Reduced Dry Periods and Varying Prepartum Diets Alter Postpartum Ovulation and Reproductive Measures

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ABSTRACT

There has been substantial recent interest in shortening dry periods; however, the effects of this management change on reproduction have not been adequately evaluated. Holstein cows ($n = 58$) were assigned in a randomized block design to 1 of 3 treatments: 1) traditional (T) dry period (~56 d) in which cows were fed a low energy diet from 56 to 29 d prepartum followed by a moderate energy diet for 28 d; 2) shortened (S) dry period (~28 d) in which cows were fed continuously a high energy diet; or 3) no planned (N) dry period in which cows were fed continuously a high energy diet. All cows received a high energy lactation diet after calving. Ovaries were evaluated by ultrasound and blood samples collected 3 times weekly beginning at d 6 or 7 postpartum until 7 d after second ovulation. Average days from calving until first detection of a 10-mm follicle were fewer in N (8.0 d) and S (8.9 d) than in T (10.5 d) cows. Time from calving to first ovulation was earlier in N (13.2 d) than in S (23.8 d) and T (31.9 d) cows. A greater percentage of follicles of the first follicular wave ovulated in N (89%; 16/18) than in T (42%; 8/19), with S (62%; 13/21) cows being intermediate. Double ovulation rate at the first ovulation was greater in T (61%) than N (16%), with S (35%) intermediate. No difference was detected in double ovulation rate at second ovulation (13/56). Number of cows with persistent corpus luteum (>30 d; 15/56) was not different among groups; however, short luteal phases were greater in N (28%; 5/18) than S (0%; 0/20) cows. Days to first artificial insemination were fewer in N (69.4 d) and S (68.0 d) than in T (75.0 d). First-service conception rate was greater in N (55%; 11/20) than in T (20%; 4/20), with S (26%; 6/23) cows being intermediate. Days open in pregnant cows were fewer in N (93.8 d) than in T (145.4 d), with S (121.2 d) cows being intermediate. Thus, shortening or eliminating the dry period leads to earlier postpartum ovulation and the results highlight the need for future large

field studies to accurately evaluate the effect of dry period length on reproductive performance of lactating dairy cows.

(Key words: reproduction, first postpartum ovulation, dry period, nutrition)

Abbreviation key: CL = corpus luteum, DP = dry period, EB = energy balance, HE = high energy, LE = low energy, ME = moderate energy, N = no planned DP, S = shortened 28-d DP, T = traditional 56-d DP management scheme.

INTRODUCTION

High-producing dairy cows typically experience a variable period of negative energy balance (EB) during early lactation due to insufficient DMI to compensate for greater milk production. Improvement in EB from its most negative nadir in early lactation toward a positive state provides an important signal for initiation of ovarian activity (Butler et al., 1981). Energy balance during the first 3 to 4 wk postpartum is correlated with interval to first postpartum ovulation (Lucy et al., 1991; Beam and Butler, 1998). Number of days to EB nadir is positively correlated with the days from calving to first postpartum ovulation (Canfield et al., 1990; Canfield and Butler, 1990; Beam and Butler, 1997). Butler et al. (1981) reported that first postpartum ovulation occurred on average 10 d after EB nadir. Ferguson (1996) combined results from 10 studies and reported that average days to first ovulation were 33.3 ± 2.1 . Days from calving to first postpartum ovulation have been evaluated in earlier studies and reported from 22 to 27 d, when anovulatory cows were not included in the calculations (Savio et al., 1990; Staples et al., 1990; Zurek et al., 1995).

Most of the literature indicates that a dry period (DP) of 40 to 60 d is needed to achieve maximum milk yield during the following lactation (Coppock et al., 1974; Sorensen and Enevoldsen, 1991; reviewed by Bachman and Schairer, 2003). Other research indicates that a DP of 30 d is sufficient to maintain milk (Lotan and Adler, 1976; Bachman, 2002; Gulay et al., 2003). Nevertheless, a shortened DP seemed to reduce milk production in primiparous, but not multiparous, cows in a

Received December 10, 2004.

Accepted February 15, 2005.

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recent study (Annen et al., 2004) and our recent study found somewhat of a reduction in milk production per day, but not in FCM production (Rastani et al., 2005). Information relating the effects of DP to subsequent reproductive performance of lactating cows is sparse. Lotan and Adler (1976) reported that days open, number of inseminations, and pregnancy rates for 18 pairs of dairy cows having DP of 30 and 60 d were numerically similar.

