulatory intervals [1, 18]. In three-wave intervals, the dominant follicle of wave 1 reached a larger diameter than for wave 2 [1, 19], and this larger size was attributed to lower progesterone concentrations during wave 1 [20], as demonstrated by administration of progesterone [21]. Except for the well-demonstrated occurrence of an FSH surge in association with the emergence

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of all waves [7, 20], the hormone differences among sequential waves have received limited study. An increase in estradiol concentrations was detected during wave 1 but not during wave 2 when waves were normalized to wave emergence [20]. No reports are available on the follicle or hormone differences associated with sequential waves when each wave is normalized to follicle deviation.

The occurrence of multiple (usually two) dominant follicles per wave may represent a change in the hormone interactions of deviation that occur in association with a single dominant follicle per wave. However, the incidence of codominant follicles in heifers is not well understood, except as indicated by an approximately 4% double-ovulation rate [22]. Codominant follicles should be useful for investigating deviation [22] because codominance represents the absence of deviation in monovular species.

The purpose of the present study was to compare the follicle and hormone characteristics of follicle deviation between waves 1 and 2. A characteristic that was not common to both waves was taken as an indication that the characteristic was not an essential component of the deviation mechanism. In addition, the follicle and hormone profiles of codominant follicles and single dominant follicles were compared.

MATERIALS AND METHODS

Heifers, Ultrasound Data, and Blood Sampling

Twenty-three nulliparous Holstein heifers ranging from 1.5 to 3.5 yr of age and weighing 450–680 kg were used for the study from June to November. The feeding program and the equipment and techniques for transrectal ultrasound scanning of the ovaries and measuring the follicles have been described [9]. Two i.m. injections of 25 mg of $PGF_{2\alpha}$ (Lutalyse; Pharmacia, Kalamazoo, MI) were given when a mature corpus luteum was present (5–17 days after ovulation). Ultrasound scanning and blood sampling were done every 24 h beginning on the day of $PGF_{2\alpha}$ treatment and continuing throughout an interovulatory interval. In addition, scanning and blood sampling were done every 8 h beginning when the largest follicle of a new wave reached or exceeded 7.0 mm and continuing until 72 h (scanning) or 40 h (sampling) after the largest follicle was 8.5 mm. The scanning and sampling at 8-h intervals were done for each wave of the interovulatory interval. The four largest follicles were tracked from examination to examination as described [1]. Three follicles were defined retrospectively, according to the maximum attained diameter as F1 (largest), F2, and F3.

For follicle and FSH data obtained at 24-h intervals throughout the interovulatory interval, the waves for all 23 heifers were evaluated by including waves with codominant follicles (defined as two follicles >10 mm). Data were plotted for F1, F2, and F3 for each of waves 1 and 2 of two-wave intervals and waves 1, 2, and 3 of three-wave intervals. For codominant follicles, the largest dominant follicle was used to represent F1, and the largest subordinate follicle was used to represent F3; F2 was not used in the analyses of data obtained at 24-h intervals. The FSH values were matched with the follicle data and normalized to the mean day of expected deviation for each wave type; deviation was normalized to the mean day of occurrence after ovulation. Data were displayed separately for two-wave and three-wave intervals.

Waves with one dominant follicle were used (n = 15 pairs of waves) for follicle, FSH, LH, estradiol, and immunoreactive inhibin (ir-inhibin) data obtained at 8-h intervals during both wave 1 and wave 2. Expected beginning of deviation in individual waves was taken as the examination when the largest follicle first reached or exceeded 8.5 mm based on previous reports [4, 6]. Observed deviation was estimated for individual waves from the profiles of diameter data of the two largest follicles and was defined as beginning at the examination preceding an increase in the difference in diameter between the two follicles [4]. The beginning of either expected or observed deviation was designated Hour 0. Data were available at 8-h intervals beginning with Hour −8. Hours −48 and −24 were included and were represented by using the closest values (Hours −56 to −40 and Hours −32 to −16). Follicle and hormone data were normalized to Hour 0 for the comparisons between waves 1 and 2.

The relationships of follicle and hormone data in the development of codominant follicles were studied for wave 1 by using the 15 waves with a single dominant follicle and 6 waves with codominant follicles. Two of eight waves with codominant follicles were excluded because the interval required for both future dominant follicles to reach 8.5 mm was prolonged (32 and 40 h), resulting in differences in diameter between F1 and F2 of 3.2 and 3.4 mm at expected deviation. The comparisons were made for only wave 1 because 80% of codominant follicles occurred during wave 1. After study of the estradiol results, it became apparent that codominant follicles were associated with high estradiol concentrations before deviation. Therefore, the hypothesis was developed (before inspecting follicle data) that the elevated estradiol at this time is attributable to higher numbers of ≥4-mm follicles in the waves with codominant follicles. For this purpose, data were evaluated for Hours −48 and −24; number of ≥4-mm follicles was not recorded after Hour −24.

