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Relationship Between Level of Milk Production and Multiple Ovulations in Lactating Dairy Cows*

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ABSTRACT

Our objective was to evaluate factors associated with spontaneous multiple ovulations in lactating dairy cows. Ovaries of cows [n = 267; >50 days in milk (DIM)] were evaluated weekly using ultrasound to determine spontaneous (i.e., no hormonal treatment) ovulation rate starting at 50 DIM and continuing until pregnancy diagnosis. Cows were fitted with a transmitter to record standing activity during estrus, and serum progesterone concentration was assessed weekly starting at wk 1 postpartum for all cows. Overall, 76 (28.5%) cows were anovular and 191 (71.5%) were ovular by 71 DIM. Incidence of anovulation was not associated with level of milk production but was associated with lower body condition. For anovular cows (n = 41) that spontaneously recovered, the multiple ovulation rate at first ovulation was 46.3%. For second and subsequent ovulations (n = 463), the level of milk production for 14 d preceding estrus was associated with increased ovulation rate. To illustrate, incidence of multiple ovulations was 1.6% (2/128), 16.9% (32/189), and 47.9% (70/146) for ovulations when cows were producing <35, 35 to <45, and ≥45 kg/d, respectively. Among cows for which estrous behavior was recorded, those with multiple ovulations (n = 48) had shorter duration of estrus (4.3 ± 0.7 vs. 9.9 ± 0.5 h) and higher production (47.2 ± 0.9 vs. 38.1 ± 0.5 kg/d) than cows with single ovulations (n = 237). Circulating concentrations of estradiol were lower (5.5 ± 0.3; n = 15 vs. 7.8 ± 0.4 pg/mL; n = 71) during periods of estrus with multiple ovulations despite a greater preovulatory follicular volume (4136 ± 123 vs. 3085 ± 110 mm^3). Similarly, serum progesterone concentration 7 d after estrus was lower for cows with multiple than single ovulations (2.5 ± 0.3 vs. 3.2 ± 0.1 ng/mL) despite a greater luteal volume (8291 ± 516 vs. 6405 ± 158 mm^3). In summary, the first spontaneous ovulation in anovular cows and a higher level of milk production for 14 d preceding estrus were associated with increased multiple ovulation rate. Additionally, cows with multiple ovulations had lower estradiol at estrus, a shorter duration of estrus, and lower progesterone at 7 d after estrus than cows with single ovulations.
(**Key words:** dairy cow, multiple ovulation, milk production, anovulation)

Abbreviation key: CL = corpus luteum/corpora lutea, $\mathbf{E_2}$ = estradiol, $\mathbf{P_4}$ = progesterone.

INTRODUCTION

The process of selection of the dominant follicle during the ovulatory wave determines the ovulation rate in cattle. This process is characterized by a deviation in the growth rate between the largest (developing dominant) follicle and the smaller (subordinate) follicles of the wave, an event that has been termed diameter deviation (Ginther et al., 1997). Occasionally, the follicular selection process is altered and 2 or more follicles within a wave become dominant, resulting in a phenomenon termed codominance (Kulick et al., 2001). Because both codominant follicles have deviated from the subordinate follicles and acquired ovulatory capacity, they are responsive to ovulatory stimuli leading to the occurrence of multiple ovulations. If oocytes from such ovulations are fertilized and pregnancy is maintained until parturition, dizygotic twins will occur. One recent study that evaluated the frequency of monozygotic twins in dairy cattle by an evaluation of polymorphic microsatellite markers reported a frequency of monozygotic twins of 3.9% (Silva Del Rio et al., 2004). Thus, it appears that the frequency of monozygous twinning is low and, therefore, nearly all twins in dairy cattle are of the dizygotic type resulting from multiple ovulations.

Twinning in dairy cattle is undesirable because it reduces reproductive efficiency and profitability (Nielen et al., 1989; Day et al., 1995; Kinsel et al., 1998). Numerous factors are associated with twinning in dairy cattle including: age of dam, season, genetics, use of antibiot-

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ics or reproductive hormones, ovarian cysts, and peak milk production (reviewed in Wiltbank et al., 2000). Because of the current trend for increased milk yield in modern cows, there is growing concern about the association between milk production and twinning (Fricke, 2001). Interestingly, some studies (Nielen et al., 1989; Kinsel et al., 1998) but not others (Deluyker et al., 1991) have reported an association between those 2 factors.

Early studies evaluating the number of corpora lutea (**CL**) by rectal palpation reported multiple ovulation rates ranging from 5 to 13% (Kidder et al., 1952; Labhsetwar et al., 1963). Unfortunately, the level of milk production was not reported in those studies. Nonetheless, recent studies with high-producing cows (35 to 45 kg/d; Fricke and Wiltbank, 1999; Santos et al., 2000; Sartori et al., 2004; López-Gatius et al., 2005) have reported greater multiple ovulation rates ranging from 14 to 39%, suggesting an increase in this reproductive measure in modern cows.

The objective of this study was to evaluate factors associated with spontaneous multiple ovulation rate and anovulation in lactating dairy cows. In contrast to previous studies, this experiment used the average milk production near the time of ovulation (during the 14 d preceding estrus) to evaluate the association between milk yield and multiple ovulations.