Remond et al. (1997) reported that BW of cows with no planned DP increased 24 kg in the first 60 DIM, whereas BW of cows with a 60-d DP lost 28 kg during same time period. We speculated that cows with a DP of 56 d would be in more negative EB than cows with no planned DP due to reduced milk production in cows with no planned DP. Thus, cows with no planned DP were expected to ovulate earlier than cows with a 56-d DP; whereas cows with a 28-d DP were expected to be intermediate in days to first ovulation based on an expected intermediate level of negative EB.

The objective of our study was to determine the effects of reducing duration of DP and simplifying feeding of transition cows on interval to first postpartum ovulation and other reproductive measures of periparturient dairy cows. It should be clearly noted that the small number of cows in this intensive study did not allow definitive evaluation of binomial reproductive measures (e.g., conception rate), but data were collected to provide a rationale for future large studies related to the effect of DP on reproductive performance.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted at the University of Wisconsin-Madison, and all animal handling and care procedures were approved by the Research Animal Resources Center of University of Wisconsin-Madison. Holstein cows ($n = 58$) were blocked by parity, average daily milk production between 67 and 60 d prepartum, and DIM. Cows were randomly assigned to 1 of 3 treatments: 1) traditional (T) DP were managed to achieve a 56-d DP in which cows were fed 28 d on a low energy (LE) diet ($NE_L = 1.50$ Mcal/kg) followed by 28 d of a moderate energy (ME) diet ($NE_L = 1.69$ Mcal/kg); 2) shortened (S) DP were managed to achieve a 28-d DP in which cows were fed continuously a high energy (HE) diet (prepartum HE diet, $NE_L = 1.75$ Mcal/kg); and 3) no planned (N) DP (0 d) in which cows were fed continuously an HE diet (Figure 1). If a cow on N dropped below 2 kg/d, milking was discontinued for the remainder of the prepartum period. All cows were fed a postpartum HE diet ($NE_L = 1.71$ Mcal/kg) from parturition through 70 DIM. The only difference between the

prepartum HE diet and the postpartum HE diet was the addition of sodium bicarbonate and a reduction in level of vitamins in the postpartum HE diet. The experimental timeline is summarized in Figure 1. Cows were fed a TMR for ad libitum intake throughout the entire experimental period. Ingredient and nutrient composition of the diets, selection criteria for the cows, and energy calculations were reported previously (Rastani et al., 2005).

Cows were housed in individual stalls in a stanchion barn during the entire DP and lactation. Cows were milked twice daily when lactating (prepartum and postpartum) and milk production was recorded at each milking. Daily milk yield, reproductive, health, and management records for each cow were collected on Dairy Comp 305 (Valley Agricultural Software, Tulare, CA). Weekly BCS was assessed for each cow by 3 individuals (Wildman et al., 1982), and weekly BW was recorded for each cow.

Blood Sampling

Blood samples for progesterone, estradiol, and FSH analyses were collected from the coccygeal vein or artery into Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) at 76, 30, 21, 14, and 7 d before expected calving date, 1 d after calving, and beginning on d 6 or 7 postpartum, 3 times weekly (i.e., Monday, Wednesday, and Friday) until 7 d after second ovulation or until 70 d postpartum. Blood was allowed to clot at 4°C for 24 h and centrifuged at $1500 \times g$ for 15 min. Serum was collected and stored at -20°C until further analysis.

Hormone Assays

Serum concentration of FSH was determined using a radioimmunoassay validated for use in cattle (Bolt and Rollins, 1983; Bolt et al., 1990). The FSH assay incorporated bFSH (USDA-bFSH-I-2) for iodination and reference standards and NIDDK-anti-o-FSH-I-2 as the primary antiserum. Hormone sensitivity, calculated as 2 standard deviations less than the mean cpm at maximum binding, was 0.08 ng/mL. Coefficients of variation for within and between assays for FSH were 3.2, 3.8% (cows in diestrus) and 6.8, 8.7% (cows near estrus), respectively.