Assays

Blood was collected by jugular venipuncture into heparinized tubes immediately before each ultrasound examination. Blood was centrifuged daily, and plasma was aspirated and then stored frozen (−20°C) until time of assay. Plasma concentrations of FSH and LH were determined using validated RIA for cattle [23, 24]. Details on the modifications and validations for use in this laboratory for FSH [7] and LH [9] have been reported previously. Mean assay sensitivity was 0.35 ng/ml for FSH (n = 2 assays) and 0.40 ng/ml for LH (n = 2 assays). Within- and between-assay coefficients of variation (CVs) were 12.6% and 16.4% for FSH and 9.5% and 11.5% for LH, respectively.

Estradiol was measured in plasma samples using a commercially available RIA kit (Ultra Sensitive Estradiol assay, DSL-4800; Diagnostic Systems Laboratory, Webster, TX) that has been validated for analysis of bovine samples [25]. Assay details as used in this laboratory have been previously described [26]. Mean assay sensitivity was 0.54 pg/ml (n = 2 assays). The mean within- and between-assay CVs were 9.8% and 7.0%, respectively.

Plasma ir-inhibin was assayed with an RIA kit (Institute of Reproduction and Development, Monash Medical Center, Clayton, Victoria, Australia). As previously described [27], the assay reagents cross-react with the full-length forms of the α-subunit and the various intact forms of inhibin. The assay has been validated for use in this laboratory, and details have been reported elsewhere [12]. Mean assay sensitivity (n = 1) was 5.2 ng/ml. The mean within- and between-assay CVs were 9.3% and 20.0%, respectively.

Statistical Analyses

The hormone but not the follicle data lacked normality; therefore, the FSH, LH, estradiol, and ir-inhibin data were transformed logarithmically. The sequential nature of the follicle and hormone data was examined using the SAS MIXED procedure with a repeated statement and a first order autoregressive structure to account for the autocorrelation between measurements [28]. Main effects of wave or group and hour and the respective interactions were determined. When a main effect or interaction was significant or approached significance, paired *t*-tests were used to determine differences between waves within an hour and within a wave between hours. Duncan multiple range tests were used for comparisons of more than two means. Paired and unpaired *t*-tests were used to examine the single-point measurements (e.g., maximum follicle diameters). The observed data (but not the transformed data) are presented as the mean ± SEM. Frequency data were examined with a Fischer exact test. A probability of <0.05 was defined as significant. Probabilities of 0.06–0.1 were considered as approaching significance.

RESULTS

Thirteen of 23 (56%) interovulatory intervals had two follicular waves, and 10 had three waves. The incidence of codominant follicles was different ($P < 0.02$) among waves (wave 1, 35%; wave 2, 4%; wave 3, 10%). Totaled over waves 1 and 2 of two-wave intervals and waves 1, 2, and 3 of three-wave intervals, the future dominant follicle at ≥4.0 mm and Hour −48 was the largest of the three follicles in 76% of the waves. The mean diameters of F1, F2, and F3 combined for single dominant and codominant waves and concentrations of FSH normalized to the day F1 was closest to 8.5 mm (expected deviation) are shown for