MATERIALS AND METHODS

Animals and Procedures

A total of 267 cows (131 primiparous and 136 multiparous) were assigned at calving to this study. During the first 3 wk of lactation, cows were housed in a tie-stall barn and fed a transition diet ad libitum. Cows were subsequently moved to a free-stall barn where they were offered a balanced TMR ad libitum and remained during the experimental period. Cows were milked at 0500 and 1700 h, and milk weights were recorded at each milking. All cows were administered bST (500 mg, Posilac; Monsanto Co., St. Louis, MO) every 2 wk, beginning at 60 to 66 DIM. The protocol used in this experiment was approved by the Animal Care Committee of the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

Weekly transrectal ultrasonography was performed for all cows with a 7.5-MHz probe (Aloka 500 ultrasound machine; Corometrics Medical Systems Inc., Wallingford, CT) starting at 50 DIM and continuing until pregnancy was diagnosed (~30 d post AI). Ultrasound measurements of follicles and CL [length (L) and width (W)] were recorded from a single frozen image of the apparent maximal area of each structure and were used to calculate the volume (V). Follicular and CL volume were 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. Multiple ovulation was retrospectively determined by presence of multiple CL and confirmed in most cows by presence of multiple dominant follicles in preovulatory ultrasound examinations. For cows with multiple preovulatory follicles and new CL after multiple ovulations, total follicular and luteal volumes were obtained by the addition of the volumes of the single corresponding structures.

Occurrence of the first ovulation postpartum was determined by analyses of serum progesterone (**P_4**; ≥1 ng/mL) from blood samples collected weekly starting 1 wk postpartum and continuing until cows were diagnosed pregnant or were 200 DIM. Anovulatory condition was defined by the absence of CL during the first 3 weekly ultrasound examinations after 50 DIM and by concentrations of serum P_4 (<1 ng/mL) from weekly samples. Cows that were anovular by 100 DIM received the Ovsynch protocol (Pursley et al., 1995) for synchronization of ovulation (n = 35). Ovulations induced by this protocol were not included in the analyses for this study.

All cows were assigned a BCS once per month. Scoring was conducted by 3 individuals using a 5-point scale with 1 = very thin and 5 = obese (Wildman et al., 1982), and the mean of the 3 scores was recorded. Body condition scoring was done for all cows on a given day; therefore, scores did not coincide with a selected day or week of lactation.

Characterization of Estrous Behavior

At 50 DIM, all cows were fitted with a pressure-sensitive radiotelemetric transmitter (HeatWatch, DDx Inc., Denver, CO) that allowed for continuous recording of standing activity. Activation of the transmitter by the weight of a mounting herdmate for a minimum of 2 s inhibits a radio-wave transmission, and generating real-time data (date, time, duration, cow number, and transmitter number). Onset of estrus was identified by the first activation of the transmitter. Duration of estrus was defined as the interval from the first to last standing event recorded during an estrous period; thus, periods of estrus consisting of only 1 standing event were excluded for this measure. All standing activity recorded by the radiotelemetry system was evaluated in relation to farm records for visual detection of estrus and AI, daily milk yield, and retrospective analyses of P_4. Additionally, ovulation was confirmed for all periods of estrus by the appearance of a new CL during the ultrasound examination after standing activity was recorded by the radiotelemetry system.

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Blood Collection and Radioimmunoassays

Weekly blood samples were obtained from each cow via venipuncture of the median caudal vein starting 1 wk postpartum and continuing until a positive pregnancy diagnosis or 200 DIM for nonpregnant cows. Samples were placed immediately on ice and arrived at the laboratory within 8 h of collection. Blood samples were centrifuged (3000 × *g* for 15 min) and serum was collected and stored at –20°C. Assay of P_4 was performed using solid-phase radioimmunoassay kits (Coat-A-Count Progesterone, DPC, Los Angeles, CA). Mean assay sensitivity, calculated as 2 SD below the mean counts per minute of maximum binding, was 0.02 ng/mL. Intra- and interassay coefficients of variation were 5.2 and 7.7%, respectively.

Follicular size was recorded and serum estradiol (**E_2**) concentrations were determined in a subset of cows on the day of estrus (n = 86; 61 to 165 d postpartum) as determined by radiotelemetry. Blood samples for E_2 were collected, processed, and stored as described above for P_4 samples. A single assay of E_2 was performed according to the procedure described by Kulick et al. (1999). Mean assay sensitivity, calculated as 2 SD below the mean counts per minute of maximum binding, was 0.4 pg/mL. Intraassay coefficient of variation was 1.2%.

Statistical Analyses

Evaluation of factors associated with the incidence of anovulation was performed by the LOGISTIC procedure of SAS (SAS Institute, 1998). The statistical model included cow, parity [treated as class with 2 levels (first and above first)], change in BCS between the closest scores recorded at 30 and 60 DIM, BCS closest to 60 DIM, average milk production during the first 70 DIM, and the average milk production from 50 to 70 DIM. Regression analysis was performed by the REG procedure of SAS (SAS Institute, 1996) to determine the best fitted line plot for the association between BCS closest to 60 DIM and the incidence of anovulation. Evaluation of the factors associated with multiple ovulations was performed by the MIXED procedure of SAS (Littell et al., 1996). For that analysis, the binomial distribution (macro GLIMMIX of SAS) was assumed for the response variable "number of ovulations" (y = 0 for single ovulation and y = 1 for more than 1 ovulation). The model included parity [treated as class with 2 levels (first and > first)], DIM, milk production during the 14 d preceding estrus, and cow, which was treated as a random effect. Additionally, the quadratic terms and interactions between main factors were included in this analysis. Using the estimates for the fixed effects and the *P* values obtained in the logistic regression analysis, the probability of multiple ovulation (y = 1) was calculated by the formula $P = 1/1 + e^{-(b_0 + b_1X_1 + b_2X_2 + \ldots + b_iX_i)}$. Duration of estrus, size of ovarian structures, concentrations of circulating E_2 and P_4, and milk production were compared between single and multiple ovulations by Student's *t*-test. The relationship between milk production and circulating E_2 and P_4, between milk production and follicular and CL volume, and between milk production and circulating E_2 and P_4 per unit volume of follicle and CL were analyzed by the REG procedure of SAS (SAS Institute, 1996). The statistical model included cow, DIM, and milk production during the 10 d preceding estrus for the evaluation of E_2 and follicular volume and during the 7 d after estrus for the evaluation of P_4 and luteal volume. The level of milk production from the 14 d before to the 14 d after estrus was evaluated for periods of estrus with single and multiple ovulations by the MIXED procedure of SAS (Littell et al., 1998; SAS Institute, 1998). Because some cows had more than 1 period of estrus recorded, the variable cow was introduced in the model as a random effect next to a fixed effect for periods of estrus with single and multiple ovulations. A probability of $P < 0.05$ was defined as significant for this study.