Serum concentration of progesterone was determined by solid-phase radioimmunoassay kits containing antibody-coated tubes and ^{125}I -labeled progesterone (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA) as previously described by Gümen and Wiltbank (2005). Hormone sensitivity, calculated as 2 standard deviations less than the mean cpm at maximum binding,

Figure 1. Summary of experimental protocol timeline showing diet, lactation period, sampling scheme, and ultrasonography in 3 treatments: traditional (T) 56-d dry period, shortened (S) 28-d dry period, and no (N) planned dry period. Cows in N and S were fed continuously a high-energy (HE) diet beginning at 56 d prepartum. Cows in T were fed a low-energy (LE) diet from 56 to 29 d prepartum followed by a moderate energy (ME) diet from 28 d prepartum to calving (d 0). All cows were fed a postpartum HE diet from parturition through 70 DIM. Blood samples were collected at 76, 30, 21, 14, and 7 d before projected calving, 1 d after calving, and thrice weekly [i.e., Monday (M), Wednesday (W), and Friday (F)] beginning at 6 or 7 d postpartum until 7 d after second ovulation or until 70 d postpartum. Transrectal ultrasound scanning to monitor follicles was performed thrice weekly beginning at 6 or 7 d postpartum until 7 d after second ovulation or until 70 d postpartum in anovulatory cows.

was 0.03 ng/mL (mean of 10 assays). Coefficients of variation for within and between the assays for progesterone were 5.4 and 7.4%, respectively, using a quality control sample with 2.0 ng/mL, and 5.3 and 5.6%, respectively, using a quality control sample with 10 ng/mL of progesterone.

Prepartum and d 1 postpartum serum concentration of estradiol were determined by a competitive ELISA as described previously (Rasmussen et al., 1996). A quality control sample was prepared from charcoal-stripped serum containing a known concentration of estradiol (250 pg/mL). The quality control sample was evaluated multiple times in each estradiol assay and the within- and between-assay coefficients of variation were 3.2 and 8.6%, respectively.

Serum concentrations of estradiol were measured in samples collected between d 6 and 14 postpartum by using modifications to a commercially available estradiol radioimmunoassay kit (Third Generation Estradiol Assay Kit, Diagnostics Systems Laboratories Inc., Webster, TX) previously validated for use in cattle (Kulick et al., 1999). The quality control sample (5 pg/mL) was evaluated multiple times in each estradiol assay and the within- and between-assay coefficients of variation were 4.8 and 6.2%, respectively.

Postpartum Ultrasound Evaluation and Reproductive Management

Ovarian ultrasonography examinations were performed using a real-time, B-mode scanner equipped with a 7.5-MHz linear-array intrarectal transducer (Aloka 500V, Corometrics Medical Systems Inc., Wallingford, CT). Measurements were made on a single frozen image of the apparent maximal area of each follicle, using the average diameter in 2 directions at right angles. Transrectal ultrasound scanning to monitor follicles was performed thrice weekly (i.e., Monday, Wednesday, and Friday) beginning on d 6 or 7 postpartum until 7 d after second ovulation or until 70 d postpartum in anovulatory cows. Ovulation was determined by ultrasound scanning based on disappearance of a large follicle followed by appearance of a resulting corpus luteum (CL) and confirmed by an increase in serum progesterone concentration.

A persistent CL was defined as a luteal structure that remained detectable in the ovary for >30 d and maintained serum progesterone concentrations >1 ng/mL (Bulman and Lamming, 1977). A short luteal phase was defined as an interval between first and second postpartum ovulation of <12 d. Anovulation was defined as no ovulation for the entire 70-d postpartum period.

Pregnancy status was determined using ultrasound at 25 to 31 and 60 to 66 d after AI. Visualization of a fluid-filled uterine horn with embryonic vesicles and a large CL (d 25 to 31) or the presence of a fetus (d 60 to 66) were used as positive indications of pregnancy. All fertility data described in the text were based on the later pregnancy diagnosis; the earlier pregnancy diagnosis was only used to determine rate of embryonic loss. First-service conception rate was calculated as the number of cows diagnosed pregnant divided by the number of cows previously inseminated. Overall conception rate was calculated as the number of cows diagnosed pregnant divided by total number of inseminations for all cows per treatment. Herd personnel were responsible for reproductive management including detection of estrus and AI. The voluntary waiting period was 50 d. For first service, cows that were detected in estrus (7 of 20 in N, 8 of 23 in S, and 3 of 20 in T cows) were inseminated by herd personnel. In the remaining cows, ovulation was synchronized by using the Ovsynch protocol (GnRH – 7d – PGF_{2α} – 56 h – GnRH – 16 to 18 h – timed AI) in which AI occurred at 70 to 76 DIM. Cows that were diagnosed not pregnant between 25 and 31 d after AI were retreated with the Ovsynch protocol for AI (98 of 117 second or later breedings). Cows that were detected in estrus were inseminated by herd personnel based on the a.m.-p.m. rule (19 of 117 second or later breedings). Fertility data was collected until 250 DIM.

