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Effects of modified FSH surges on follicle selection and codominance in heifers

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Abstract

Follicular Wave 1 emerges near ovulation and Wave 2 emerges during mid-diestrus and are stimulated by FSH Surges 1 and 2, respectively. During a follicular wave, one follicle becomes dominant and continues to grow, and the others become subordinates. The eminent and recognizable event during this selection process is diameter deviation. The relationships between the patterns of the wave-stimulating FSH surge and deviation were studied by altering the FSH surge, using follicle ablations. Codominance was used as the primary indicator of altered deviation. In waves with codominant follicles, the day of deviation was based on the beginning of regression of the third-largest follicle. The following groups (n=8/group) were used: 1) controls, 2) 6.5-mm group; all follicles ≥ 5.0 mm ablated except F1 (largest) and F2 when F1 was ≥ 6.5 mm, 3) Day-4 group; all follicles ≥ 5.0 mm ablated on Day 4 (ovulation=Day 0) and 4) Day-7 group; all follicles ≥ 5.0 mm ablated on Day 7. Ablations were done during the decreasing FSH concentrations in Surge 1, during the minimal FSH concentrations between Surges 1 and 2, and during the increasing FSH concentrations in Surge 2 in the three ablation groups, respectively. Concentration of FSH at the beginning of deviation was 38% greater ($P < 0.03$) in the 6.5-mm group than in the controls. The greater FSH concentrations did not interfere with deviation, as indicated by the unaltered interval from emergence to deviation and an unaltered incidence of codominant follicles ≥ 10.0 mm. Ablations on Day 4 induced the most prominent surge as indicated by a 74% greater ($P < 0.003$) FSH peak and 31% greater ($P < 0.05$) FSH concentration at deviation than in controls. The Day-4 group had a greater ($P < 0.05$) rate of codominance (6 of 8 waves, 75%) than in each of the other groups (25%). The day at the beginning of deviation between F1 and F3 in codominant waves was not different from the day of deviation between F1 and F2 in single dominant waves. Ablations on Day 7 induced a surge with a peak intermediate between the peak for the control and Day-4 groups ($P < 0.01$) but with no difference from controls in FSH concentrations near deviation and in the rate of codominance. There was no difference among groups in the length of the interval between wave emergence and the beginning of deviation, despite the experimentally increased FSH concentrations encompassing deviation in the 6.5-mm and Day-4 groups. This result indicated that the initiation of deviation was more dependent on attained follicle diameters than on the concentrations of FSH reached during the decline in the FSH surge. Follicle ablation during the low FSH concentrations between surges induced the most prominent FSH surge and was the only group with altered deviation as indicated by a high incidence of codominance.

Keywords: codominant follicles, follicle development, follicle selection, follicle-stimulating hormone, luteinizing hormone

Introduction

Selection of the dominant follicle in cattle occurs from a cohort of growing antral follicles, termed a follicular wave (reviewed in Ginther *et al.,* 2003a). Two or three successive follicular waves occur during the estrous cycle. The first wave (Wave 1) appears around the time of ovulation and the second during mid-diestrus (Wave 2). Following emergence of a group of follicles at 4 or 5 mm, the follicles develop in a common-growth phase for about 2.5 days. When the largest follicle reaches a mean of about 8.5 mm, the common-growth phase ends and deviation in diameter begins. Diameter deviation is considered to be the eminent event in follicle selection and is characterized by a continuation in growth rate of the largest follicle (developing dominant follicle) and a reduction in growth rate or regression of the smaller follicles (subordinate follicles).

Emergence of each follicular wave is stimulated by a surge in FSH which reaches a peak when the follicles are about 5.0 mm (reviewed in Ginther *et al.,* 2003a). The surges are designated Surge 1 and Surge 2 for Wave 1 and Wave 2, respectively. The decrease in FSH following the peak is a function of multiple growing follicles of the wave until diameter deviation begins. At that time or shortly thereafter, the developing dominant follicle alone continues the role of FSH suppression. Deviation is initiated toward the end of the FSH decline. The requirement of a declining or low concentration of FSH when the most advanced

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follicle reaches the appropriate stage can be overridden with exogenous FSH (Adams *et al.,* 1993) or by maintaining endogenous FSH concentrations with an anti-inhibin (Kaneko *et al*., 1997); as a result, deviation is experimentally delayed or the number of dominant follicles increased. Apparently, at the appropriate stage the most advanced follicle is able to utilize the declining FSH concentrations through an increase in responsiveness to gonadotropins, owing to the differential production among follicles of enabling follicular-fluid factors (reviewed in Ginther *et al*., 2003a). Although the wave-stimulating FSH surge must decline from peak concentrations for deviation to occur, a study of natural surges and waves suggested that the time of the beginning of deviation was more dependent on the diameter reached by the future dominant follicle than on the extent of the FSH decline (Haughian *et al*., 2004). This observation has not been tested experimentally.

A transient elevation in concentrations of LH encompasses deviation during Wave 1 (Bergfelt *et al*., 2000) and is utilized for maximum development of the dominant follicle (Ginther *et al*., 2001b). In this regard, infusion of LH in hourly pulses was associated with greater diameter and duration of the dominant follicle of Wave 1 in postpartum beef heifers (Duffy *et al*., 2000). The similarities in follicle diameters and gonadotropin concentrations between Waves 1 and 2 have been studied by centralizing data for each wave to the beginning of deviation (Kulick *et al*., 2001). No differences in follicle diameters or FSH concentrations were found between Waves 1 and 2, except that the dominant follicle was smaller for Wave 2 after the beginning of deviation; this was attributable to lower LH concentrations. A transient elevation in LH seemed to occur during deviation in Wave 2, but the increase was not significant. This aspect of the LH/deviation relationship during Wave 2 needs further study.

Codominant follicles are defined as two follicles reaching a diameter of at least 10 mm (Kulick *et al*., 2001; Beg *et al*., 2003). The incidence of spontaneous codominance in Holstein heifers during Wave 1 has been reported as 28% (Beg *et al*., 2003) and 35% (Kulick *et al.,* 2001). Two deviations were observed in 9 of 11 heifers with codominant follicles combined for the two studies. The first deviation occurred when the largest follicle was about 8.5 mm and was indicated by reduced growth of the third-largest follicle. The second deviation between the two dominant follicles occurred at means of 36 or 50 hours after the first deviation and was associated temporally with a more precipitous decrease in FSH after the beginning of the first deviation in both studies. Codominance may be a useful model for studies on the mechanism of deviation and the role of characteristics of the FSH surge because codominance in monovular species can be considered to be a defect in the deviation mechanism (Ginther *et al*., 2003a). In the initial study (Kulick *et al*., 2001), codominant follicles were associated with greater concentrations of FSH and LH before deviation and a greater reduction in FSH after the beginning of deviation. In a subsequent study (Beg *et al*., 2003), FSH and LH concentrations before deviation did not differ between waves with codominants versus single dominants, but FSH did decrease to lower concentrations after deviation in the codominant waves. In contrast to these studies, no differences in plasma FSH concentrations were found between beef cattle selected for twin versus single ovulations, and twinning was associated with greater concentrations of serum and follicular fluid insulin-like growth factor-1 (Echternkamp *et al*., 1990; 2004). The inconsistencies among studies in the concentrations of FSH and LH associated with one versus two dominant follicles indicates the need for further study in this area.