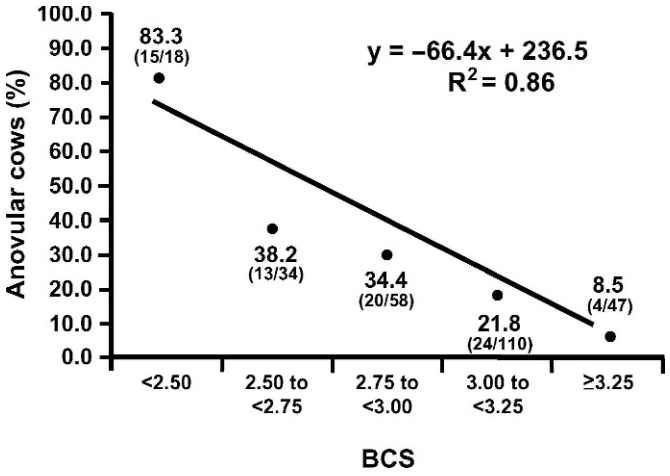

Figure 1. Negative linear relationship ($P < 0.05$) between BCS closest to 60 d postpartum and incidence of anovulation at 71 d postpartum.

RESULTS AND DISCUSSION

Anovulation and First Postpartum Ovulation

All 267 cows were included in the analyses of anovulation. Incidence of anovulation at 71 DIM was associated with a greater change in BCS between the closest scores recorded at 30 and 60 DIM ($P < 0.005$) and greater change in BCS between the scores closest to 30 and 60 DIM ($P < 0.0001$; Figure 1). In contrast, parity (P =

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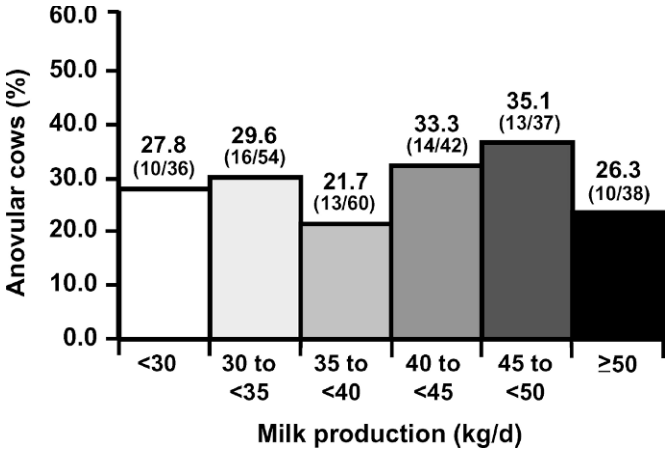

Figure 2. Incidence of anovulation at 71 d postpartum for cows assigned to milk production groups based on mean milk production from 50 to 70 d postpartum.

0.77), average milk production during the first 70 DIM (P = 0.65), and average milk production from 50 to 70 DIM (P = 0.72; Figure 2) were not associated with the incidence of anovulation at 71 DIM. Cows that lost BCS between 30 and 60 DIM had a greater incidence of anovulation by 71 DIM than cows that maintained or gained BCS during the same period [38.0 (38/100) vs. 22.8% (38/167); P = 0.008] despite having similar milk production during the first 70 DIM (35.1 ± 0.8 vs. 36.6 ± 0.6 kg/d; P = 0.16). A linear relationship was found between BCS closest to 60 DIM and the incidence of anovulation at 71 DIM with the frequency of anovular cows decreasing as BCS increased (Figure 1). Similar to our results, Gümen et al. (2003) found a linear relationship between BCS at 47 to 53 DIM (range ≤2.25 to ≥3.25) and the incidence of anovulation by 53 DIM. However, Moreira et al. (2001) reported a quadratic effect of BCS at 60 DIM (range 2.0 to 4.5) and Santos et al. (2004) at 70 DIM (range 2.25 to 3.75) on the incidence of anovulation by 60 and 70 DIM, respectively. In the Santos study as in ours, a loss in BCS from 37 to 70 DIM, but not milk production during the first 3 mo of lactation, was associated with increased incidence of anovulation at 70 DIM. In spite of the strong association between lower BCS and delayed first postpartum ovulation, it is intriguing that at 71 DIM most [63.1%; (48/76)] anovular cows had good BCS (≥2.75). Similarly, in the study by Gümen et al. (2003), 62.5% (40/64) of the anovular cows at 60 DIM had BCS ≥2.75. Those observations suggest that reasons other than low BCS might preclude most of the anovular cows from ovulating by 60 to 70 DIM.

The incidence of anovulation at 71 DIM did not differ between primiparous and multiparous cows [29.0% (38/131) vs. 27.9% (38/136); P = 0.84]. The 2 parity groups had similar BCS closest to 60 DIM (3.02 ± 0.02 for primiparous and 2.97 ± 0.03 for multiparous; P = 0.11) even though multiparous cows produced more milk during the first 70 DIM (41.5 ± 0.6 vs. 30.4 ± 0.5 kg/d; P < 0.0001). A similar rate of anovulation in primiparous and multiparous cows differs from some reports (Gümen et al., 2003; Santos et al., 2004) but agrees with others (Cartmill et al., 2001; Bartolome et al., 2002). Unfortunately, BCS or milk production was not reported in the previous studies for primiparous and multiparous cows.

