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Reproductive Hormones and Follicular Growth During Development of One or Multiple Dominant Follicles in Cattle[1]

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ABSTRACT

The mechanisms regulating ovulation rate under natural conditions are not yet defined, particularly for monovular species. In the present study, we evaluated ovarian structures (every 12 h by ultrasonography) and circulating hormones (every 6 h) to determine the differences between cows that developed one (single dominant; n = 16), two (double dominant; n = 8), or three (triple dominant; n = 3) dominant follicles. The four largest follicles were tracked retrospectively, and the data were normalized to the time of expected follicular deviation (F1 ≥ 8.5 mm; hour 0). Follicular dynamics from emergence to deviation were similar, whereas after deviation, expected subordinate follicles continued to grow at a rate similar to the dominant follicle. Triple dominants had greater FSH than double dominants (hour −24 to hour −12) and single dominants (hour −42 to hour −6), and double dominants had greater FSH than single dominants (hour −24 to hour −12). Increased circulating estradiol but lower inhibin were observed in cows that developed multiple follicles. In addition, double dominants had greater LH than single dominants (hour −42 to hour −24 and hour −6 to hour 0) and lower progesterone than single dominants (hour −12 and hour −6). Luteal volume was similar between groups, but milk production was greater for codominant than for single-dominant cows. Thus, selection of multiple dominant follicles during high milk production is related to a transient increase in circulating FSH and LH during the 24 h before follicular selection, producing continued postdeviation growth of follicles that ordinarily would have regressed. Increased FSH and LH probably result from decreased circulating inhibin and progesterone in cows that develop codominant follicles.

follicle-stimulating hormone, ovary

INTRODUCTION

The selection of a dominant follicle in monovular species (e.g., cattle, horses, and humans) determines the ovulation rate in females. In cattle and horses, the selection process is characterized by a deviation in the growth rate between the largest (developing dominant) follicle and the smaller (subordinate) follicles of a wave, which is an event termed diameter deviation [1, 2]. Despite substantial research, the mechanisms responsible for the selection of a single dominant follicle in monovular species have not been elucidated completely, although the intrafollicular and hormonal changes associated with follicular selection are becoming increasingly understood [3, 4].

Follicular development in cattle [5, 6] and horses [7, 8] occurs in a wave-like pattern. In both species, emergence of each follicular wave is stimulated by an FSH surge [9, 10]. After emergence, follicles enter a common-growth phase, and the FSH surge begins to decline, reaching nadir levels near the time of deviation [10, 11]. This decline in FSH has been identified as a key component of the selection process, because experimental administration of FSH during the common-growth phase of the wave in cattle [12] and horses [13] prevented deviation and allowed the development of multiple dominant follicles.

A transient increase in circulating LH surrounding deviation has been reported in cattle [14, 15] and mares [10]. Additionally, in both species, the expression of granulosa LH receptors has been reported to increase near deviation in the future dominant follicle [16–18]. The acquisition of LH receptors in the granulosa cells of future dominant follicles might allow the transient LH increase to have a functional effect in the selection process. In this regard, LH is known to stimulate an increase in estradiol concentrations in follicular fluid, which is involved in the continuing depression of FSH to concentrations lower than those required by the smaller follicles, thus facilitating the establishment of dominance [19].

Occasionally, the selection mechanism is altered, and two or more follicles within the same wave become dominant, resulting in a phenomenon that has been termed codominance [15, 20–23]. Codominant follicles can result in multiple ovulations and, in some cases, dizygotic twin births. In lactating dairy cattle, an increase in the frequency of multiple ovulations has been reported, and increased milk production has been identified as the primary factor related to the increase in the incidence of this phenomenon [23, 24]. Multiple births in dairy cattle are undesirable, because cows calving twins have greater risk of peripartum complications than cows calving singletons [25]. Additionally, twin pregnancies have increased risk of embryonic and fetal mortality, and twin calves have reduced birth weight and increased neonatal calf mortality compared to singleton calves [26]. In contrast, in beef cattle maintained under intensive management conditions, multiple births might be desired to increase profitability. In this regard, a cattle population selected for increased twinning rate [27] has been developed and maintained at the U.S. Department of Agriculture (USDA) Meat Animal Research Center, since 1981. Using this population of cattle, ovarian structures and reproductive hormone concentrations have been studied in cows both selected and unselected for twin births [28, 29]. No differences were found in circulating FSH and LH, but

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estradiol was greater in cows selected for high twinning rate. These studies were designed to evaluate the genetics of this twinning population and are somewhat difficult to interpret in a broader sense because of a lack of normalization to follicular profiles and use of all selected cattle in a single group rather than selection of only those cattle that actually developed multiple dominant follicles. Nonetheless, it seems that understanding the mechanisms involved in double ovulation in cattle clearly could have practical implications in agriculture or human medicine.