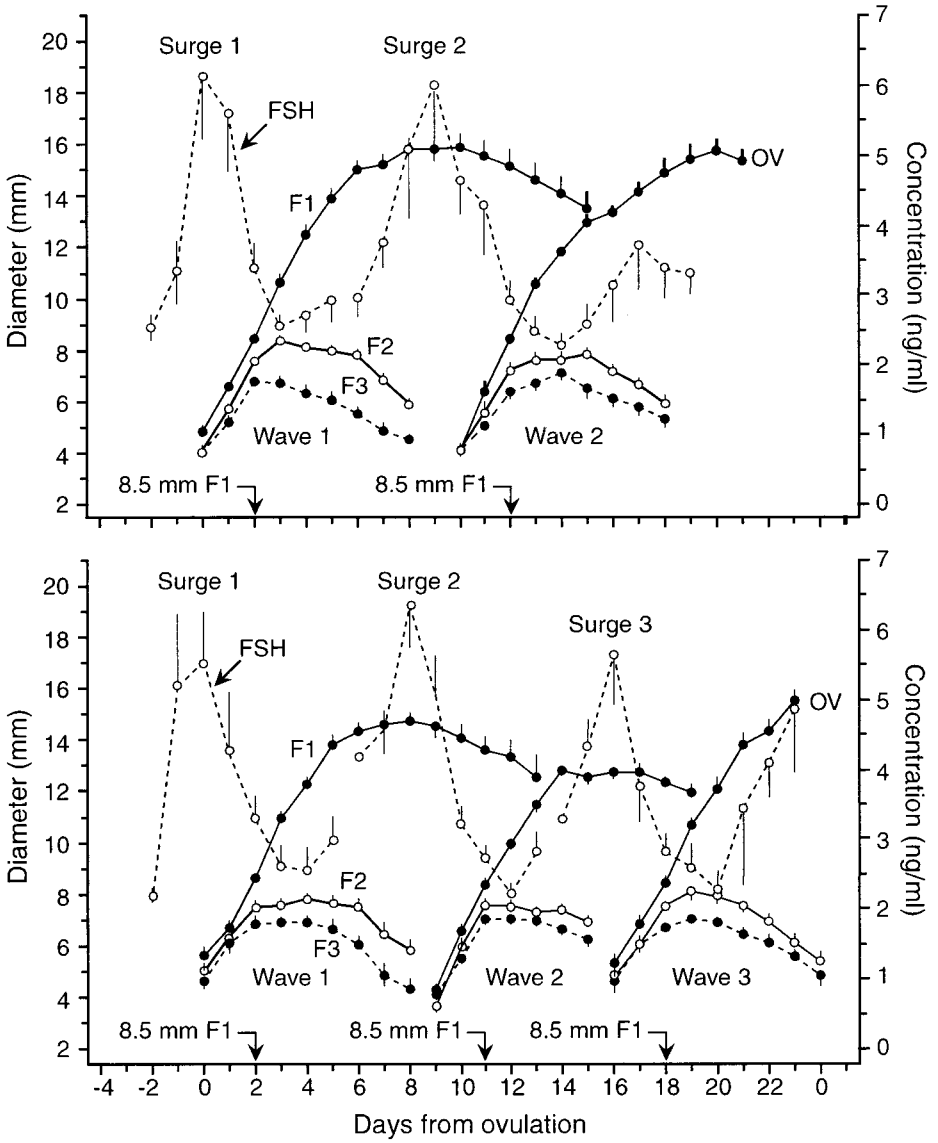

FIG. 1. Mean (±SEM) diameters of follicles and concentrations of circulating FSH for two-wave (n = 13) and three-wave (n = 10) interovulatory intervals. Follicles are identified by maximum attained diameter as F1 (largest), F2, and F3. Waves with codominant follicles (>10 mm) were included by using the largest follicle as F1 and the largest subordinate follicle as F3 and omitting F2. The FSH values were matched with follicle values and normalized to the mean day of expected deviation (F1, 8.5 mm) for each wave; the mean day was positioned on the interovulatory day scale. OV, Ovulation.

each of the five waves (Fig. 1). For all waves, concentration of FSH decreased (*P*s of <0.03 to <0.002) after expected deviation, except the decrease approached significance ($P < 0.07$) in wave 1 of three-wave intervals.

For the 15 heifers with only a single dominant follicle in each wave, wave 2 did not differ between two-wave (n = 8) and three-wave (n = 7) interovulatory intervals for any of the follicle profiles or follicle characteristics; thus, data were combined. Discrete data on the characteristics of deviation in wave 1 versus wave 2 and the results of the statistical analyses are shown in Table 1. There were no differences between waves in the frequency with which the dominant follicle developed from the follicle that first reached 7.0 or 8.5 mm (88%–100%).

Mean diameters of F1, F2, and F3 for Hours −48 to 72 and the statistical probabilities are shown in Figure 2 for the following three groups: 1) single dominant follicle, wave 1, n =15; 2) single dominant follicle, wave 2, n =15; and 3) codominant follicles, wave 1, n =6. The main effects of group and hour and the interaction were significant for F1 and F2, whereas only the hour effect was significant for F3. The mean diameter of F1 in the single-dominant wave 1 group was greater ($P < 0.02$) than that for the other two groups on each of Hours 32–72. Diameter of F2 was greater ($P < 0.01$) for the codominant group than for either of the single dominant groups for Hours 16–72. The maximum diameter of F1 during Hours 0–72 of wave 1 was less ($P < 0.02$) for the codominant group (13.2 ± 0.5 mm) than for the single dominant group (14.4 ± 0.3 mm). For wave 1, there were more follicles ≥4 mm in the codominant group than in the single dominant group at Hour −48 (7.7 ± 2.2 follicles versus 4.4 ± 0.9 follicles; $P < 0.06$) and Hour −24 (10.2 ± 1.0 follicles versus 7.8 ± 0.6 follicles; $P < 0.03$). In waves with codominant follicles, F3 deviated near the expected time (F1, approximately 8.5 mm), whereas F2 continued to grow to >10 mm. However, in four of six waves, F2 displayed an apparent deviation when F2 was >10 mm at 16, 56, 64, and 64 h, respectively, after the deviation that involved F3 (Fig. 3), i.e., F2 did not maintain dominant status. In the remaining two waves, F1 and F2 grew to similar maximum diameters (<2.0 mm difference between F1 and F2).