In the present study 53.9% (41/76) of the anovular cows at 71 DIM spontaneously recovered from the condition before 100 DIM. On average, those cows maintained or gained body condition between 60 and 100 DIM, whereas those cows that remained anovular were still losing condition during this period (0.1 ± 0.0 vs. –0.2 ± 0.1 BCS units; P < 0.001). Nevertheless, milk production was similar from 70 to 100 DIM for cows that recovered and for those that were still anovular by 100 DIM (41.9 ± 1.1 vs. 39.1 ± 1.4 kg/d, respectively; P = 0.10).

Multiple ovulation rate was 46.3% (19/41) for first ovulations of cows that spontaneously recovered from the anovulatory condition between 70 and 100 DIM. No differences were detected in change in BCS between 60 and 100 DIM (–0.1 ± 0.1 BCS units vs. 0.1 ± 0.1 BCS units; P = 0.16) or in milk production from 70 to 100 DIM (42.8 ± 1.7 vs. 41.1 ± 1.6 kg/d; P = 0.45) between cows ovulating 1 or multiple follicles at first postpartum ovulation. Anovular cows that reached 100 DIM [46.1% (35/76)] had ovulation synchronized (Ovsynch), and most of those cows [88.6% (31/35)] had the first P_4 increase detected by 125 DIM after they received Ovsynch.

Multiple Ovulations in Cycling Cows

For second and subsequent ovulations, multiple ovulation rate was 22.4% (104/463), and average milk production during the 14 d preceding estrus was 40.7 ± 0.4 kg/d. This multiple ovulation rate is within the range of 15 to 39% reported recently for high-producing lactating cows (35 to 45 kg/d; Santos et al., 2000; Sartori et al., 2004; López-Gatius et al., 2005). In our experiment, the level of milk production for the 14 d preceding estrus and DIM were associated with increased incidence of multiple ovulations. Figure 3 shows the actual incidence of multiple ovulations in relation to milk production. Additionally, a tendency for an interaction between the level of milk production during the 14 d preceding estrus and DIM (P = 0.07), and parity (P = 0.08) on increased incidence of multiple ovulation was de-

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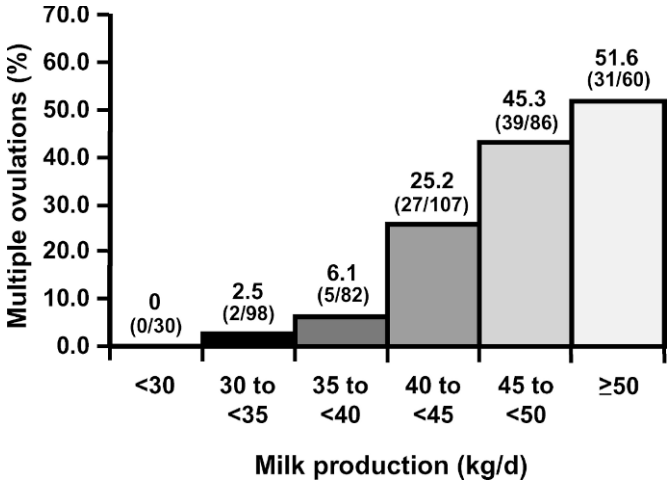

Figure 3. Incidence of multiple ovulations for cows assigned to milk production groups based on mean milk production during the 14 d preceding estrus.

tected. The estimates for the fixed effects and the corresponding P values for the regression used to evaluate those associations were: intercept (–49.13), parity = 1 (–0.96; P = 0.08), DIM (–0.16; P = 0.0001), quadratic effect of DIM (0.0004; P = 0.002), average milk production for the 14 d before estrus (2.18; P < 0.0001), quadratic effect of average milk production for the 14 d before estrus (–0.02; P < 0.0001), and interaction between average milk production for the 14 d before estrus and DIM (0.001; P = 0.07). When those estimates were used to calculate the probability (P) of multiple ovulation (y = 1) by replacing them in the formula $P = 1/1 + e^{-b_0 + b_1X_1 + b_2X_2 + \ldots + b_iX_i)}$; the probability of multiple ovulation increased as the level of milk production during the 14 d preceding estrus increased (Figure 4). The

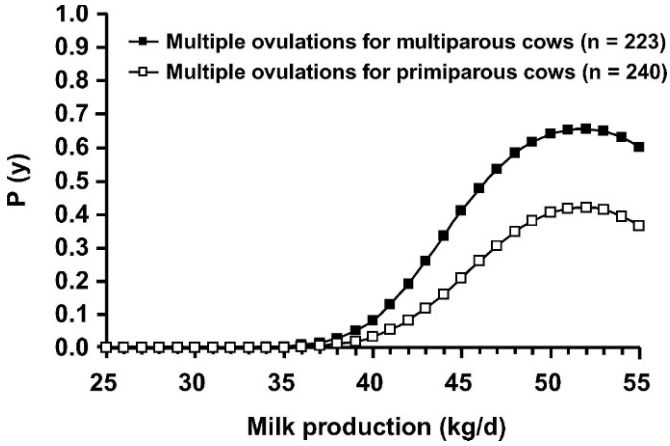

Figure 4. The relationship between the probability (y) of multiple ovulation and level of milk production during the 14 d preceding estrus.

model for the probability of multiple ovulation is: P (y) = EXP (–49.13) + (–0.96 X_1) + (–0.16 X_2) + (0.0004 X_3) + (2.18 X_4) + (–0.02 X_5) + (0.001 X_6); where X_1 = parity (first or above first), X_2 = DIM, X_3 = quadratic effect of DIM, X_4 = average milk production for the 14 d before estrus, X_5 = quadratic effect of average milk production for the 14 d before estrus, and X_6 = interaction between average milk production for the 14 d before estrus and DIM.