The cow offers an intriguing opportunity for studying follicular selection, because it allows the use of frequent ultrasound evaluations that can be correlated to circulating hormones. It has been postulated that the deviation mechanism in cattle can be established in less than 8 h [30]. Therefore, intensive collection of data surrounding deviation is required to monitor accurately the follicular development and circulating hormones. Additionally, other authors have reported that the deviation mechanism is studied more effectively when data are normalized to the beginning of deviation because of variation in the interval from emergence to deviation [2]. The high spontaneous codominance and multiple ovulation rate observed in lactating dairy cows provide an opportunity to evaluate the hormonal profiles associated with follicular selection, because codominance represents a natural aberration in the follicular deviation process. Two previous studies provided some information regarding circulating hormones in heifers with codominant follicles [15, 20]. One study [20] found no differences in FSH concentrations before deviation and a tendency toward lower FSH after follicular deviation in heifers with codominant (n = 5) compared to single dominant follicles, but that study provided no further hormonal or follicular comparisons. In contrast, another study [15] found that heifers with codominant follicles (n = 6) tended ($P < 0.1$) to have higher circulating FSH before deviation and lower circulating FSH after deviation. Additionally, in that study, heifers with codominant follicles had differences in LH (treatment × time interaction; $P < 0.05$) and estradiol (higher at most times; $P < 0.01$) compared with single-dominant-follicle heifers. However, it was somewhat difficult to associate these intriguing hormonal changes with follicular selection, because hormonal concentrations were compared only three times before follicular deviation. Interestingly, reproductive hormones and follicular development during selection have not been compared between lactating cows that develop one dominant follicle (single dominant) and multiple dominant (codominant) follicles. Therefore, the purpose of the present study was to determine the circulating reproductive hormones and follicular growth profiles associated with one or multiple dominant follicles during the first follicular wave after ovulation in lactating dairy cows, an experimental model that has an increased incidence of codominant follicles.

MATERIALS AND METHODS

Cows, Ultrasound Data, and Blood Sampling

Thirty-nine lactating Holstein cows (age, 41–64 days) were used in the present study. The Ovsynch protocol as described previously [31] was used to synchronize ovulation in 22 of these cows. Briefly, each cow received 100 μg i.m. of GnRH (Cystorelin; Merial Limited, Iselin, NJ), followed 7 days later by 25 mg i.m. of prostaglandin (PG) $F_{2\alpha}$ (Lutalyse; Pharmacia & Upjohn Co., Kalamazoo, MI), followed 2 days later by a second i.m. treatment of 100 μg of GnRH. For the remaining 17 cows, ovulation was synchronized by the use of 100 μg i.m. of GnRH, followed 7 days later by 25 mg i.m. of $PGF_{2\alpha}$. Cows were housed in a stanchion barn at the Dairy Cattle Research Center in the University of Wisconsin, Madison, where they were fed a total mixed ration ad libitum, had free access to water, and were milked twice daily, with weights recorded at each milking.

Ovarian structures were monitored by transrectal ultrasonography using a 7.5-MHz, linear-array probe (Aloka 900V; Corometrics Medical Systems, Inc., Wallingford, CT). Ultrasonography was performed daily (0800 h) starting on the day of first GnRH treatment and then twice daily (0800 and 2000 h) starting on the day of $PGF_{2\alpha}$ treatment and continuing until the emergence of the second follicular wave. Measurements of ovarian structures were made on a single, frozen image of the apparent maximal area for each structure. Length (L) and width (W) of structures were used to calculate follicular diameter (D) and corpus luteum (CL) volume (V). Follicular diameter was calculated with the formula $D = (L + W)/2$, and CL volume was calculated with the formula $V = 4/3 \times \pi \times R^3$, using a radius ($R$) calculated by the formula $R = (L/2 + W/2)/2$. For CL with a fluid-filled cavity, the volume of the cavity was calculated and subtracted from the total CL volume. Follicles were identified as F1 (largest), F2, F3, and F4 based on retrospective determination of maximum follicular diameter. Follicular and CL profiles as well as hormone concentrations were normalized to the time of expected follicular deviation (F1 ≥ 8.5 mm; hour 0), and data were analyzed from 48 h before (hour −48) to 48 h after deviation (hour 48).

Follicular wave emergence was based on first detection of the future dominant follicle at 4.0 mm. Follicular deviation was defined as occurring during the retrospectively identified ultrasound examination in which the beginning of the greatest difference in growth rates between the largest follicle (i.e., dominant follicle) and the second largest follicle (i.e., largest subordinate follicle) was identified. Codominant follicles were defined as two or more follicles of 10 mm in diameter that had not yet undergone diameter deviation. For codominant cows, deviation was defined as the beginning of the greatest difference in growth rates between the two (double dominants) or the three (triple dominants) largest follicles (i.e., codominant follicles) and the third (double dominants) or fourth (triple dominants) largest follicle (i.e., largest subordinate follicle).