For the comparisons of the single dominant groups between wave 1 and wave 2, the concentrations of FSH showed an effect of hour, and the interaction approached significance (Fig. 4). Concentrations for wave 2 were higher ($P < 0.02$) at Hour −48 and lower ($P < 0.02$) at Hour 40. The concentrations decreased ($P < 0.05$) after Hour 0

TABLE 1. Mean (±SEM) for discrete end points for waves 1 and 2 with a single dominant follicle.

End point	Wave 1	Wave 2
Expected deviation (F1, 8.5 mm)*		
F1 actual diameter (mm)	8.6 ± 0.1	8.6 ± 0.1
F2 actual diameter (mm)	7.8 ± 0.2	7.6 ± 0.3
Hours after emergence†	52.9 ± 3.8	52.3 ± 0.4
Observed deviation		
F1 diameter (mm)	8.5 ± 0.1	8.7 ± 0.1
F2 diameter (mm)	7.9 ± 0.2	7.8 ± 0.2
Hours after emergence	52.9 ± 3.5	53.5 ± 2.3
Maximum diameter (mm)‡		
F1	14.4 ± 0.3	13.1 ± 0.2§
F2	8.6 ± 0.2	8.5 ± 0.2
Hour of maximum diameter		
F1	69.6 ± 1.2	69.6 ± 1.2
F2	30.4 ± 7.8	28.0 ± 6.8

* Follicles are identified by maximum attained diameter as F1 (largest) and F2.
† Emergence based on first detection of future dominant follicle at 4.0 mm.
‡ Maximum diameter between Hours 0 (F1, 8.5 mm) and 72.
§ Difference between means ($P < 0.05$). No significant difference was observed for other end points, but the difference in diameter of F1 at observed deviation approached significance ($P < 0.08$).

for each wave. Concentrations of LH were higher for wave 1 than for wave 2 (main effect), with a main effect of hour but without a significant interaction. Concentrations were different ($P < 0.05$) between Hours −48 and 0 and between Hours 0 and 40 for each wave, except for between Hours −48 and 0 of wave 2 ($P < 0.07$). Because the increase between Hours −48 and 0 for wave 2 was not definitive, the LH concentrations of wave 2 were also examined by regression analyses. The data were represented by a quadratic ($P < 0.008$) and not a linear ($P < 0.2$) regression model, with the highest point of the curve at Hour 0. The effect of hour for estradiol was significant, and the effect of wave approached significance; the interaction was not significant. Within waves, an increase ($P < 0.05$) occurred between Hours −8 and 0 for wave 1 and between Hours 0 and 40 for wave 2. Between waves within hours, differences were found at Hour 24 ($P < 0.05$) and Hour 32 ($P < 0.001$). There was an effect of hour for ir-inhibin and an interaction that approached significance. Concentrations decreased ($P < 0.008$) between Hours −24 and 0 and continued to decrease until Hour 16. Between waves within hours, concentrations were higher for wave 1 at Hour −48 ($P < 0.02$), Hour −24 ($P < 0.003$), Hour 24 ($P < 0.03$), and Hour 32 ($P < 0.04$).

For the hormone comparisons between the single dominant and codominant groups of wave 1 (Fig. 5), there were hour effects for FSH, LH, and estradiol and a group effect for estradiol. There was an interaction for LH and an interaction that approached significance for FSH and estradiol. Hormone concentrations were higher in the codominant group than in the single dominant group for the following hormones and hours: FSH, Hour −24 ($P < 0.06$); LH, Hour −24 ($P < 0.03$); and estradiol, Hour −48 ($P < 0.002$), Hour −24 ($P < 0.04$), and Hour −8 ($P < 0.0001$). For waves with FSH values beginning before Hour −40, an apparent transient FSH elevation, based on inspection, occurred at Hour −32 or Hour −24 in 4 of 6 waves with codominant follicles compared with 3 of 14 waves with a single dominant follicle. Concentrations of FSH were lower