Similar to the findings for this study, one experiment evaluating double ovulation rate in lactating cows that received Ovsynch reported a positive association between higher level of milk production and double ovulations (Fricke and Wiltbank, 1999). In that study, cows with greater than average milk production 2 d before timed AI (>40.0 kg/d; n = 94) had a greater incidence of double ovulation (20.2 vs. 6.9%; P < 0.05) than cows with less than average milk production 2 d before timed AI (≤40.0 kg/d; n = 102). In contrast, another study evaluating multiple ovulation rate for synchronized and natural ovulations (López-Gatius et al., 2005) reported an antagonistic association between higher level of milk production and the incidence of multiple ovulations.

Several factors might explain the variation in the results from those studies. For example, first postpartum ovulations may have been included in the analyses, the cows had different levels of production [(40.5 ± 0.8 kg/d) Fricke and Wiltbank, 1999; (35.6 ± 9.7 kg) López-Gatius et al., 2005; (40.7 ± 0.4 kg/d) for the present study], and milk production data differed (2 d before AI: Fricke and Wiltbank, 1999; day of AI: López-Gatius et al., 2005; average of 14 d before estrus: present study). Based on the results discussed above, first postpartum ovulation is associated with an increased incidence of multiple ovulations that is unrelated to level of milk production. In addition, it appears that the dramatic increase in multiple ovulation rate occurs at levels of milk production above 40 kg/d, a level that may be achieved by relatively few cows in some studies. Further, the present (data presented below) and some previous studies (Lewis and Newman, 1984; Walton and King, 1986) show a reduction in milk production around the time of naturally occurring estrus that is not consistent between lower and higher producing cows. The effects of milk production on hormonal changes that cause selection of a single or multiple dominant follicles probably occur a few days before estrus when follicular deviation occurs (Lopez et al., 2005). Thus, future studies of multiple ovulation rate should consider using an average milk production during the few days before estrus/ovulation, use a complete regression analyses of the data because the relationship primarily is observed above 40 kg/d of milk production,

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and should analyze first ovulation separate from other ovulations.

In contrast to the limited number of studies that have evaluated the association between milk production and ovulation rate, there is abundant evidence suggesting a positive relationship between higher milk yield and twinning rate (Nielen et al., 1989; Kinsel et al., 1998). One epidemiological study of twinning (Kinsel et al., 1998) using 52,362 lactations from 260 dairies from 1983 to 1992 reported that twinning rate increased steadily during the experimental period from 1.4 to 2.4% (range 0 to 9.6%). In that study, cows calving twins had, on average, 2.7 kg greater peak milk production [33.9 (n = 1276) vs. 31.2 kg/d (n = 51,086)] than cows calving singletons.

Multiple ovulation rate [17.9 (43/240) vs. 27.3% (61/223); $P = 0.01$] and milk production during the 14 d preceding estrus (39.2 ± 0.5 vs. 42.0 ± 0.5 kg/d; $P < 0.0001$) were lower for ovulations recorded from primiparous than multiparous cows. Additionally, a potential effect of parity ($P = 0.08$) was detected by the regression analysis evaluating factors associated with multiple ovulations. When average milk production during the 14 d preceding estrus was used to divide data into ovulations that occurred when milk production was higher or lower, multiple ovulation rate was greater ($P < 0.001$) for ovulations from high-producing primiparous [33.0% (37/112); 46.0 ± 0.4 kg/d] and multiparous cows [41.1% (53/129); 47.6 ± 0.3 kg/d] than for ovulations from low-producing primiparous [4.7% (6/128); 33.3 ± 0.3 kg/d] and multiparous cows [8.5% (8/94); 34.9 ± 0.5 kg/d].

Similarly, Fricke and Wiltbank (1999) reported that the incidence of double ovulation tended to increase ($P = 0.09$) with increasing parity. Additionally, in that study when cows were classified by average milk production 2 d before timed AI (≤40.0 or >40.0 kg/d), double ovulation rate was greater for primiparous [22.2% (2/9)] and multiparous high producers [20.0% (17/85)] than for primiparous [7.4% (4/54)] and multiparous low producers [6.2% (7/102)]. Likewise, López-Gatius et al. (2005) reported an increase in double ovulation with increasing parity from 6.7% (46/689) for first-lactation cows to 16.6% (89/536) for second-lactation cows, and to 25.0% (142/567) for third- or higher-lactation cows.

Potential Mechanisms for Multiple Ovulations

The mechanisms regulating ovulation rate under natural conditions are not defined in cattle. Nonetheless, some intriguing hormonal differences between single dominant and codominant heifers and cows have been reported. Kulick et al. (2001) found that heifers with codominant follicles had higher predeviation FSH, LH, and E_2 concentrations than heifers with a single dominant follicle. Lopez et al. (2005) found greater predeviation FSH and LH, and lower inhibin and P_4 in cows with codominant compared with single dominant follicles. Consistent with our current results, in this last study, cows with codominant follicles produced more milk than cows with single dominant follicles (47.9 ± 1.0 vs. 37.8 ± 0.7 kg/d). Results for these studies suggest that differences in the endocrine environment before deviation might play a role in the occurrence of codominance. Another common observation of studies evaluating follicular development and ovulation rate in cattle is the high incidence of codominance and multiple ovulations in animals that developed the ovulatory follicular wave in low or undetectable concentrations of P_4. One study in beef heifers (Bailey et al., 2004) in which the ovulatory wave was synchronized to emerge and grow in the presence (high P_4) or absence (low P_4) of CL found multiple ovulation rates of 37.5 vs. 0% for waves that grew in the low vs. high P_4 environment, respectively. Similarly, Matsui et al. (2004) reported incidences of codominance and multiple ovulations in both cows and heifers of 75 and 60%, respectively, for waves that grew in a low P_4 environment. Extending this concept to the current study, in cows that spontaneously recovered from the anovular condition and ovulated for the first time, the ovulatory wave likely grew in the absence of P_4 and, this scenario could be the reason for the increased multiple ovulation rate observed in those cows.