Daily blood samples were collected via coccygeal venipuncture using evacuated tubes (Vacutainer; Becton-Dickinson, Rutherford, NJ) starting the day of first GnRH treatment and then every 6 h starting on the day of $PGF_{2\alpha}$ treatment and continuing until the emergence of the second follicular wave. Samples were centrifuged at 1600 × g for 15 min, and serum was collected and stored frozen at −20°C for later RIA of progesterone, estradiol, FSH, LH, and inhibin. The protocol used in the present experiment was approved by the Animal Care Committee of the College of Agricultural and Life Sciences at the University of Wisconsin, Madison.

Hormone Assays

Assay of progesterone was performed using a solid-phase RIA kit (Coat-A-Count Progesterone; Diagnostics Products Corporation, Los Angeles, CA). Mean assay sensitivity, calculated as 2 SD less than the mean counts per minute of maximum binding, was 0.008 ng/ml. Intra- and interassay coefficients of variation were 7.2% and 4.2%, respectively. Assay of estradiol was performed according to the procedure previously described by Kulick et al. [14]. Mean assay sensitivity and intra- and interassay coefficients of variation were 0.15 pg/ml, 6.4%, and 4.3%, respectively. Serum FSH and LH were measured by RIA validated for cattle [32, 33]. The FSH assay incorporated the primary antibody NIDDK-anti-oFSH-I-2 and the radiolabeled and standard antigen USDA-bFSH-I-2. Mean assay sensitivity and intra- and interassay coefficients of variation were 0.05 ng/ml, 5.8%, and 2.5%, respectively. The LH assay incorporated USDA-bLH-B-6 for iodination and reference standards and USDA-309-684p as the primary antiserum. Mean assay sensitivity and intra- and interassay coefficients of variation were 0.11 ng/ml, 4.8%, and 3.1%, respectively. Serum immunoreactive (ir)-inhibin was determined using a RIA kit (Institute of Reproduction and Development, Monash Medical Center, Clayton, VIC, Australia) as previously described by Beg et al. [16]. The assay cross-reacts with the full-length forms of the α subunit as well as the various intact forms of inhibin [34]. The assay was considered to detect total inhibin plus free α subunit. Mean assay sensitivity and intra- and interassay coefficients of variation were 4.13 ng/ml, 5.5%, and 2.4%, respectively.

Statistical Analysis

Follicular diameter, CL volume, hormone concentrations, and milk production were compared by the MIXED procedure of SAS with a repeated statement and a first-order autoregressive structure to account for the cor-

relation between measurements [35]. Main effects of group and hour and their interaction were determined. Differences between continuous data such as follicular diameter on a specific hour were compared between groups by Student *t*-test.

RESULTS

After retrospective evaluation of follicular profiles for the 39 cows, 12 were removed from further analysis, either because they were not identified clearly as either single or codominant cows by ultrasonography or because they had missing values for follicular diameter. Of the remaining 27 cows, 16 developed one dominant follicle (single dominant), and 11 developed multiple dominant follicles (codominant) during the first follicular wave. Of the codominant cows, three developed three dominant follicles (triple dominant), and eight developed two dominant follicles (double dominant). Figure 1 shows individual follicular profiles and circulating FSH concentrations for representative cows that developed triple, double, and single dominant follicles from 48 h before to 48 h after expected deviation. Although some general uniformity to the profiles was observed, clear variation between individual cows in both circulating FSH and follicular profiles also was observed. Figure 2 shows the average follicular profiles for cows with triple, double, or single dominant follicles from 48 h before to 48 h after expected deviation. Time from emergence to deviation (ranges are ± SEM throughout) was similar ($P > 0.10$) for all groups (60 ± 7, 62 ± 4, and 62 ± 2 h for triple-, double-, and single-dominant cows, respectively). Similarly, mean diameter and growth rates of F1, F2, and F3 were similar ($P > 0.10$) between groups from hour −48 until deviation (hour 0). Diameters at de-