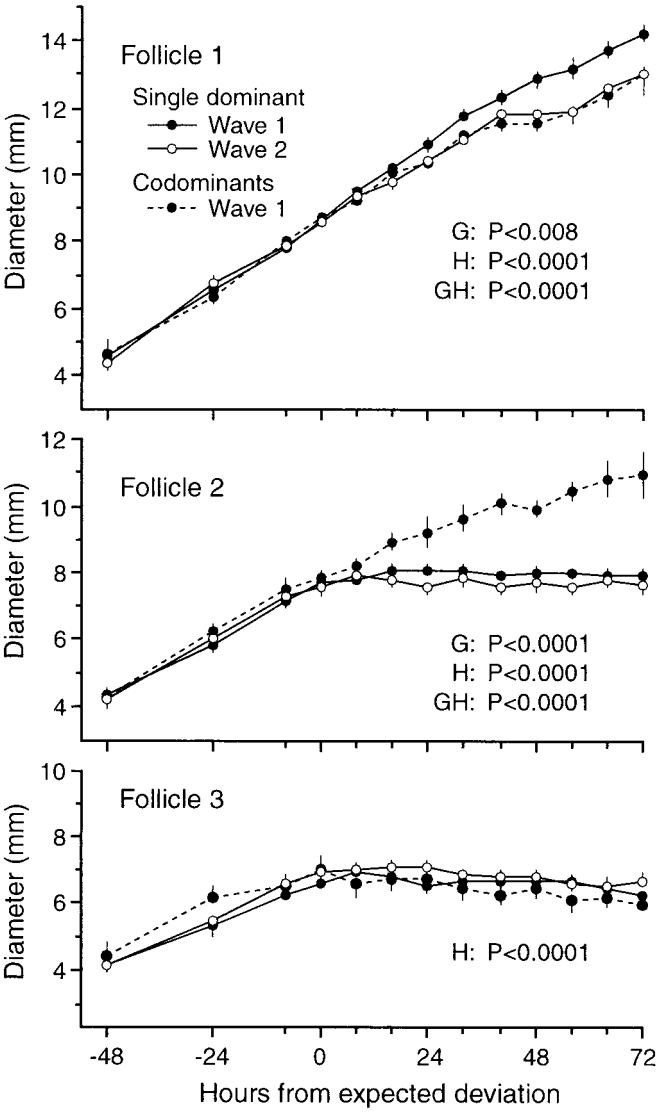

FIG. 2. Mean (±SEM) diameters of F1, F2, and F3 (ranked according to maximum diameter) for three groups of waves. Data are normalized to the expected beginning of deviation (F1, 8.5 mm). Significant main effects (G, group; H, hour) and interactions (GH) are shown.

($P < 0.05$) in the codominant group at Hours 32 and 40 and for LH at Hour 32. There were no significant group or hour effects or an interaction for ir-inhibin (data not shown) between the single dominant and codominant groups.

DISCUSSION

The mechanisms of deviation are more effectively studied by normalizing data to the beginning of deviation because of variation in the interval from follicle emergence to deviation [4]. The beginning of expected deviation (examination when the largest follicle first reaches 8.5 mm) has been used when experimental manipulations interfere with the deviation mechanism [3, 8, 9]. In the present study, expected deviation was used to remove the subjectivity associated with estimating the time of observed deviation and to facilitate comparisons of waves with single versus codominant follicles. The time of expected versus observed deviation was not different within either wave 1 or wave 2.

The results of daily examinations of 23 interovulatory intervals were similar to those of previous reports on the

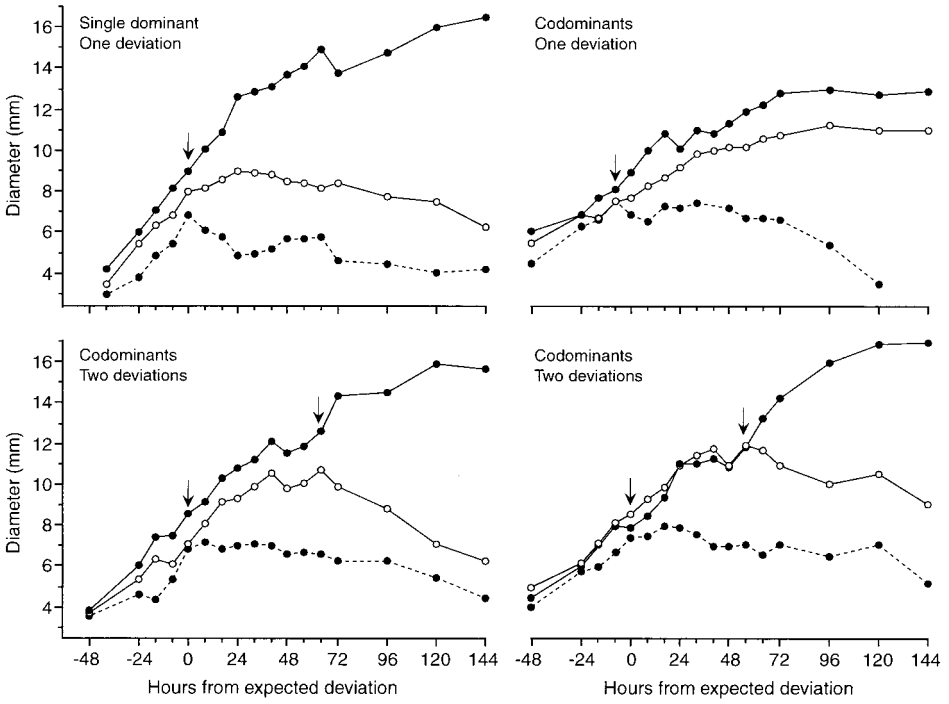

FIG. 3. Diameters of the three largest follicles for four individual waves chosen to illustrate patterns of single-dominant and codominant follicles. The arrows indicate hours of observed deviation, including a second deviation for waves in the lower two panels.