A high incidence of codominance (43%) was reported to occur in Holstein heifers after ablation of all follicles ≥ 5 mm 5 days after ovulation (Gibbons *et al*., 1997). However, control (nonablated) heifers were not included. In this regard, it was noted in an abstract (Mussard *et al.,* 2002), that aspiration of all follicles ≥ 5 mm 4 to 5 days postovulation and administration of PGF2α was followed by a 47% double-ovulation rate in beef cows. In a study in beef heifers (Bergfelt *et al*., 1994), the incidence of codominance (defined as two follicles ≥ 11 mm) was 18% following ablation of all follicles at random days of the estrous cycle, compared to 0% in controls (difference approached significance, $P < 0.07$). None of these studies considered the effects of ablating at various times relative to a follicular wave or FSH surge on the characteristics of the induced FSH surge and follicle deviation and on the incidence of codominance.

The purpose of the present experiment was to determine if follicle ablation at differing times relative to natural FSH surges alters the characteristics of the induced FSH surge and follicle deviation or alters the incidence of codominance. Hypotheses were: 1) deviation begins when the follicles reach a critical diameter without regard to the extent of the decline in the FSH surge; 2) follicle ablation at the nadir between FSH surges stimulates a more prominent induced surge; and 3) the more prominent induced FSH surge increases the incidence of codominance. In addition, because of inconsistencies and tentative conclusions in the literature, follicle and gonadotropin comparisons were made between Waves 1 and 2 and between waves with single dominant and codominant follicles.

Materials and Methods

Heifers and Ultrasonography

The University of Wisconsin Animal Care and use Committee approved all animal handling and sample-collection procedures. The husbandry and

feeding program, transrectal and transvaginal ultrasound equipment, and ultrasound techniques for monitoring for ovulation and measuring and tracking follicles (maintaining day-to-day identity) have been described (Ginther *et al.,* 1999). Holstein heifers (450 to 670 kg) from 2-3 yr of age were injected with a luteolytic dose of PGF2α (25 mg, Lutalyse; Pharmacia, Kalamazoo, MI), following confirmation of the presence of a mature corpus luteum (5-17 days after ovulation). Heifers were checked once daily for ovulation (Day 0) following PGF2α injection. Follicles of the follicular wave that emerged near Day 0 (Wave 1) were ablated on designated days. The experimental groups (n=8/group) were: 1) control group (no ablations); 2) 6.5-mm group (ablation of all follicles ≥ 5.0 mm, except the two largest follicles when the largest was ≥ 6.5 mm; 3) Day-4 group (ablation of all follicles ≥ 5.0 mm on Day 4); and 4) Day-7 group (ablation of all follicles ≥ 5.0 mm on Day 7). The intention was to ablate the designated follicles during the decreasing concentrations of FSH in Surge 1 (6.5-mm group), during minimal concentrations between surges 1 and 2 (Day-4 group), and during the increasing concentrations of Surge 2 (Day-7 group).

Heifers received an injection of PGF2α 4 days after the largest follicle reached ≥ 8.2 mm during Wave 1 (6.5-mm group) or Wave 2 (control, Day-4, and Day-7 groups). The PGF2α was given to induce luteolysis to determine if the codominant follicles would ovulate. Codominant follicles in a wave were defined as two follicles that reached a diameter ≥ 10 mm (Kulick *et al*., 2001). Follicle and gonadotropin data in the 6.5-mm group were compared to Wave 1 and Surge 1 in the controls. Data for the induced Wave 2 following ablation of all follicles on Days 4 or 7 (Day-4 or Day-7 groups) were compared to Wave 2 and Surge 2 of the controls. Follicle ablations were done by ultrasound-guided transvaginal aspiration of follicle contents, using an 18 gauge needle connected to a vacuum pump as described (Ginther *et al.,* 1999). An additional ultrasound scanning was done 12 h following ablation, and another aspiration procedure was done at follicle sites with a fluid collection of ≥ 5.0 mm. The three largest follicles were tracked daily for each wave and the diameters were recorded.

The first, second, and third largest follicles according to maximum attained diameter were identified as F1, F2, and F3. Follicular-wave emergence was defined as the first retrospective identification of F1 at ≥ 4 mm. The beginning of expected deviation was defined by the day that F1 reached ≥ 8.2 mm. Because of the 1-day interval between ultrasound scans, the 8.2-mm reference for F1 was used so that the actual mean diameter would approximate the expected mean diameter at the beginning of deviation, according to previous studies (8.5 mm; Ginther *et al.,* 1999; 2000). Expected deviation was used for objectivity in the comparisons among groups. However, for comparisons of follicle dynamics between heifers with single and codominant follicles, observed deviation was used because of inadequate available information on the characteristics of the first and second deviation in heifers with codominant follicles. Observed deviation for singletons was defined retrospectively by the beginning of an apparent difference in growth rates between the two largest follicles as described (Ginther *et al*., 1997). For heifers with codominant follicles, the beginning of the first observed deviation was defined by an apparent difference in growth rates between F1 and F3, and a second observed deviation was defined by a subsequent apparent difference in growth rates between the two dominant follicles (F1 and F2; Kulick *et al*., 2001; Beg *et al.,* 2003).

Hormone assays

Blood samples (10 ml) were taken from the coccygeal vein once daily. Following collection of blood samples, plasma was separated by centrifugation at 1500 g for 20 min, decanted into storage vials, and frozen (-20^{o} C) until assayed. Plasma concentrations of FSH and LH were determined by RIAs that have been described and validated for bovine plasma in our laboratory (Adams *et al*., 1992; Ginther *et al.,* 1999). The intra- and interassay coefficients of variation and mean assay sensitivity, respectively, were 8.7%, 3.2%, and 0.04 ng/ml for FSH, and 7.5%, 7.3%, and 0.15 ng/ml for LH.