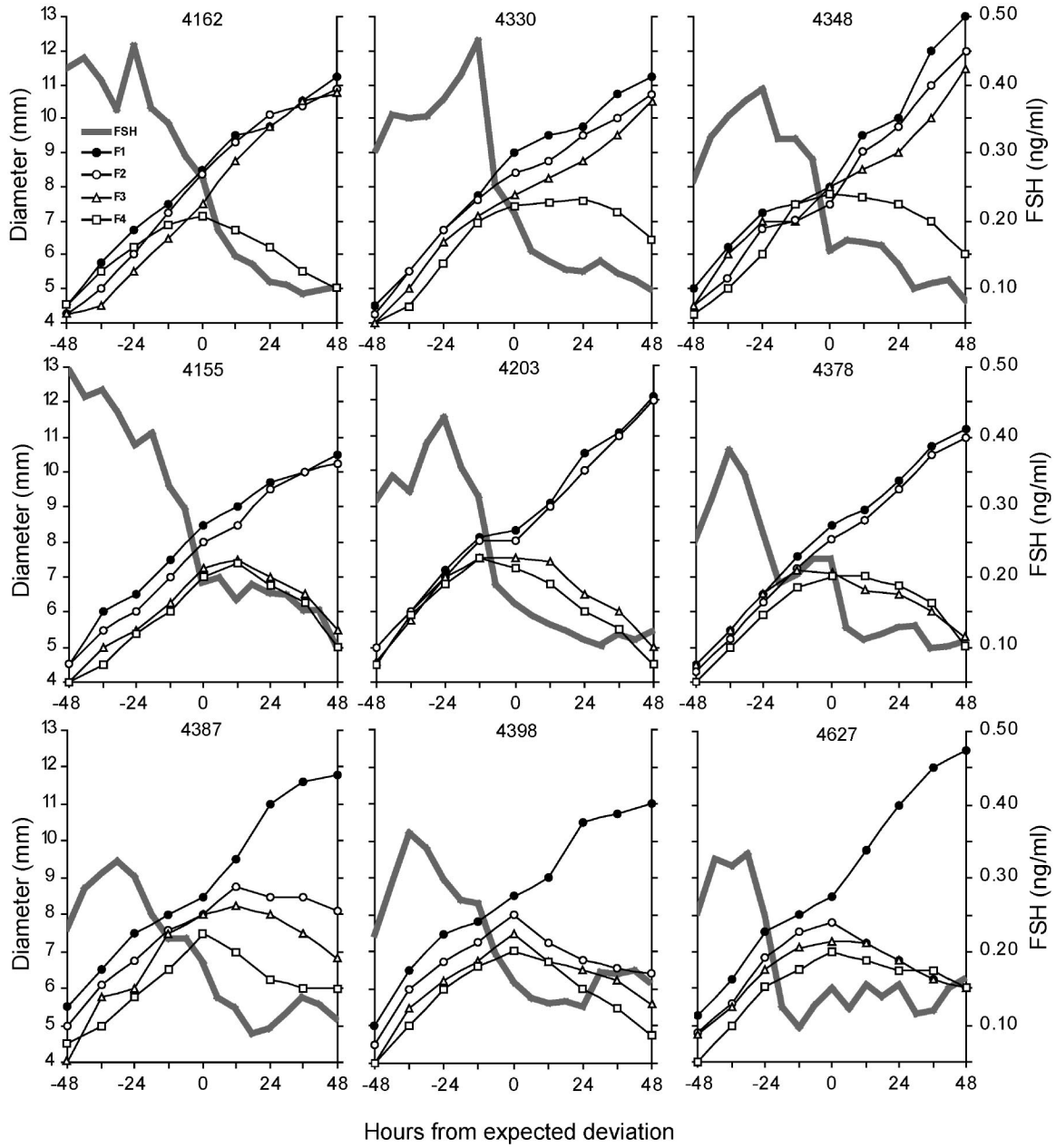

FIG. 1. Follicular profiles and circulating FSH concentrations for triple-dominant (n = 3), double-dominant (n = 3), and single-dominant (n = 3) cows. Diameters of F1, F2, F3, and F4 are based on retrospective determination of maximum follicular diameter. Data are normalized to the beginning of expected deviation (F1 ≥ 8.5 mm; hour 0).

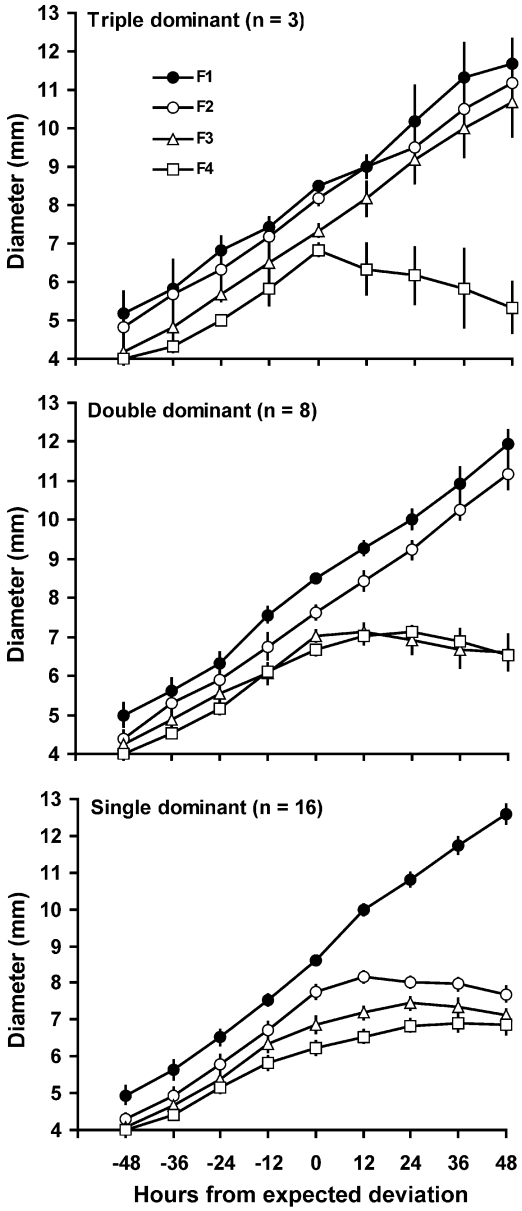

FIG. 2. Follicular profiles for triple-dominant (n = 3), double-dominant (n = 8), and single-dominant (n = 16) cows. Diameters (mean ± SEM) of F1, F2, F3, and F4 are based on retrospective determination of maximum follicular diameter. Data are normalized to the beginning of expected deviation (F1 ≥ 8.5 mm; hour 0).