characteristics of follicular waves [1] and temporal associations between an FSH surge and emergence of a wave [7]. However, in the previous studies the waves were normalized to follicle emergence, whereas the present study focused on the diameter at deviation by normalizing to the beginning of expected deviation. The resulting distinct deviation in mean diameter of both F2 and F3 from F1 supports the use of an 8.5-mm largest follicle to define expected deviation. Follicle-deviation characteristics in waves 1 and 2 of two-wave intervals and waves 1, 2, and 3 of three-wave intervals were similar to those previously reported for wave 1, including the following: l) F1 and F2 grew approximately in parallel during the day preceding the beginning of deviation [4], 2) mean deviation began on average when F1 was 8.5 mm [6], and 3) FSH concentrations continued to decline after the beginning of deviation [3]. The future dominant follicle was larger than the other two follicles at Hour −48 in 76% of waves; this finding is consistent with reports that the future dominant follicle of wave 1 emerged 6–7 h on average before emergence of the future largest subordinate follicle [4, 26]. Deviation appeared to involve the two largest subordinate follicles (F2 and F3) at a common time, although this was not clear for wave 3 in the three-wave intervals. An average common day of deviation for the two largest subordinate follicles has not been previously reported.

More detailed comparisons of waves resulted from ovarian examination and blood sampling at 8-h intervals for waves 1 and 2 in 15 heifers with a single dominant follicle for each wave. No differences were found in hour or follicle characteristics of deviation between waves 1 and 2 for either expected or observed deviation. The similarities were

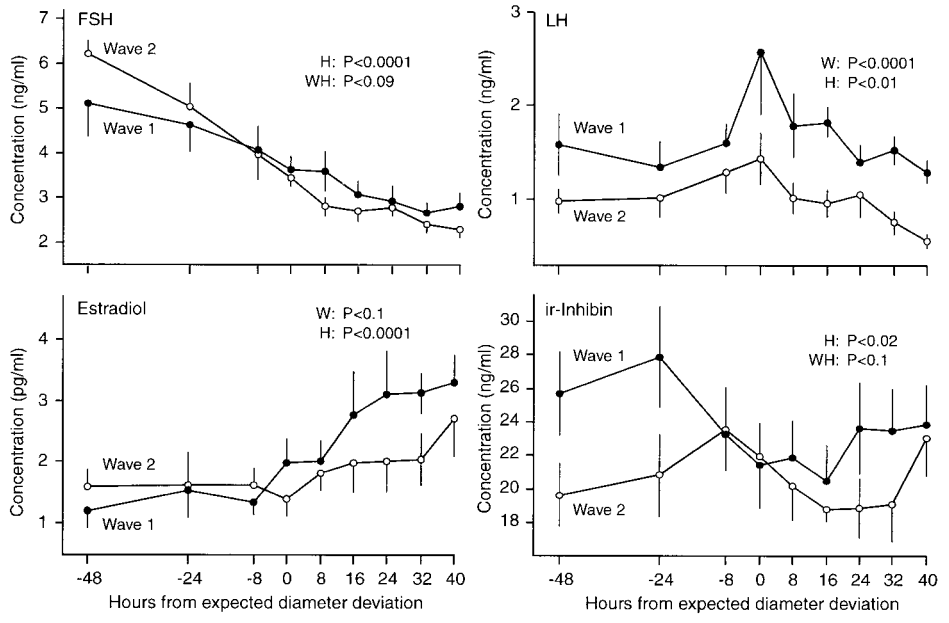

FIG. 4. Mean (±SEM) circulating concentrations of FSH, LH, estradiol, and ir-inhibin for waves with a single dominant follicle during wave 1 (n = 15) and wave 2 (n = 15). Data are normalized to the expected beginning of deviation (largest follicle, 8.5 mm). Main effects (W, wave; H, hour) and interaction (WH) that were significant or that approached significance are shown.