Statistical Analyses

Analyses of hormonal data were conducted using the mixed models procedure of SAS with repeated measures in time as a subplot (SAS Inst. Inc., Cary, NC). The model was:

$$Y_{ijkl} = \mu + \text{trti} + \text{cowj}(\text{trti}) + \text{timek} + \text{trti} \times \text{timek} + \varepsilon_{ijkl}$$

where Y_{ijkl} = dependent observations (serum progesterone, estradiol, and FSH); μ = overall mean; trti = effect of treatments [i = N, S, T (fixed effect)]; cowj = cows [j = 1, 2, 3, ..., 20 (random effect)]; timek = effect of time [k = days (fixed effect)]; $\text{trti} \times \text{timek}$ = interaction of treatments and time (fixed effect); and ε_{ijkl} = random error. To account for auto-correlated errors due to repeated measures and unequal time intervals for the same experimental unit $\text{cowj}(\text{trti})$, the spatial power structure was used (Littell et al., 1998). Prepartum and postpartum periods were analyzed separately.

Single mean comparisons, including days to first ultrasonography, days to first ovulation, days to 10-mm follicle size, days to first AI, and days open in pregnant

cows were analyzed using the mixed models procedure. The model was:

$$Y_{ijkl} = \mu + B_i + P_j + \text{trtk} + P_j \times \text{trtk} + \varepsilon_{ijkl}$$

where Y_{ijkl} = dependent observations (mean comparisons); μ = overall mean; B_i = effect of blocks [i = 1, 2, 3, ..., 23 (random effect)]; P_j = effect of parity [j = primiparous or multiparous (fixed effect)]; trtk = effect of treatments [k = N, S, T (fixed effect)]; $P_j \times \text{trtk}$ = interaction of parity and treatments (fixed effect); and ε_{ijkl} = random error.

When treatment was significant in the model, differences between treatments were determined using the PDIF option (SAS Inst. Inc.). Significance was declared when $P < 0.05$, and trends were discussed when $P < 0.15$. Services per conception were analyzed by the nonparametric Kruskal-Wallis test (SAS Inst. Inc.). One-way ANOVA was used to compare EB, milk production, BCS (average value for the 3 evaluators), and BW among treatments (N, S, and T). Outliers were determined by the Univariate procedure (SAS Inst. Inc.). Cows were considered outliers when data were outside of 1.5 interquartile ranges. Correlation coefficients were calculated using PROC CORR (SAS Inst. Inc.). Binomial response data were analyzed by Fisher's Exact test, using all possible 2×2 comparisons from the 3×2 table.

RESULTS AND DISCUSSION

Prepartum Measures

Actual days dry was similar to the planned days dry: 55.3 ± 1.1 , 28.5 ± 0.9 , and 4.3 ± 1.0 d dry for the T, S, and N treatments, respectively. Some cows with no planned DP spontaneously became dry (<2 kg/d) before calving. Gestation length was similar across treatments (277 ± 1.1 , 279 ± 0.8 , and 280 ± 1.0 d for T, S, and N treatments, respectively). Two cows were found to be outliers based on days dry (31 d in T and 25 d in N treatment) and were not included in subsequent analyses.

Regardless of treatments, prepartum serum progesterone and FSH concentration decreased ($P < 0.001$) as calving approached (Figure 2). Serum estradiol concentration increased ($P < 0.001$) in all cows as calving approached regardless of treatment (Figure 2). Serum estradiol concentration was not different among treatments until 14 d before expected day of calving (Figure 2). At 14 and 7 d before expected day of calving serum estradiol was greater ($P < 0.05$) in T than N cows, with S cows being intermediate (Figure 2). To our knowledge, no previous studies have evaluated the changes in pre-

Figure 2. Mean ($\pm$ SEM) serum concentrations of A) FSH, B) estradiol, and C) progesterone in cows having traditional (T) 56-d dry periods, shortened (S) 28-d dry periods, or no (N) planned dry period. Pre- and postpartum periods are separated by a dashed line. Pre- and postpartum periods were analyzed separately. The asterisks indicate differences ($P < 0.005$) among treatments on a given day(s).