Statistical analyses

Due to the lack of a normal distribution as determined by the Kolmogorov-Smirnov test, FSH and LH data were transformed logarithmically. The sequentialnature of the follicle and hormone data was examined using the MIXED procedure of SAS with a repeated measures statement and a first order autoregressive structure to account for the autocorrelation between measurements (SAS Institute Inc., Cary, NC). Main effects of group and day and their interaction were determined. When an interaction was significant or approached significance, paired t-tests were used to detect differences between days within a group and unpaired *t*-tests were used between groups within a day. The differences between groups in the incidence of codominance were analyzed by Chi-square tests. A probability of $P \leq 0.05$ indicated that a difference was significant and probabilities between $P > 0.05$ and ≤ 0.1 indicated that a difference approached significance.

Results

Diameters of F1 or F2 did not differ significantly or approach a difference between the beginning of expected deviation and the beginning of observed deviation within any of the waves. Follicle and gonadotropin data on the characteristics of expected deviation in follicular Wave 1 versus Wave 2 in the

control group and the results of statistical analyses are shown (Table 1). There were no differences between waves for the follicle, FSH, and LH end points, except that circulating concentration of LH on the day before and the day of the expected beginning of deviation and maximum diameter of F1 were greater in Wave 1 than in Wave 2. The diameters of F1 and F2 and changes in circulating concentrations of FSH and LH for Wave 1 and Wave 2 in the control group and main effects and interactions that were significant or approached significance are shown (Fig. 1). The interaction of wave by day for F1 reflected smaller diameters ($P < 0.05$–0.02) for Wave 2 during 3 to 5 days after the beginning of expected deviation. The interaction of wave by day for plasma LH concentrations involved greater ($P < 0.05$) concentrations in Wave 1 at -4, -1, and 0 days from the beginning of deviation and in Wave 2 at 5 days. Concentrations of LH increased between -2 and 0 days from the beginning of deviation for Wave 1 ($P < 0.04$) and Wave 2 ($P < 0.001$) and decreased between 0 and 3 days for wave 1 ($P < 0.05$) and between 0 and 2 days for wave 2 ($P < 0.008$).

Table 1. Mean ± SEM for end points for the indicated waves in the control group and in groups with all but two largest follicles ablated when largest was 6.5 mm or all follicles ablated on Day 4 or Day 7.

	Control group		6.5-mm group	Day-4 group†	Day-7 group†
End points	Wave 1	Wave 2	(Wave 1)	(Wave 2)	(Wave 2)
	A	B	C	D	E
Codominance (No. heifers)	2 of 8	2 of 8	2 of 8	6 of 8*	2 of 8
Follicle ≥ 4.0 mm (No.)	10.4±0.9	9.3±0.8	9.2±0.6	11.4±0.8*	9.8±1.1
At emergence**					
Day†	-1.0±0.3	8.3±0.4	-0.9±0.3	4.5±0.0*	7.8±0.2
FSH (ng/ml)	0.20±0.03	0.24±0.03	0.17±0.02	0.32±0.04*	0.29±0.03
LH (ng/ml)	0.62±0.10	0.52±0.11	0.65±0.09	0.65±0.08	0.53±0.06
Emer. to deviation†† (d)	2.8±0.2	2.6±0.3	2.4±0.3	2.5±0.2	2.8±0.2
One day before deviation					
FSH (ng/ml)	0.16±0.02	0.21±0.03	0.18±0.02	0.30±0.05*	0.25±0.04
LH (ng/ml)	0.67±0.08	0.47±0.06*	0.71±0.07	0.65±0.08*	0.53±0.06
At deviation					
Day	1.8±0.3	10.8±0.5	1.6±0.3	6.8±0.4*	10.3±0.2
F1 diameter (mm)	8.7±0.2	8.7±0.2	8.6±0.2	8.5±0.3	8.7±0.2
F2 diameter (mm)	8.0±0.2	7.8±0.3	7.7±0.2	8.4±0.3#	8.0±0.2
FSH (ng/ml)	0.13±0.01	0.16±0.03	0.18±0.02*	0.21±0.02#	0.18±0.04
LH (ng/ml)	0.74±0.09	0.55±0.06*	0.76±0.01	0.68±0.05#	0.52±0.06
Maximum					
F1 diameter (mm)	16.5±0.4	15.2±0.4*	17.5±0.2*	15.9±0.4	16.6±0.4*
F2 diameter (mm)	9.2±0.5	8.9±0.5	9.9±0.4	11.2±0.9*	9.4±0.4
FSH (peak; ng/ml)	0.22±0.03	0.27±0.03	0.24±0.03	0.47±0.04*	0.37±0.04*

* End points in column B that differ ($P < 0.05$) from column A, in C that differ from A, in D that differ from B, and in E that differ from B.
\# Means that approach a difference ($P < 0.07$) from column B.
† Day 0=day of ovulation.
** First retrospective detection of the future largest follicle (F1) at ≥ 4.0 mm.
†† Expected beginning of deviation (largest follicle ≥ 8.2 mm).