A similar scenario could occur in cycling high-producing cows that also were found to have a reduction in circulating P_4 (discussed below). This could be caused by increased metabolism of this hormone due to the increased feed consumption and liver blood flow associated with high milk production (Sangsritavong et al., 2002). A reduction in circulating P_4 would be expected to increase GnRH and LH pulse frequency (Adams et al., 1992) and this could be the reason for the observed predeviation increase in LH reported in animals with codominant follicles (Kulick et al., 2001; Lopez et al., 2005). This increase in LH may be involved in the deviation mechanism because LH stimulates the production of E_2 and insulin-like growth factor 1 (Ginther et al., 2001). These intrafollicular factors, among others, might increase responsiveness of the largest follicle(s) to the decreasing concentrations of FSH at deviation, thereby allowing more than 1 follicle to become dominant. The elevation in GnRH pulses may also increase circulating FSH before follicular deviation in a similar fashion to that observed in animals with codominant follicles (Kulick et al., 2001; Lopez et al., 2005). Alternatively, or in addition to the above hormonal changes, increases in growth hormone, either endogenous or exogenous, could also increase circulating IGF-I and this

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could have a role in increasing number of dominant follicles. Obviously, our study has highlighted the intriguing relationship between double ovulation rate and milk production but future intensive and manipulative studies will be needed to differentiate the numerous possible mechanisms that could be causing this relationship.

Thus, we would speculate that the absence of circulating P_4 during development of the preovulatory wave in anovular cows might have allowed for an increase in predeviation LH concentrations, perhaps coupled with increased FSH, and this might have lead to an increased number of selected follicles and multiple ovulations. Similarly, lower circulating concentrations of P_4 during development of the preovulatory wave in cows with high milk production might have allowed similar predeviation hormone alterations and this might have lead to an increased number of selected follicles and multiple ovulations in higher producing cows. Nevertheless, manipulative studies on the physiology of codominance in lactating dairy cows are required to validate these speculations.

Duration of Estrus, Circulating Steroids, and Ovarian Structures in Multiple-Ovulating Cows

Among cows for which estrous behavior was recorded, those with multiple ovulations (n = 48) had shorter ($P < 0.0001$) duration of estrus [4.3 ± 0.7 (0.4 to 19.2 h) vs. 9.9 ± 0.5 h (0.4 to 26.5 h)] and higher production (47.2 ± 0.9 vs. 38.1 ± 0.5 kg/d; $P < 0.001$) than cows with single ovulations (n = 237). Data on duration of estrus, ovarian structures, circulating hormones, and milk production associated with multiple and single ovulations for a subset of cows in which all this information was collected on the day of estrus and 7 d after estrus are included in Table 1. In this subset of cows, duration of estrus was also lower for cows with multiple compared with single ovulations, and circulating concentrations of E_2 were lower for periods of estrus associated with multiple ovulations despite a greater preovulatory follicular volume. Similarly, 7 d later, serum P_4 concentrations for cows with multiple ovulations were less than for cows with single ovulations despite a greater CL volume.

The poor correlation observed between ovarian structures and circulating hormones in this analysis might be caused by either a reduced production of E_2 and P_4 by the follicles and CL of the cows evaluated or by an increased metabolic clearance rate of these hormones leading to circulating concentrations that were less than expected. To our knowledge, no studies have validly evaluated whether follicles and CL of high-producing lactating cows have lower E_2 and P_4 production than low-producing cows or heifers. Current evidence suggests that the low circulating concentrations of these hormones observed in high-producing cows might be caused by increased steroid metabolism associated with higher milk production and feed consumption (Sangsritavong et al., 2002).

Data from this study on single and multiple ovulations were used to evaluate the association between milk production and size of ovarian structures and circulating steroid hormones (Figure 5). Milk production during the 10 d preceding estrus and 7 d after the day of estrus was negatively related to circulating concentrations of E_2 and P_4, respectively (Figure 5A and B). Milk production during the 10 d preceding estrus was positively associated with follicular volume on the day of estrus (Figure 5C). Luteal volume was also positively correlated with milk production during the 7 d after estrus (Figure 5D). Multiple ovulators had some of the lowest circulating E_2 and P_4 concentrations despite having greater follicular and luteal volumes. We also analyzed the relationship between milk production vs. circulating concentrations of E_2 and P_4 by follicular and luteal unit volumes. This analysis showed the highest relationship with milk production (R^2 = 0.44 to 0.47). Thus, these results are consistent with peak concentration of E_2 (or P_4) and peak follicular or luteal volume being primarily related to milk production perhaps due to increasing steroid metabolism with increasing milk production. Surprisingly, circulating concentrations of E_2 and P_4 were not correlated with follicular volume on the day of estrus ($r = -0.24$; $P = 0.24$) or with CL volume 7 d after estrus ($r = -0.17$; $P = 0.11$), respectively. Duration of estrus was positively associated with circulating concentrations of E_2 ($r = 0.57$; $P < 0.0001$) but negatively associated with level of milk production during the 10 d before the day of estrus ($r = -0.60$; $P < 0.0001$).