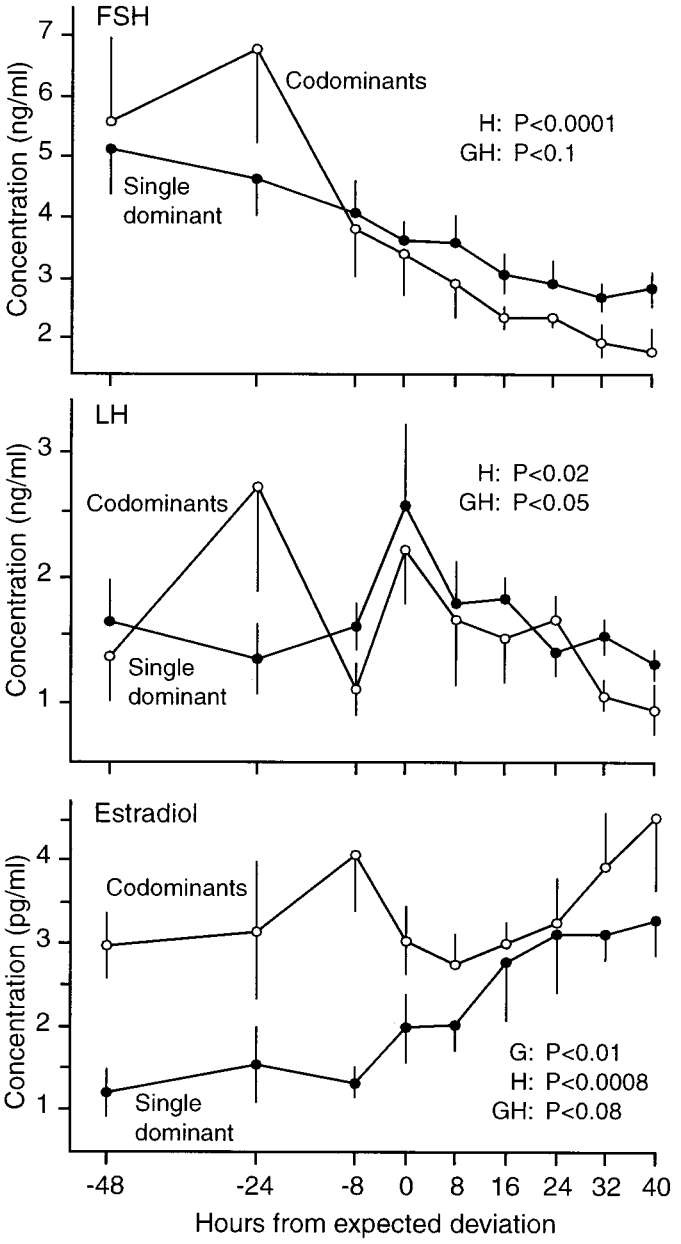

FIG. 5. Mean (±SEM) circulating concentrations of FSH, LH, and estradiol during wave 1 for single dominant follicle waves (n = 15) and codominant follicle waves (follicles >10 mm; n = 6). Data are normalized to the expected beginning of deviation (largest follicle, 8.5 mm). Main effects (G, group; H, hour) and interaction (GH) that were significant or approached significance are shown.

indicated by diameters of F1 and F2 at deviation, the hour of occurrence of deviation relative to wave emergence, and the growth and regression profiles of F2 and F3. There appeared to be differences in mean FSH concentrations between waves 1 and 2; however, the wave effect was not significant and the interaction only approached significance. The hour effect included a decrease in concentrations of FSH after the beginning of deviation for both waves 1 and 2 as shown by the examinations at 8-h intervals and for all waves as shown by the examinations at 24-h intervals. These results agree with those of previous reports for wave 1 [3, 8, 9]. The postdeviation decline in FSH in wave 1 has been temporally associated with increasing secretion of estradiol [26] by the largest follicle [8], beginning at Hour 0. A similar temporal FSH/estradiol relationship was found for both wave 1 and wave 2 in the present study; estradiol concentrations increased as the FSH concentrations continued to decline after the beginning of deviation. The increase in estradiol concentrations appeared to begin 8 h earlier and reached higher levels for wave 1 at Hours 24 and 32; however, the interaction and wave effect were not significant. In a previous study of waves 1 and 2, estradiol concentrations were higher during wave 1 than during wave 2, but a significant increase was not detected in association with wave 2 [20]. These reported results contrast with those of the present study, perhaps because waves were normalized to emergence in the previous study and to deviation in the present study.

A decrease in ir-inhibin concentrations over the 24 h preceding deviation has not been reported previously. The concentrations continued to be low during the postdeviation FSH decline. Predeviation ir-inhibin was lower in wave 2 than in wave 1, but this conclusion was weakened by an interaction that only approached significance. The present study involved total inhibin. However, the role of systemic inhibins in deviation will not likely be clarified until assays are used that distinguish between immunoreactive and bioactive forms [29] and data are normalized specifically to the beginning of deviation.

The only distinctive hormone difference between waves 1 and 2 involved the transient elevation in LH concentrations that reached a maximum mean at deviation. The concentrations were higher for wave 1, but the shape of the elevation did not differ between waves, as indicated by nondetection of an interaction. For both waves, the maximum concentrations occurred at Hour 0 with an increase and decrease on each side of the peak, although only the increase for wave 2 approached significance. In this regard, however, the significant fit of a quadratic regression model for wave 2 indicated both an increasing and a decreasing component. A less pronounced LH elevation encompassing wave 2 compared with wave 1 is attributable to higher systemic progesterone concentrations during wave 2. Although progesterone was not assayed in the present study, an association between higher progesterone concentrations and lower LH concentrations has been demonstrated [20, 30, 31]. A transient LH elevation at deviation has been previously reported for wave 1 [10, 11, 26]. The increased elevation may be involved in the deviation mechanism by increasing the follicular concentrations of estradiol and IGF-1, especially in the largest follicle [11, 12]. However, the transient LH elevation apparently is not essential for the occurrence of diameter deviation in a majority of waves [11]. Cattle with a history of twin births had higher concentrations of follicular fluid and plasma IGF-1 than did controls [32], but the wave of origin and status of the follicles was not given. Cattle with a history of twins had higher concentrations of plasma IGF-1 [33].