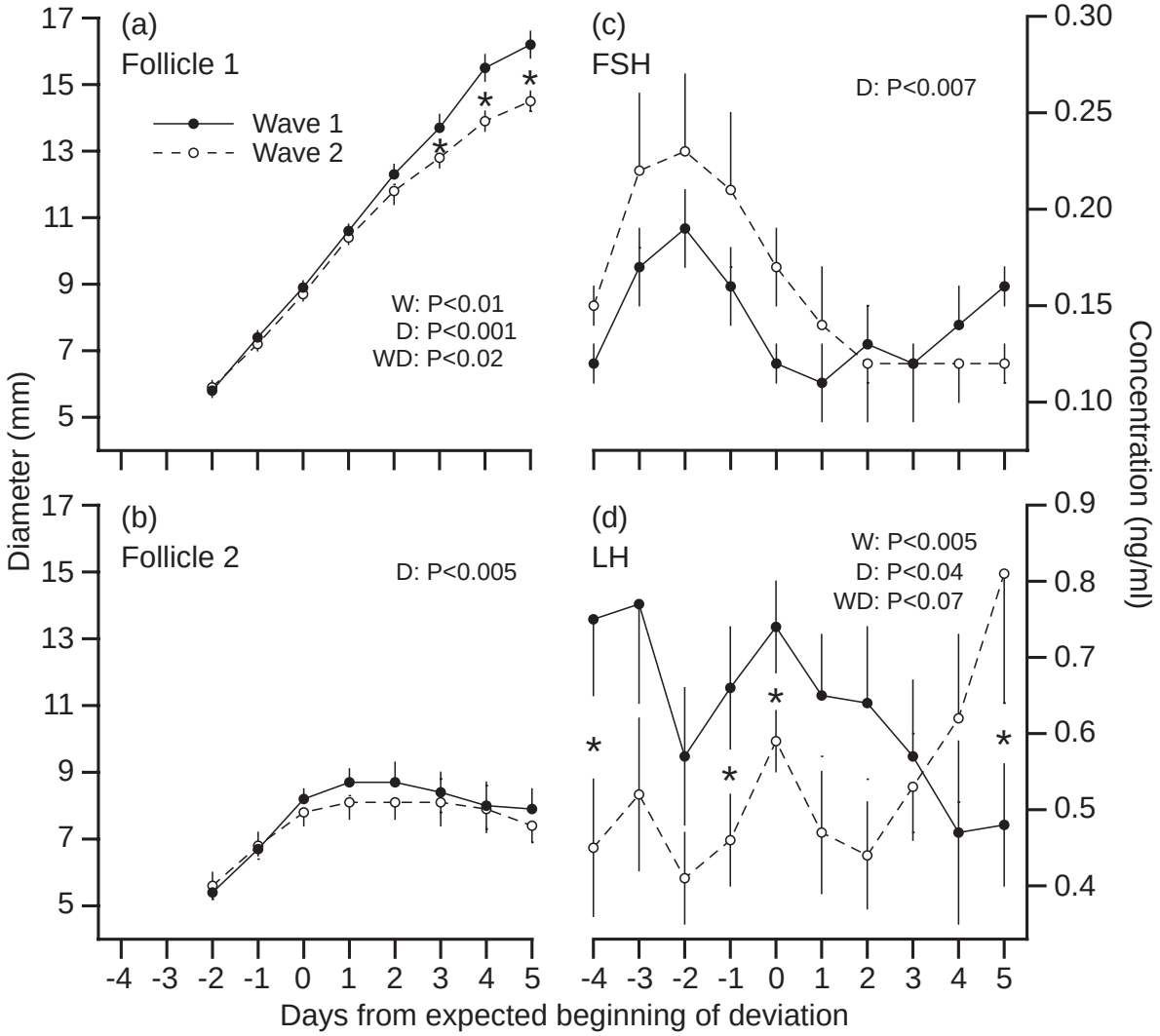

Figure 1. Mean ±SEM diameter of the two largest follicles (a,b; Follicle 1 or F1=largest) and concentrations of circulating FSH (c) and LH (d) during follicular wave 1 versus 2. Data were normalized to the expected beginning of diameter deviation (F1 ≥ 8.2 mm). Probabilities for main effects (W, wave; D, Day) and interaction (WD) are shown. The asterisks indicate a difference ($P < 0.05$) between waves within a day. Concentrations of LH increased ($P < 0.05$) between –2 and 0 days and decreased ($P < 0.05$) after 0 day for each wave.

Follicle and gonadotropin data for the 6.5-mm group (ablation of all follicles except the two largest) during Wave 1, were compared to follicular Wave 1 and FSH Surge 1 of the controls by centralizing to the day of ablation (largest follicle ≥ 6.5 mm). Single-point data and differences between groups are shown (Table 1). Concentration of FSH at expected beginning of deviation and maximum diameter attained by F1 was greater in the 6.5-mm group than in the control group.

Means and results of statistical analyses for F1, F2, FSH, and LH over days are shown for the two groups (Fig. 2). There were no significant group effects or interactions for any end point, but the day effect was significant for all end points. Although there were no interactions, a greater diameter of F2 at 4 days ($P < 0.06$) and 5 days ($P < 0.07$) after ablation approached significance. Concentration of FSH was 42% greater ($P < 0.03$) 1 day after ablation in the 6.5-mm group (0.17 ± 0.02 ng/ml) than in the controls (0.12 ± 0.01 ng/ml) and was 38% greater ($P < 0.05$) at the beginning of deviation (Table 1).

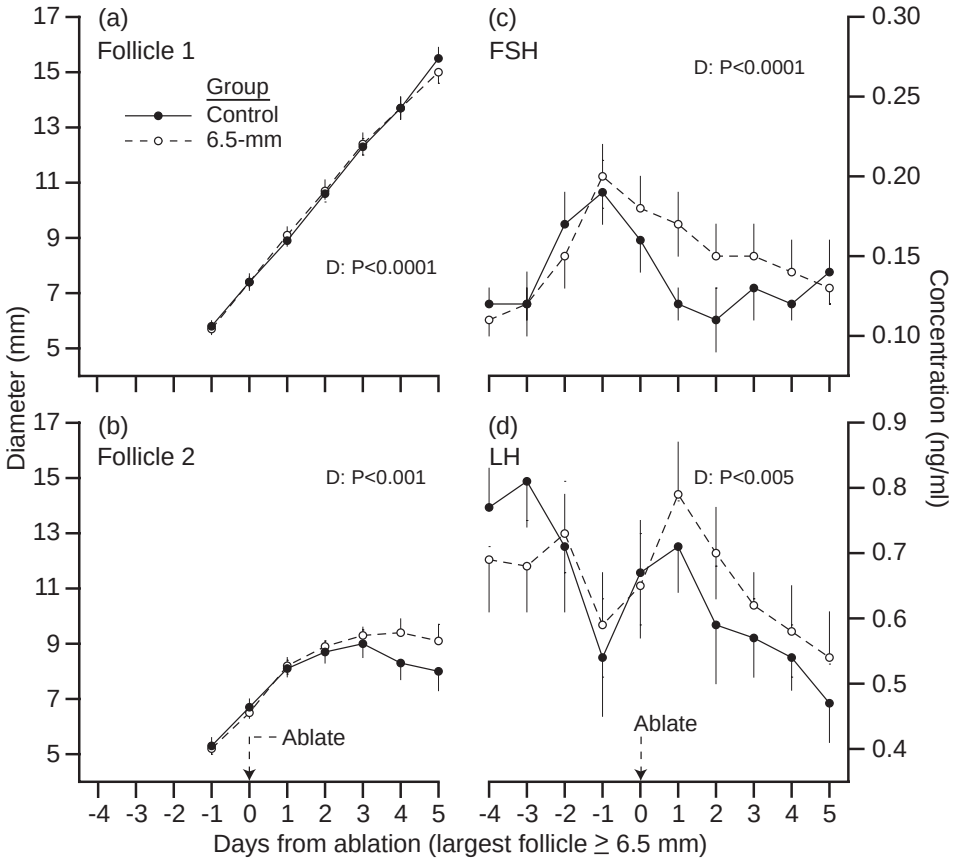

Figure 2. Mean ± SEM diameter of the two largest follicles (a,b; Follicle 1 or F1=largest) and concentrations of circulating FSH (c) and LH (d) for the control group and 6.5-mm group during Wave 1. In the 6.5-mm group, all follicles ≥ 5.0 mm, except F1 and F2, were ablated when F1 reached ≥ 6.5 mm. Only the day effect was significant for each end point.