Previous authors have also reported a poor correlation between ovarian structures and circulating hormones in dairy cows with natural ovulations (Inbar et al., 2001; Sartori et al., 2002; Sartori et al., 2004). For example, Sartori et al. (2004) reported that lactating cows (single and double ovulators) despite having greater ovulatory size follicles than heifers had lower circulating E_2 concentrations from luteolysis to ovulation. Similarly, 7 d after ovulation, P_4 concentrations were lower in cows than heifers despite greater CL volume in cows. Differences in P_4 persisted from 7 d after ovulation until luteolysis even though total CL volume was greater for cows during this period.

Therefore, results from the present and previous studies suggest that there is a poor correlation between

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Table 1. Milk production, duration of estrus, ovarian structures, and circulating hormones [mean ± SEM (range)] for a subset of cows in which blood samples and ultrasonography of ovarian structures was conducted on the day of estrus and 7 d later.

	Multiple ovulations n = 15	Single ovulations n = 71	*P* value
Day of estrus			
Duration of estrus,[1] h	4.0 ± 1.0 (0.4 to 15.7)	9.8 ± 0.9 (0.4 to 26.5)	0.004
Follicular volume,[2] mm^3	4136 ± 123 (3363 to 5198)	3085 ± 110 (1436 to 6795)	<0.0001
Estradiol, pg/mL	5.5 ± 0.3 (4.1 to 9.1)	7.8 ± 0.4 (2.2 to 14.6)	0.005
Milk production,[3] kg/d	49.9 ± 0.8 (45.6 to 55.3)	39.9 ± 1.0 (20.5 to 56.1)	<0.0001
Days postpartum,[4] d	90 ± 8 (61 to 165)	97 ± 3 (61 to 157)	0.40
Seven days after estrus			
Luteal volume,[2] mm^3	8291 ± 516 (5198 to 12,370)	6405 ± 158 (2144 to 8181)	<0.0001
Progesterone, ng/mL	2.5 ± 0.3 (1.1 to 4.5)	3.2 ± 0.1 (1.3 to 6.3)	0.04
Milk production,[5] kg	49.3 ± 0.5 (45.5 to 55.2)	38.5 ± 0.4 (18.9 to 57.0)	<0.0001

[1]Number of hours between the first and the last standing events of a period of estrus.

[2]Total follicular and luteal volumes were obtained by the addition of the volumes of the single corresponding structures.

[3]Average milk production for the 14 d preceding estrus.

[4]DIM when information on estrous behavior was collected by radiotelemetry.

[5]Average milk production for the 7 d after the day of estrus.

ovarian structures (follicle and CL) and circulating hormones (E_2 and P_4) in high-producing cows and that this alteration might be caused by increased metabolism of those hormones during high milk production. Furthermore, such an alteration in circulating hormones might play an important role in the incidence of codominance and multiple ovulations.

Milk Production at Estrus

The level of milk production was evaluated from 14 d before to 14 d after estrus for second and subsequent ovulations. In general, multiple ovulations occurred during higher milk production than single ovulations (47.5 ± 0.2 vs. 38.8 ± 0.1; $P < 0.001$). Single ovulations were further classified by the average milk production of the cow at the time of estrus as single ovulations occurring during higher (≥38.8 kg/d) or lower milk production (<38.8 kg/d). Milk production was greater ($P < 0.0001$) for cows with multiple ovulations than for cows with single ovulations (Figure 6). In the same analysis, a reduction in milk production was observed around the time of estrus. To estimate this reduction, the difference between average milk production during the 10 d preceding estrus (used as a baseline) and milk production on the day of estrus was calculated for all periods of estrus. Overall, there was a reduction of 2.7 ± 0.3 kg of milk (6.4%) on the day of estrus. Interestingly, reduction in milk was less ($P < 0.05$) for periods of estrus associated with multiple ovulations [reduction = 1.6 ± 0.5 kg (2.6%)] and single ovulations in cows producing ≥38.8 kg/d [reduction = 2.4 ± 0.4 kg (4.8%)] than for periods of estrus associated with single ovulations for cows producing <38.8 kg/d [reduction = 3.5 ± 0.4 kg (9.6%)].

Previous studies have reported a reduction in milk production around the time of spontaneous estrus (Lewis and Newman, 1984; Walton and King, 1986) but no previous studies have evaluated the effect of milk yield or double ovulation on this reduction. This reduction might be caused by a decrease in feed intake (Walton and King, 1986) for cows in estrus because they spend less time feeding than herdmates not in estrus (Esslemont and Bryant, 1976). For example, Walton and King (1986) reported a decrease in milk yield (from 7 to more that 35%) and in feed intake (>15%) on the day of estrus. For the present study, smaller reductions in milk production observed for periods of estrus occurring during higher milk production might be related to shorter and perhaps less intense expression of estrus due to reduced circulating concentrations of E_2 in high-producing cows. Conversely, the greater reduction in