Higher levels of LH occurred and higher levels of FSH tended to occur at Hour −24 in the codominant group than in the single-dominant group. Because the study focused on deviation, adequate data were not available to determine whether the apparent FSH peak at approximately Hour −24 represented a delayed peak of the wave-stimulating FSH surge or a secondary peak. In this regard, multiple fluctuations of FSH during the FSH decline between emergence and deviation have been described [34]. The transient increase in FSH and LH concentrations close to the beginning of deviation may have interfered with the establishment of deviation between F1 and F2, thereby increasing the incidence of codominant follicles. The present observations

provide rationale for developing hypotheses on the relationship between the development of codominant follicles and FSH and LH concentrations before the time of expected deviation. Near the hour of deviation, the concentrations of FSH and LH were similar for the single and codominant groups. However, the postdeviation decline in both gonadotropins was greater in the codominant group, reaching lower concentrations of LH and tending to reach lower concentrations of FSH by Hour 32. In individuals with adequate data, the postdeviation concentrations of FSH decreased to <2 ng/ml for more than one examination in 4 of 5 waves with codominant follicles compared with 3 of 14 waves with a single dominant follicle. The apparently greater FSH decline in the codominant group may account for the occurrence of a second deviation-like phenomenon in most (67%) of these waves after both follicles reached >10 mm. Apparently, the balance between FSH requirements and the prevailing diameter of follicles is tightly coupled for follicles as large as 10 mm, as has been proposed for follicles that are 8.5 mm at the beginning of the first deviation [9]. In a previous study [33], altered concentrations of FSH and LH were not found in cattle with a history of twin births; however, the data were normalized to estrus rather than to deviation. In another study [35], cattle with a history of twins had greater LH release, but not FSH release, in response to GnRH.

Circulating estradiol concentrations were elevated in the codominant group over Hours −48 to −8. The hypothesis was supported that the elevated concentrations resulted from more follicles of ≥4.0 mm. The feedback relationships between estradiol and the gonadotropins before deviation are not clear, but a negative effect of estradiol on FSH [8] and a positive effect of LH on estradiol [11] near or soon after the beginning of deviation have been demonstrated. Higher estradiol after deviation in waves with codominant follicles could account for the greater reduction in FSH, but in the present study the higher estradiol concentrations at Hours 32 and 40 were not significant.

Follicle 1 was smaller during Hours 32–72 (end of study) when it was a single dominant follicle of wave 2 or a codominant follicle of wave 1 than when it was a single dominant follicle of wave 1. These results indicate that when F1 reaches >10 mm, adequate concentrations of LH are needed for maximal growth. The LH concentrations apparently are inadequate for maximal growth during the later portion of the transient elevation associated with wave 2 and during the period associated with codominant follicles. In this regard, F1 (single dominant follicle) of wave 1 was smaller in diameter by Hour 31 when concentrations of LH were experimentally reduced [11], and suppression of LH prevented growth beyond 9 mm [36]. As indicated by the study at 24-h intervals, F1 of wave 2 in two-wave intervals eventually reached a maximum diameter equivalent to that of F1 of wave 1 after 72 h. This implies that the slower average growth rate of a single dominant follicle of wave 2 between Hours 32 and 72 was compensated for by a longer period for reaching maximum diameter and ovulation (Fig. 1).

In conclusion, no follicle or hormone differences were found between waves 1 and 2 that would cast doubts on current postulates on the mechanism of deviation as developed previously from the study of wave 1 [2, 5]. The only detected follicle difference between waves involved a smaller dominant follicle for wave 2, beginning 32 h after the expected beginning of deviation. The only detected hormone difference between waves involved a less pronounced transient LH elevation encompassing deviation for wave 2. The lower LH elevation in wave 2 likely accounted for the smaller dominant follicle 32 h after deviation. Codominant follicles occurred more frequently during wave 1 than during other waves. The development of codominant follicles was preceded on average by a transient increase in concentrations of FSH and LH 24 h before the expected beginning of deviation. Either or both of these gonadotropins could have interfered with the deviation of F2, thereby leading to two dominant follicles. Codominant follicles were sometimes associated with an apparent second deviation characterized by cessation of growth of the F2 dominant follicle when both follicles were >10 mm. The second deviation may have reflected a greater postdeviation decline in FSH concentrations in the codominant group than in the single-dominant group.

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