Means for single-point data and significant differences between the control group (Wave 2) and the Day-4 group and between the control group and the Day-7 group are indicated (Table 1). Heifers in the Day-4 group had more follicles for Wave 2 and more codominant follicles than for any other group. Maximum diameter of F2 and maximum peak concentration of FSH were also greater in the Day-4 group than in the control and Day-7 groups. Differences in concentration of FSH and differences in concentrations of LH at the beginning of deviation approached significance ($P < 0.07$) between the Day-4 group and the control group. Compared to the controls, maximum diameter of F1 and peak FSH concentration were greater in the Day-7 group. Concentrations of FSH and LH for the three groups over Days 0 to 14 are shown (Fig. 3). The group-by-day interaction was significant for FSH, owing to the earlier FSH surge for the Day-4 group (peak at Day 5) than for the other two groups (peak at Day 8). Only the day effect was significant for LH. Diameters of F1 and F2 for -2 to 6 days from the beginning of deviation in the three groups are shown (Fig. 4). Follicle 1 showed an interaction that approached significance, and F2 showed an interaction that was significant. Follicle 1 was larger ($P<0.03$–0.05) in the Day-4 and Day-7 groups at 3 to 6 days after expected deviation than in the control group, and F2 was larger ($P < 0.03$–0.01) in the Day-4 group than in the controls at 0 to 6 days.

Number of heifers with a single dominant follicle was too few (n=2) in the Day-4 group, and the number of heifers with codominant follicles in Wave 2 was too few (n=2) in all groups, except the Day-4 group, to permit a comparison of the characteristics associated with single and double dominants within any group. Inspection of data seemed to indicate that the follicle and gonadotropin characteristics within single and within double dominant follicles were similar among groups. Therefore, data for single (n=14) versus double (n=10) dominants during Wave 2 were combined for the three groups. Gonadotropin data normalized to the expected beginning of deviation are shown (Fig. 5). The group-by-day interaction was significant for both FSH and LH. Within days, FSH concentrations were greater in the

codominant group one day before the beginning of the first deviation than in singletons. The FSH and LH concentrations after the beginning of deviation did not differ between the singleton and codominant heifers. However, the percentage decrease for FSH was greater after deviation in the codominant group, so that by 2 days after deviation the difference between groups was significant ($P < 0.04$). The percentage decrease in LH after deviation was not different between groups (not shown). The peak FSH concentration for Surge 2 was greater for heifers with codominants than for heifers with singletons (Table 2).

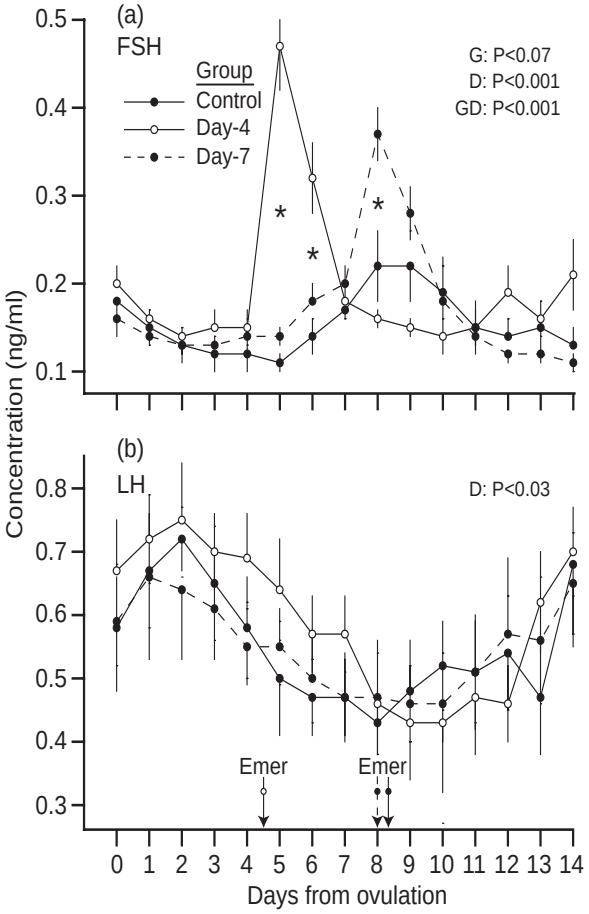

Figure 3. Mean ± SEM concentrations of circulating FSH (a) and LH (b) for the control group and two groups in which all follicles ≥ 5.0 mm were ablated on Day 4 (ovulation=Day 0) or Day 7. Probabilities for main effects (G, group; D, day) and the interaction (GD) are shown for each end point. Asterisks indicate a difference ($P < 0.05$) in FSH concentrations between the control group and the Day-4 group on Days 5 and 6 and between the control group and the Day-7 group on Day 8. Emer=the mean day of emergence of follicular Wave 2 for each group.

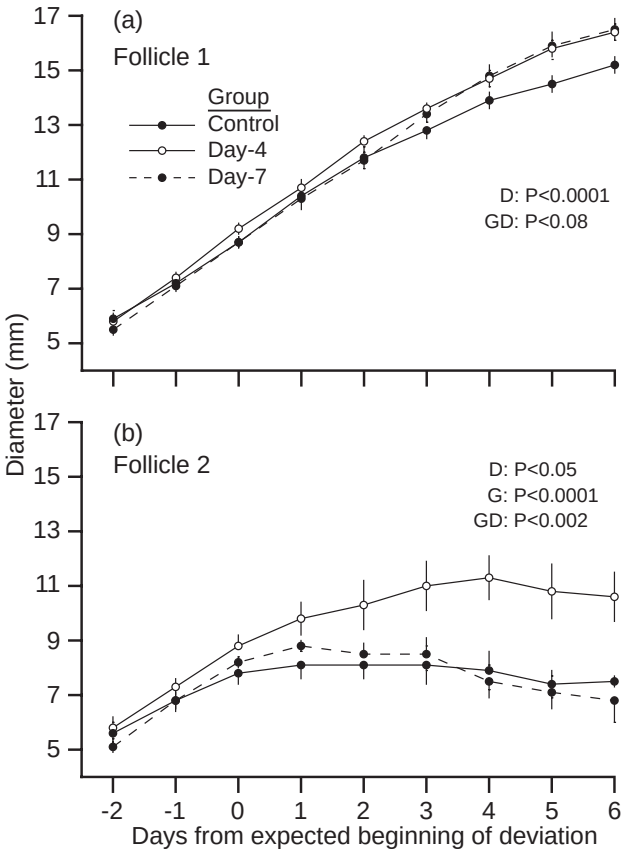

Figure 4. Mean ± SEM diameter of the largest follicle (a; Follicle 1 or F1) and the second largest follicle (b; Follicle 2 or F2) of Wave 2. Data were normalized to the expected beginning of deviation (F1 ≥ 8.2 mm). Probabilities for main effects (G, group; D, day) and interaction (GD) are shown. For Follicle 1, the Day-4 and Day-7 groups were different ($P < 0.05$) from the control group on each of 3–6 days after the beginning of deviation. For F2, the Day-4 group was different ($P < 0.05$) from the control group on each of days 0–6.