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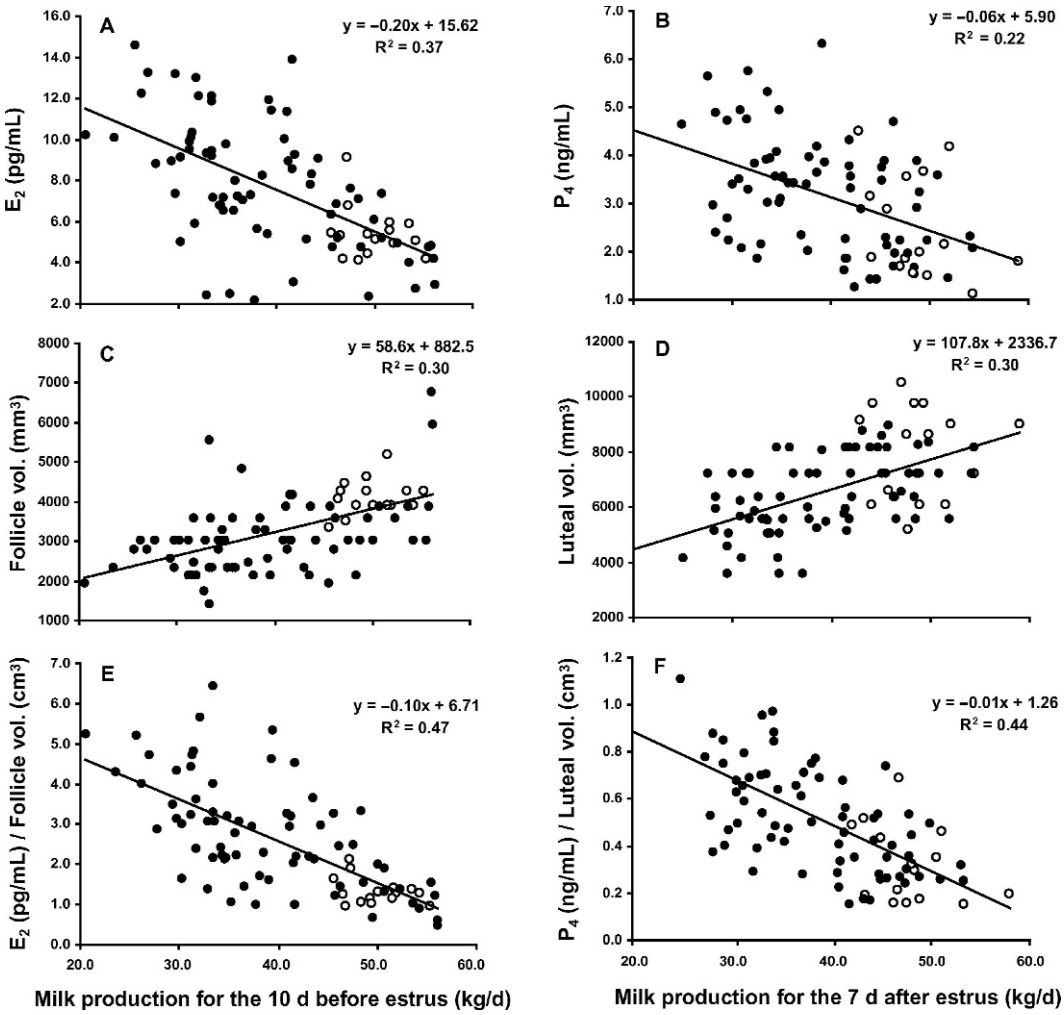

Figure 5. Relationship between circulating concentrations of estradiol (E_2) and progesterone (P_4) and the level of milk production (A, B), between follicular and luteal volume and the level of milk production (C, D), and between circulating concentrations of E_2 and P_4 by unit volume of follicle and corpus luteum (CL) and the level of milk production (E, F). Solid circles represent single ovulations (n = 71); open circles represent multiple ovulations (n = 15). A linear relationship ($P < 0.001$) was identified for all analyses.

milk production observed for periods of estrus during lower production might be related to longer and perhaps more intense expression of estrus due to greater circulating concentrations of E_2 in low-producing cows.

CONCLUSIONS

In summary, the first spontaneous ovulation occurring in anovular cows and a higher level of milk production for 14 d preceding estrus were associated with increased multiple ovulation rate. Results for this study might partially explain the increasing incidence of multiple ovulations and twinning currently observed in high-producing cows. Additionally, based on the association between higher milk production and multiple ovulation rate observed in this study, it might be expected that twinning rates will continue to increase along with milk production in future years. Thus, research needs to be conducted to develop management strategies for improving the health and performance of periparturient twin-carrying cows or alternatively to modify the hormonal changes that are producing the increased multiple ovulation rate caused by high milk production.

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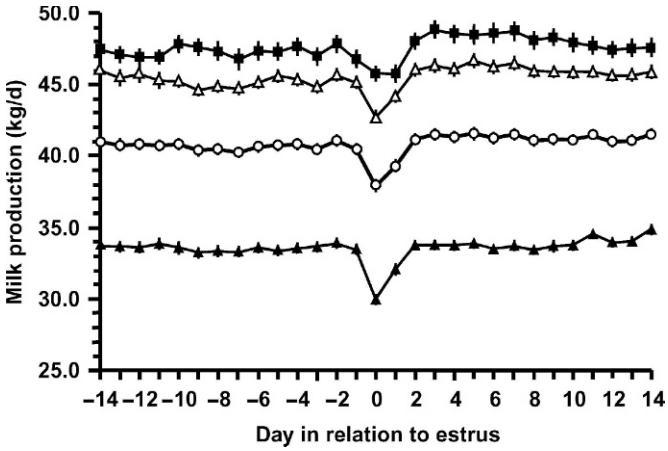

Figure 6. Mean (± SEM) milk production of Holstein cows for 14 d before to 14 d after periods of estrus associated with multiple vs. single ovulations. Solid squares represent milk production for periods of estrus associated with multiple ovulations (n = 104), open triangles represent milk production for periods of estrus with single ovulations during higher milk production (n = 160), solid triangles represent milk production for periods of estrus associated with single ovulations during lower milk production (n = 199), and open circles represent milk production associated with all ovulations.

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