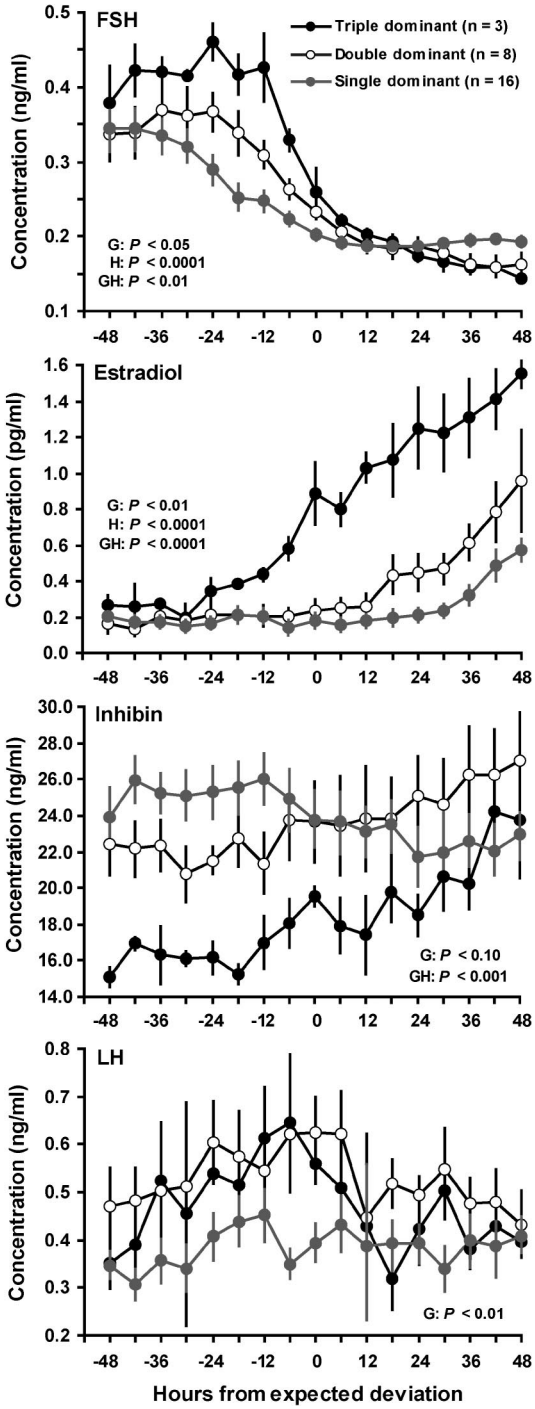

FIG. 3. Circulating concentrations (mean ± SEM) of FSH, estradiol, inhibin, and LH for triple-dominant (n = 3), double-dominant (n = 8), and single-dominant (n = 16) cows. Data are normalized to the beginning of expected deviation (F1 ≥ 8.5 mm; hour 0). Significant main effects of group (G), hour (H), and their interaction (GH) are shown.

viation were 8.5 ± 0.0, 8.5 ± 0.0, and 8.6 ± 0.1 mm for F1 follicles; 8.2 ± 0.2, 7.6 ± 0.2, and 7.8 ± 0.2 mm for F2 follicles; and 7.3 ± 0.2, 7.0 ± 0.1, and 6.9 ± 0.2 mm for F3 follicles for triple-, double-, and single-dominant cows, respectively. Following deviation, cows with codominant follicles had larger ($P < 0.005$) F2 from hour 24 to hour 48 than single-dominant cows, and triple-dominant cows had larger ($P < 0.005$) F3 from hour 24 to hour 48 than double- and single-dominant cows.

Figure 3 shows circulating reproductive hormone profiles for single-, double-, and triple-dominant cows. Concentrations of FSH showed an effect of group ($P < 0.05$), hour ($P < 0.0001$), and an interaction ($P < 0.01$). Mean FSH concentrations declined over time, reaching nadir at hours 24, 42, and 48 after expected deviation for single-, double-, and triple-dominant cows, respectively. Triple-dominant cows had greater ($P < 0.05$) FSH concentrations than double-dominant cows at hours −24, −18, and −12 and than single-dominant cows from hour −42 to hour −6. Double-dominant cows had greater ($P < 0.05$) FSH concentrations than single-dominant cows at hours −24, −18, and −12.