All heifers with codominants had two observed deviations, and only F1 ovulated after the PGF2α treatment. A comparison of the profiles for diameters of F1, F2, and F3 for singletons and codominants is shown (Fig. 6). Follicle 2 was larger ($P < 0.003$) in the codominant heifers than in the singletons on each day after the beginning of observed deviation, but F1 and F3 did not differ between singleton and codominant heifers. The interval between the first and second observed deviations in heifers with codominants was 1.8 ± 0.3 days. The follicle characteristics of the first observed deviation in heifers with codominants were not significantly different from those of observed deviation in singletons (Table 2). Concentrations of both FSH and LH were greater in the codominant heifers at the beginning of observed deviation.

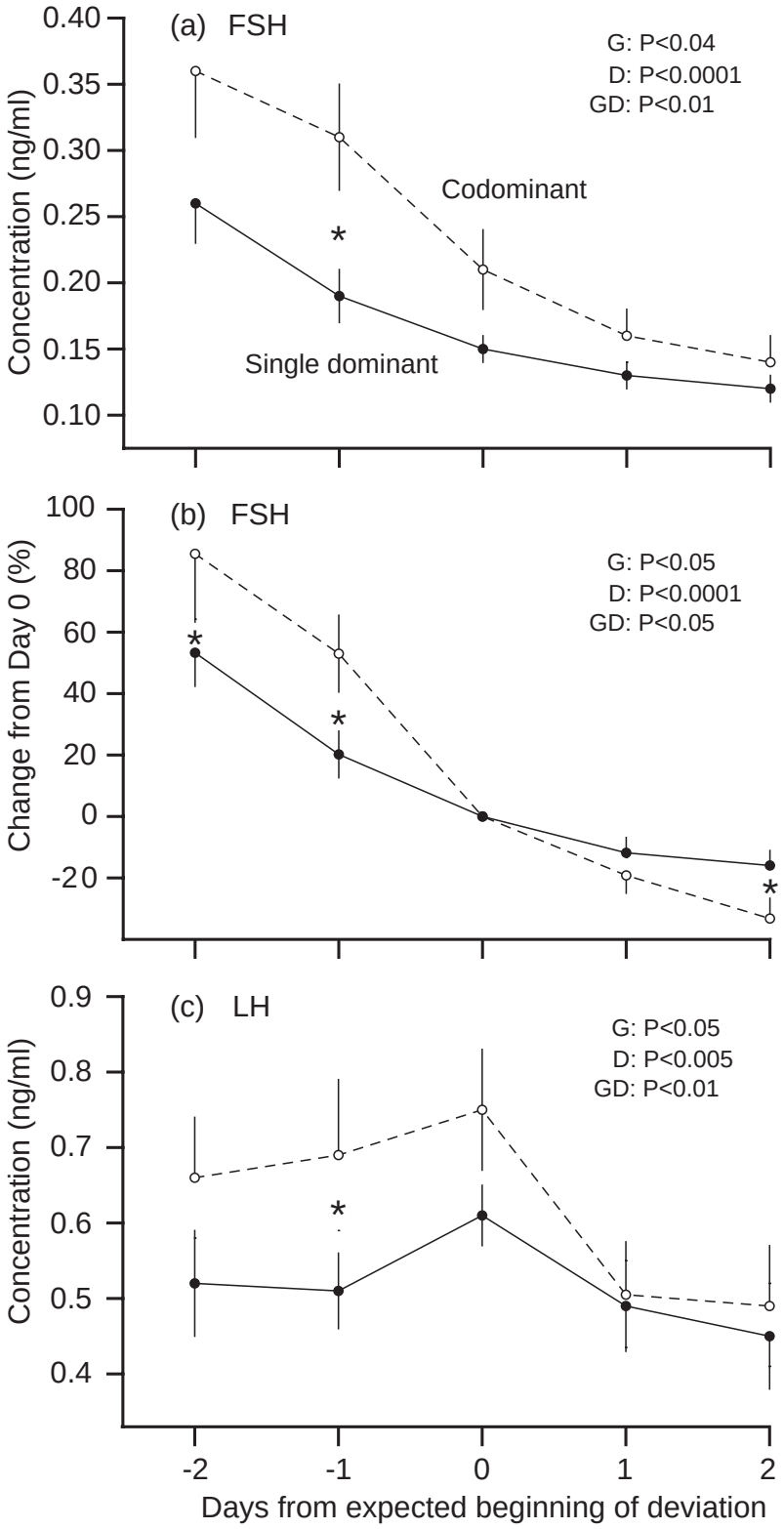

Figure 5. Mean ± SEM concentrations of circulating FSH (a), percentage change in FSH (b), and concentrations of LH (c) in heifers with single dominant (n=14) and double dominant (n=10) follicles. Data were normalized to the day of expected beginning of deviation (Follicle 1 or F1 ≥ 8.2 mm). Probabilities for main effects (G, group; D, day) and the interactions (GD) are shown. An asterisk indicates a difference ($P < 0.05$) between groups within a day.

Table 2. Mean ± SEM for characteristics of observed deviation for single dominants and the first observed deviation for codominants for Wave 2 and Surge 2 totaled over controls and heifers with follicles ablated on Day-4 or Day-7

End points	Single dominant	Codominant
No. heifers	14	10
Total follicles ≥ 4.0 (No.)	9.0 ± 0.6	11.8 ± 0.7*
Emergence to beginning of observed deviation (d)**	2.9 ± 0.2	2.9 ± 0.2
At beginning of observed deviation†		
Diameter largest follicle (mm)	8.6 ± 0.1	8.7 ± 0.2
Diameter second-largest follicle (mm)	7.8 ± 0.2	8.2 ± 0.2
FSH (ng/ml)	0.15 ± 0.01	0.21 ± 0.03*
LH (ng/ml)	0.61 ± 0.04	0.74 ± 0.08*
FSH peak (ng/ml)	0.32 ± 0.03	0.44 ± 0.04*

*Difference (P< 0.05) between groups within a row.
**Emergence refers to first detection of F1 at ≥ 4.0 mm.
† The single deviation in single-dominant heifers and the first deviation in codominant heifers.