Concentrations of estradiol showed a main effect of group ($P < 0.01$), hour ($P < 0.0001$), and an interaction ($P < 0.0001$). Triple-dominant cows had greater ($P < 0.05$) estradiol concentrations than both double-dominant and sin-

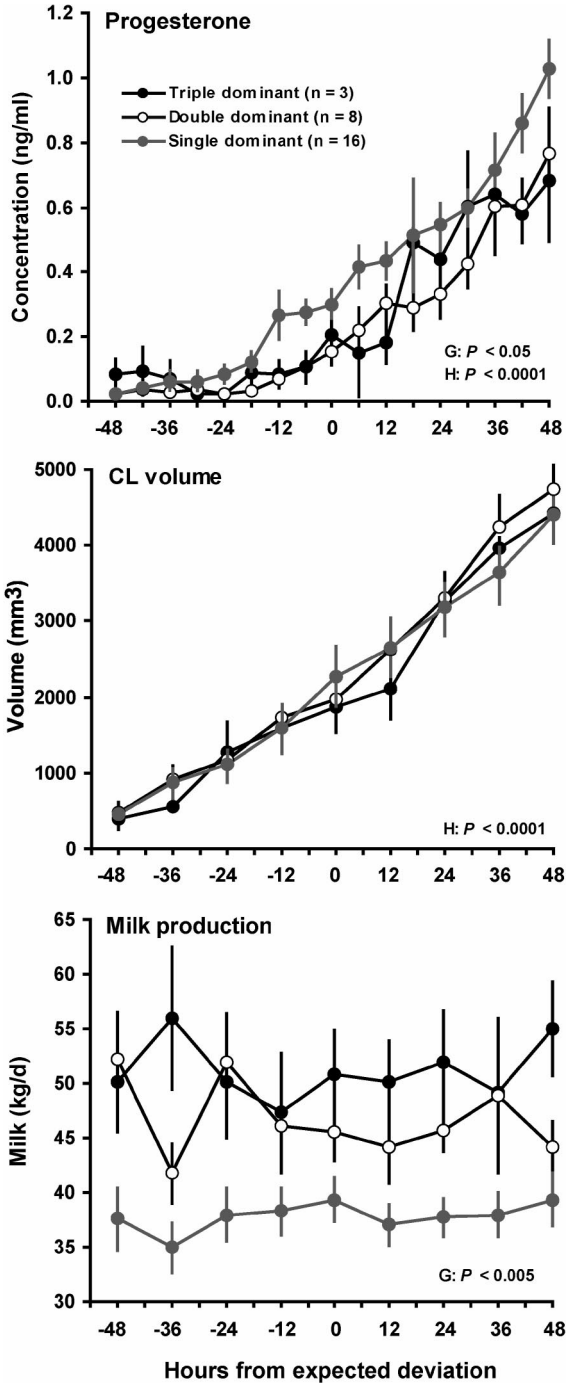

FIG. 4. Concentrations (mean ± SEM) of progesterone, CL volume, and milk production for triple-dominant (n = 3), double-dominant (n = 8), and single-dominant (n = 16) cows. Data are normalized to the beginning of expected deviation (F1 ≥ 8.5 mm; hour 0). Significant main effects of group (G), hour (H), and their interaction (GH) are shown.

gle-dominant cows from hour −12 to hour 48. Double-dominant cows had greater ($P < 0.05$) estradiol concentrations than single-dominant cows from hour 18 to hour 48.

Concentrations of ir-inhibin showed a main effect of group ($P < 0.10$) and an interaction of group with time ($P < 0.001$). Triple-dominant cows had lower ($P < 0.10$) ir-inhibin concentrations than double-dominant cows at hours −48, −42, −30, −24, −18, 12, and 24 than single-dominant cows ($P < 0.05$) from hour −48 to hour −12. Double-dominant cows had lower ($P < 0.10$) ir-inhibin concentrations than single-dominant cows at hours −30, −24, and −12.

Concentrations of LH showed a main effect of group ($P < 0.01$). Double-dominant cows had greater ($P < 0.05$) LH concentrations than single-dominant cows from hour −42 to hour −24 and from hour −6 to hour 6, whereas triple-dominant cows had greater ($P < 0.05$) LH concentrations than single-dominant cows only at hour −6.

Figure 4 shows circulating progesterone concentrations, CL volume, and milk production for cows that developed single, double, and triple dominant follicles. Concentrations of progesterone showed a main effect of group ($P < 0.05$) and hour ($P < 0.0001$). Triple-dominant cows had lower ($P < 0.05$) progesterone concentrations than single-dominant cows at hours 6, 12, 42, and 48. Double-dominant cows had lower ($P < 0.05$) progesterone concentrations than single-dominant cows at hours −12, −6, 6, 18, 24, 30, 42, and 48. Luteal volume increased over time for all groups, showing only a main effect of hour ($P < 0.0001$). Milk production was greater ($P < 0.05$) in codominant than in single-dominant cows. Triple-dominant cows had greater ($P < 0.05$) milk production than single-dominant cows from hour −48 to hour −24 and from hour 0 to hour 48. Milk production was greater ($P < 0.10$) in double-dominant cows than in single-dominant cows from hour −48 to hour −12 and from hour 12 to hour 36. Average milk production was different ($P < 0.01$) between the three groups, with production of 51.2 ± 1.4, 46.7 ± 1.3, and 37.8 ± 0.7 kg/day for triple-, double-, and single-dominant cows, respectively.