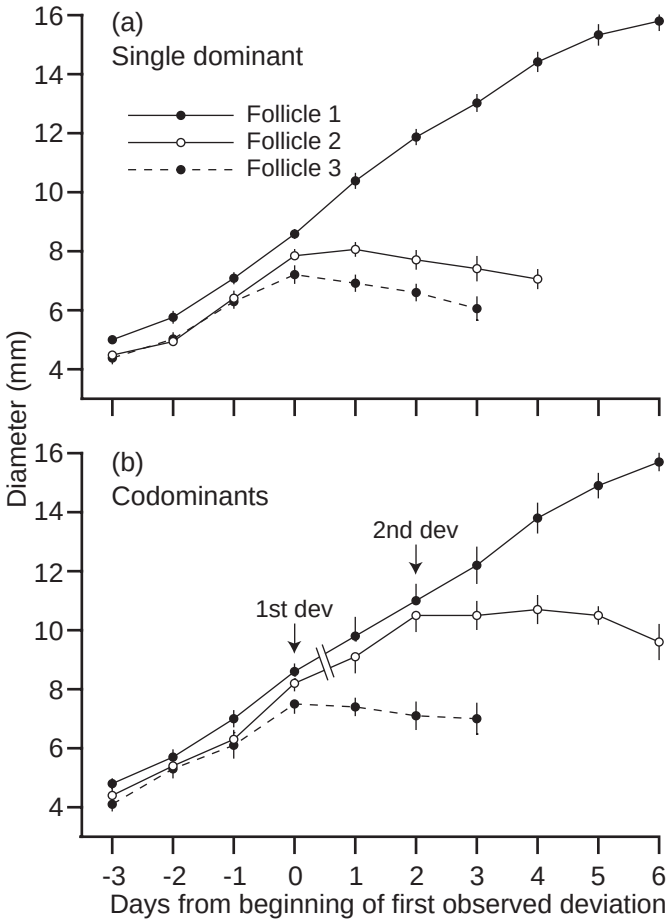

Figure 6. Mean ± SEM diameters of three largest follicles for heifers with a single dominant follicle (a; n=14) and codominant follicles (b; n=10). Data were normalized to the beginning of observed deviation for singletons and to the first observed deviation (1st dev) for codominants. In the codominants, the second deviation (2nd dev) is normalized to the mean day on which it occurred relative to the first deviation. Follicle 2 (F2) was larger (P < 0.0003) in the codominant heifers on each day after the beginning of observed deviation (group-by-day interaction; P < 0.0001), but F1 and F3 did not differ between groups.

Discussion

Wave 1 and Wave 2 were similar in number of follicles per wave, interval between emergence and the beginning of deviation, diameter of F1 and F2 at the beginning of deviation, and FSH concentrations, but diameter of postdeviation F1 and LH concentrations were smaller for Wave 2 than for Wave 1, as previously reported (Kulick *et al*., 2001). The smaller diameter of the dominant follicle of Wave 2 was one of the earliest reported characteristics of bovine follicular waves (Sirois *et al.,* 1988; Ginther *et al.,* 1989). The lower concentrations of LH encompassing the Wave-2 deviation can be attributed to higher progesterone concentrations at mid-diestrus (Ginther *et al*., 2001a). The lower LH may account for the smaller post-deviation diameter of F1 during Wave 2 (Gong *et al*., 1995; Ginther *et al*., 2001a), although the reduced diameter was not apparent until near the end of the differences between waves in LH concentrations. The reduced post-deviation growth of the dominant follicle of Wave 2 may result from reduced intrafollicular estradiol and IGF-1. This assumption is based on the increase in these factors differentially in F1 versus F2 at the beginning of deviation during Wave 1 (Beg *et al*., 2001; 2002; Ginther *et al*., 2002a; 2003b) and the reduction in follicle diameter and follicular-fluid concentrations of these factors when systemic LH is experimentally reduced by exogenous progesterone (Ginther *et al*., 2001a). In the previous study (Kulick *et al*., 2001), a mean transient elevation in LH (increase in concentrations followed by a decrease) with the maximal concentration at the beginning of deviation was found for Wave 1, but the LH elevation during Wave 2 was not significant. In the present study, both an LH increase and decrease from a maximum concentration at the beginning of deviation were documented for each wave, thus demonstrating for the first time an LH elevation in association with the beginning of deviation in Wave 2.

In the 6.5-mm group, all follicles ≥ 5.0 mm, except the two largest, were ablated when the largest was ≥ 6.5 mm (actual diameter, 7.1 ± 0.2 mm). The two largest follicles were retained to assess the follicle response to the expected increase in FSH. As shown retrospectively, the ablation occurred as expected during the declining portion of FSH Surge 1 or between the FSH peak and the beginning of deviation. Ablation of the smaller follicles caused greater FSH concentrations at the expected beginning of deviation, consistent with a report that multiple (> 2) growing follicles ≥ 5.0 mm contribute to the FSH decline (Gibbons *et al*., 1997). The experimental increase in FSH concentrations in the 6.5-mm group resulted in concentrations that were about 38% greater than in controls at the beginning of deviation. However, the greater concentrations did not interfere with deviation as indicated by no difference in the interval from emergence to deviation or in the incidence of codominants. These results indicate that greater FSH concentrations than what occurs normally were accommodated without altering deviation, supporting Hypothesis 1. This result is consistent with the assumption that the time of the beginning of deviation is more dependent on the attainment of critical follicle diameters than on concentrations of FSH reached during the decline in the FSH surge. Thus, the present result provides experimental support for the recent observation of the lack of a relationship between variability in the characteristics of the FSH surge and the length of the interval from emergence to deviation (Haughian *et al*., 2004). Follicle 2 tended to be larger in the 6.5-mm group beginning about 3 days after the beginning of deviation, and F1 attained greater maximum diameter than in the controls. This may have been a delayed manifestation of the greater FSH concentrations, encompassing deviation; there was no indication that LH was involved.

Ablation of follicles on Day 4 during the nadir between Surge 1 and Surge 2 was associated with the most prominent induced FSH surge, in which FSH concentration at the peak was greater than for all other surges and 74% greater than in controls; this result supported Hypothesis 2. Apparently, the low FSH concentrations between surges reflected maximum production of FSH suppressors by the follicles (Ginther *et al*., 2003a), and therefore a more prominent FSH rebound occurred when the suppressors were removed by follicle ablation. The modified FSH surges after ablating follicles during the inclining (Day-7 group) and declining (6.5-mm group) portions of a natural surge were ineffective in stimulating codominance. The incidence of codominance, as well as the prominence of the induced FSH surge, was greater for the Day-4 group (75% incidence) than for the control and Day-7 groups during Wave 2 or for the control and 6.5-mm groups during Wave 1 (25% for each group). Thus, the Day-4 ablations increased the incidence of codominance three-fold over the spontaneous incidence, supporting Hypothesis 3. The greater incidence of codominance occurred only when FSH concentrations were increased at both the peak and at deviation (Day-4 group), but not when concentrations were increased only at the peak (Day-7 group) or only at deviation (6.5-mm group). In this regard, administration of FSH every 12 hours for 2 days beginning at a ≥ 6.0-mm largest follicle resulted in a greater maximum diameter of the subordinate follicles (Adams *et al*., 1993) as in the present Day-4 group. A later study (Ginther *et al*., 2002b) indicated that the reported (Adams *et al*., 1993) dose of FSH maintains FSH concentrations that are as great or greater than the concentrations at the peak of a natural surge.