DISCUSSION

The design of the present study allowed determination of the differences in circulating reproductive hormones and follicular growth between cows that developed single or codominant follicles during the first follicular wave after ovulation. In addition, because three of the cows developed three dominant follicles, it was possible, to our knowledge for the first time, to characterize the follicular dynamics and circulating hormones in a monovular species with naturally occurring triple follicular dominance. We normalized all data to the time when the largest follicle first reached a diameter of 8.5 mm or larger, which is the expected beginning of follicular deviation. This produced a common normalization point to analyze follicular dynamics and circulating hormonal concentrations between cows that had selection of differing numbers of dominant follicles. Follicular dynamics up to the time of expected deviation was surprisingly similar between groups; however, clear differences were observed in circulating reproductive hormones before deviation that likely are important for changing the follicular selection process, resulting in selection of multiple rather than one dominant follicle.

The initial analysis of follicular dynamics up to the time of expected deviation produced many values that would have been expected based on previous scientific literature. For example, intervals from emergence to deviation did not differ between groups (60–62 h) and were similar to those previously reported in cattle (60.4 ± 4.2 h [11] and 61.0 ± 3.7 h [14]). Because of the normalization process, it was known that the mean diameter of F1 at hour 0 would be similar between groups (~8.5 mm); however, the mean diameters of F2 and F3 also were similar between groups at hour 0 and were similar to those of previous reports. For example, Ginther [36] summarized several reports in cattle and determined that the mean diameters of the two largest

follicles at the onset of deviation were 8.5 and 7.7 mm, with deviation beginning, on average, 2.5 days after first detection of the future dominant follicle at 4 mm. Our data regarding cows with single dominant follicles were similar to these values. However, two scenarios could have occurred in codominant cows that would have been consistent with very different physiological models. In one scenario, deviation could have been delayed in cows with codominant follicles and, perhaps, would have occurred at the time that the smallest dominant follicle reached 8.5 mm. This scenario would have been consistent with a given size of dominant follicle being important for continued growth of the follicle after deviation. However, in the present study and in a previous study of codominant heifers [15], deviation of the subordinate follicles occurred at the time the largest dominant follicle reached, on average, 8.5 mm, regardless of the number of dominant follicles that were being selected. This suggests that the F2 follicle was physiologically different in the cows with double dominant or triple dominant follicles despite this follicle being similar in size to the future subordinate follicle in the single-dominant-follicle group. Similarly, the F3 follicle was a similar diameter in the three groups (~7 mm) despite the finding that by the next ultrasound examination, this follicle would be regressing in the single-dominant and double-dominant cows but continuing to grow in the triple-dominant cows. This leads to the speculation that in codominant cows, the smaller follicles (F2 in double dominants and F2 and F3 in triple dominants) have undergone the changes that result in a dominant follicle that can continue to grow with low circulating FSH, not that codominance resulted from a delay in the time of follicular deviation. It seems likely that predeviation changes in circulating reproductive hormones produced these postdeviation changes in follicular dynamics.

The most dramatic and consistent hormonal difference between the three groups was the elevation in circulating FSH at 24, 18, 12, and 6 h before deviation. Some previous studies have not detected this difference in circulating FSH in heifers with codominant follicles [20] or in cows with a history of twinning [28]. However, those studies either had low numbers of codominant animals [20] or normalized data to time of estrus (not deviation) and did not specifically use only cows that had codominant follicles [28]. However, consistent with the present results, a previous study [15] found a tendency for greater FSH concentrations in codominant than in single-dominant heifers 24 h before expected deviation. Near the time of deviation and for approximately 30 h after deviation, FSH concentrations reached minimal concentrations in all groups despite the differences in number of dominant follicles that continued to grow after this time. After 36 h, a tendency toward reduced FSH was observed in cows with double or triple dominant follicles, which is consistent with the findings of other studies [15, 20] and with the elevated estradiol in cows with multiple dominant follicles.

Previous studies in cattle that measured circulating estradiol during follicular development reported that the time of estradiol increase is in close proximity with the onset of deviation [14, 19]. We observed a premature increase in circulating estradiol in codominant cows that was particularly apparent in cows that developed triple dominant follicles. This may reflect a greater number of follicles involved in the production of estradiol and, perhaps, an earlier differentiation of follicles into the dominant follicle phenotype. Nevertheless, the elevation in estradiol in cows with codominant follicles seems to rule out our previous speculation [23] that increased FSH is the result of reduced circulating estradiol.