The greater number of total follicles ≥ 4 mm in association with the more prominent FSH surge in the Day-4 group is consistent with the finding that elevating the FSH peak with exogenous FSH 24 h after ablation of follicles increased the number of follicles in the

resulting wave (Gibbons *et al*., 1997). In the Day-4 and Day-7 groups, the peak FSH concentrations were means of 74% and 37% higher, respectively, than in Wave-2 of the controls. The intermediate peak concentrations in the Day-7 group and intermediate doses of exogenous FSH in the reported study (Gibbons *et al*., 1997) did not increase the number of follicles. Apparently, in both studies the intermediate peak of the induced or manipulated FSH surge was below the threshold needed for stimulating more follicles. The increase in follicle numbers when exogenous FSH is given or endogenous concentrations are increased experimentally seem to contradict the report (Haughian *et al*., 2004) that heifers with more follicles ≥ 5.0 mm during natural follicular waves have less prominent FSH surges. Under natural conditions, however, when increasing numbers of follicles reach 5.0 mm, they increasingly suppress FSH (Gibbons *et al*., 1997), whereas this mechanism may be less effective when FSH concentrations are experimentally increased.

The comparisons between heifers with a single dominant versus codominant follicles during Wave 2 were made by combining the three groups (Wave 2 of control, Day-4, and Day-7 groups). The characteristics of codominance were similar among the three groups, except for the higher incidence of codominance in the Day-4 group. For example, on the day of the greatest difference in the FSH means between codominants and single dominants (1 day before observed deviation), the concentrations in the control, Day-4, and Day-7 groups were 80, 80, and 50% greater, respectively, in the codominant heifers than in the single-dominant heifers. The FSH Surge 2 in the codominant heifers compared to the single-dominant heifers was more prominent throughout the surge as indicated by a 40% greater mean concentration both at the peak and at the beginning of deviation. Greater predeviation concentrations of FSH in heifers with codominant follicles is consistent with a higher incidence of codominance with the prominent FSH surge in the Day-4 group. In previous studies of codominance in heifers, predeviation FSH concentration was not greater for codominance (Beg *et al*., 2003) or only tended to be greater ($P < 0.06$; Kulick *et al*., 2001), but the number of codominant heifers was limited (n=5 or 6). In a recent report (Echternkamp *et al*., 2004), no difference in FSH concentrations was found on days 18–21 of the estrous cycle between cows selected versus unselected for twins. The contrasting result in the present study of greater FSH concentrations in association with codominant follicles than with single dominant follicles can be attributed to the centralization of data to the beginning of deviation. The more prominent FSH surge likely interfered with deviation between F1 and F2 thereby increasing the incidence of codominant follicles (F1 and F2). This interpretation is consistent with the conclusion (discussed in Introduction) that the occurrence of deviation between F1 and F2 in heifers that develop one dominant follicle occurs during declining FSH concentrations.

Greater LH concentrations preceding and at the day of deviation were also associated with the development of codominant follicles. In previous studies (Kulick *et al*., 2001; Beg *et al*., 2003), there were nonsignificant predeviation LH increases in association with codominance. The apparent divergent results can be attributed, at least in part, to more codominant heifers in the present study (n = 14) than in the previous studies (n = 5 or 6). The relative contributions of elevated FSH versus LH to codominance was not determinable in this study.

In heifers with a single dominant follicle, the reduced growth rate of F2 beginning at observed deviation was demonstrated previously to occur in unison with reduced growth rate of F3 (Ginther *et al*., 2001a). For this reason, the first deviation in the codominant heifers was based on a retrospective comparison of diameters of F1 and F3. The characteristics of deviation in heifers with single dominants were similar to those of the first deviation in heifers with codominants. The similarities involved length of the interval from emergence to deviation, diameters of F1 and F2 at deviation, and pattern of regression of F3. The difference involved a post-deviation growth of F2 at an apparently continuing rate in codominants but not in single dominants. The second deviation in the codominant heifers occurred on average 2 days after the first deviation and involved the F1 and F2 dominant follicles. In the present study, a second deviation occurred in all heifers and therefore only one dominant follicle survived. In some codominant heifers, a second deviation does not occur, resulting in full development of the two dominant follicles (Kulick *et al*., 2001). Treatment with PGF2α did not cause ovulation of F2, but the treatment, in retrospect, was given after the second deviation had begun. If the PGF2α had been given earlier, it is likely both follicles would have ovulated. In a study on the acquisition of ovulatory capacity, administration of a high dose of LH when the dominant follicle reached 10 mm resulted in ovulation in 80% of cows (Sartori *et al*., 2001).

In previous studies (Kulick *et al.,* 2001; Beg *et al*., 2003), the second deviation in waves with codominant follicles was associated with a more precipitous post-deviation decrease in FSH than for single dominants. The precipitous decline resulted in lower FSH concentrations in the codominant heifers by about the time the second deviation began. In the present study, significant post-deviation differences in FSH concentrations were not obtained, but the relative or percentage decrease after deviation was greater 2 days after deviation in the codominant group. Apparently, the balance between FSH requirements and the prevailing diameter of follicles is tightly coupled when a codominant follicle is as large as 10 mm, as has been proposed for follicles in which the largest was 8.5 mm (Ginther *et al*., 2000).

In conclusion, ablating all but the two largest follicles after the peak of FSH Surge 1 (F1 ≥ 6.5 mm) resulted in a slower rate of decline in the FSH surge, indicating that the smaller follicles contributed to the FSH decline. The FSH concentrations at the beginning of deviation were 40% greater than in controls, but did not result in codominance or interfere with the day of occurrence of deviation. Our interpretation is that the beginning of deviation between the two largest follicles when the largest reaches about 8.5 mm is more dependent on the attained follicle diameters than on the concentrations of FSH reached during the decline in the FSH surge. Ablating follicles ≥ 5.0 mm during the low FSH concentrations between surges (Day-4) induced the most prominent FSH surge and a greater incidence (75%) of codominant follicles (≥ 10.0 mm) than in controls (25%) or after ablating follicles during the inclining (Day-7; 25%) or declining (6.5 mm F1; 25%) portions of an FSH surge. Day-4 ablations also resulted in more total follicles ≥ 4.0 mm during the common-growth phase of the induced follicular wave.

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