The relationship between growing follicles and the decline in FSH has been described in a two-way functional coupling model in which inhibin has been identified as the primary FSH suppressant before deviation [3, 37]. In the present study, some caution should be exercised in interpreting the ir-inhibin information, because our assay measured only the α subunit of inhibin. Notwithstanding, the simplest explanation for the elevated predeviation FSH concentrations in cows with codominant follicles is the observed depression in ir-inhibin concentrations during the predeviation period. This depression was particularly apparent in the cows with triple dominant follicles. Because of the well-known suppressive action of inhibin on FSH secretion, it is tempting to propose that lower circulating concentrations of inhibin at these times might have allowed the higher FSH concentrations observed in the codominant group. In this regard, reduction of circulating inhibin by treatment with an inhibin antiserum has been shown to increase plasma FSH concentrations and ovulation rate in heifers [38], cows [39], sheep [40], goats [41, 42], and mares [43]. Conversely, administration of inhibin, either in the form of steroid-free bovine follicular fluid or in a purified form, to intact or ovariectomized heifers resulted in suppressed levels of circulating FSH and, in intact heifers, inhibition of follicle growth greater than 5 mm in diameter [44–46]. In humans, it has been suggested that inhibin B limits the duration of the FSH rise through negative feedback at the pituitary level and, therefore, may be critical in the selection of a single dominant follicle [47]. A potential model that might explain the age-related increase in risk of dizygotic twins in women has been proposed [48, 49]. In that model, the increased circulating FSH concentrations and the corresponding increased incidence of multiple ovulations observed in ovulating premenopausal women relate to the decrease in inhibin secretion caused by a diminished follicular pool in older women [50]. Extending that model to the present study, lower inhibin would lead to elevated FSH concentrations in cows with codominant follicles. However, the reasons for the depressed inhibin concentrations in the codominant cows are not clear. Perhaps, as in aging women, reduced numbers of follicles are present in a follicular wave in codominant cows. The present study did not quantify the absolute number of follicles in a follicular wave and, therefore, cannot rule out this explanation. Alternatively, perhaps inhibin metabolism is elevated in high-producing dairy cows, as previously shown for metabolism of estradiol and progesterone [51].

The role of LH in follicular growth is well described, and changes in LH also may be important in the increasing rate of codominance. It has been reported that LH has a role in the growth and function of the largest follicles after the beginning of deviation [52] and that LH pulse frequency during this period is influenced negatively by an increased level of circulating progesterone [53]. In the present study, circulating progesterone was lower in cows with codominant compared with single dominant follicles. This reduction in progesterone results from the elevated milk production in codominant cows and the increased metabolism of progesterone [51]. A reduction in circulating progesterone would be expected to elevate LH pulse frequency, and this could be reflected in the highly variable but statistically significant elevation in predeviation mean LH. Previous studies in cattle [52, 54] and horses [10] have found a tran-

sient increase in LH concentrations surrounding the time of deviation. It has been postulated that this increase in LH might be involved in the deviation mechanism, because LH is known to stimulate the production of estradiol and insulin-like growth factor (IGF)-I [3, 52]. These intrafollicular factors, among others, might increase responsiveness of the largest follicles to the decreasing concentrations of FSH at deviation. The numerous effects of free IGF-I include stimulation of granulosa and theca cell proliferation, increased numbers of FSH and LH receptors, and enhanced steroidogenesis by granulosa and theca cells [4, 55]. A previous study found greater follicular fluid and plasma IGF-I concentrations in beef cows selected for twinning than in unselected control cows [56]. Another study in dairy heifers found a transient increase in LH concentrations at the time of deviation for both the first and second follicular waves [15]. The profile for elevated LH was similar between the two waves. However, the concentrations were greater for wave 1 than for wave 2, and these differences were attributed to the expected lower progesterone concentrations during wave 1. Interestingly, in that study, the incidence of codominant follicles was greater for wave 1 (35%) than for wave 2 (4%). Obviously, sampling LH concentrations at 6-h intervals is not sufficient to detect accurately changes in LH pulse frequency, and future studies are needed to determine if codominant cows have elevated LH pulse frequency and/or amplitude. Nevertheless, an elevation in predeviation LH concentrations, perhaps coupled with elevated FSH, could be important in stimulating the changes that are necessary to allow a follicle to change from a subordinate to a dominant phenotype. It also is tempting to speculate that the elevation in GnRH that may be necessary to increase circulating LH concentrations may be important for the elevation in circulating FSH during the predeviation period in cows that develop codominant follicles.

In summary, the process of deviation was preceded, on average, by increased FSH and LH concentrations and lower ir-inhibin and progesterone concentrations in cows that developed multiple dominant follicles compared with cows that developed a single dominant follicle. Lower circulating inhibin may produce the increased circulating FSH, whereas lower progesterone may allow increased LH concentrations in codominant cows. These hormonal differences might have promoted development of a dominant phenotype in the future largest subordinate follicle and allowed continued growth despite depressed FSH and deviation of other subordinate follicles. The lack of elevation in circulating FSH and LH in codominant cows after deviation argues that the number of dominant follicles was already determined by the circulating hormones and intrafollicular changes occurring before deviation. Increased level of milk production might be a factor contributing to the increased incidence of multiple ovulation observed in lactating dairy cows, possibly through increased metabolism of progesterone. The results of the present study provide a framework for future manipulative studies regarding the physiology of codominance in lactating dairy cows.

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