partum hormonal concentrations during manipulation of DP. Nevertheless, hormonal concentrations during a traditional DP are similar to a previous report (Rasmussen et al., 1996). Before analysis of these samples, we hypothesized that circulating estradiol and progesterone would be decreased in cows with shortened DP due to the greater feed consumption and milk production in these cows. This hypothesis was based on a previous study (Sangritavong et al., 2002) in which elevated DMI increased metabolism of steroids in lactating cows. Although circulating progesterone was not altered, the decrease in circulating estradiol was consistent with this hypothesis and could be due to the greater DMI in N than T cows (Rastani et al., 2005). The physiological implications of reduced circulating estradiol concentrations during the final prepartum days are intriguing. Circulating estradiol dramatically increases as parturition approaches and this increase may be important for changes in the reproductive tract that are important for normal parturition. Despite the reduced concentrations of estradiol, no problems with parturition were detected in the N cows (Rastani et al., 2005). In addition, increased circulating estradiol near parturition may be important for changes in the mammary gland that are essential for maximal milk production after parturition. Milk production was less in the N cows compared with S and T cows (Rastani et al., 2005). Based on our results, we propose that a reduced prepartum circulating estradiol should be included among the numerous possible explanations for reduced milk production in cows with no DP or shortened DP.

Early Postpartum Measures

Serum FSH concentration was not different among treatments 1 d after calving. Serum FSH concentration, however, was greater ($P < 0.005$) in T than N cows, with S cows being intermediate at 6 d after calving. In addition, FSH concentration was greater ($P < 0.005$) in T than N and S cows at 8 d after calving (Figure 2). It seems likely that the postpartum FSH surge was earlier in N cows (based on follicle data described below) and therefore, the lack of blood samples between d 1 and 6 might have not allowed detection of the peak of the FSH surge in this treatment.

Average days from calving to first ultrasonography were similar among groups (between 6.3 and 6.6 d postpartum; Table 1). Average diameter of follicles at first ultrasonography was greater ($P < 0.05$) in N than T cows, with S cows being intermediate. Average days from calving until first detection of a 10-mm follicle were less ($P < 0.05$) in N and S than T cows. Beam and Butler (1997) reported that average days from calving until the first 10-mm follicle ranged from 8.5 ± 0.6 to

Table 1. Effects of varying dry period and prepartum diet on traits.

	Treatments ¹		
	No DP	Shortened	Traditional
Cows, no.	18	21	19
Actual days dry	4.3 ± 1.1	28.5 ± 0.9	55.3 ± 1.2
Average days to first ultrasonography	6.3 ± 0.2	6.6 ± 0.2	6.5 ± 0.2
Follicle diameter at first ultrasonography, mm	9.5 ± 0.7 ^a	8.2 ± 0.5 ^{a,b}	6.3 ± 0.6 ^b
Days to detection of first 10-mm follicle	8.0 ± 0.5 ^a	8.9 ± 0.4 ^a	10.5 ± 0.6 ^b
Days to first postpartum ovulation	13.2 ± 1.2 ^a	23.8 ± 3.4 ^b	31.9 ± 4.4 ^b

^{a,b}Means within row having different superscript letter differ ($P < 0.05$).

¹No DP = no planned dry period; shortened = 28-d dry period; and traditional = 56-d dry period.

10.5 ± 0.5 d in cows with varying EB (-2.8 ± -6.7 Mcal/d). In another report, postpartum interval to detection of the first dominant follicle was 11.6 ± 8.9 d (Savio et al., 1990). In our experiment, follicles of cows in N reached 10 mm in diameter 2.5 d earlier than in T cows. In addition, although days to first ultrasonography were not different among treatment, follicular diameter was greater in N (9.5 ± 0.7 mm) than T (6.3 ± 0.6 mm) cows at first ultrasonography. Larger follicular diameter in N at 7 d postpartum supports the idea that emergence of the first postpartum follicular wave occurred earlier in N than in T cows.

Time to First Ovulation

Average days from calving to first postpartum ovulation were less ($P < 0.05$) in N (13.2 ± 1.2 d) than S (23.8 ± 3.4 d) and T (31.9 ± 4.4 d) cows (Table 1). One cow each from S and T did not ovulate during the experimental period (70 d postpartum). Day of ovulation for these 2 cows was assigned to be 70 d. Even without these cows, the range for ovulation was more variable in S (9 to 51 d) and T (9 to 63 d) than N (7 to 27 d) cows (Figure 3). The results for the T and S cows were similar to those in previous studies in which cows had a traditional DP. Savio et al. (1990) reported that days from calving to first postpartum ovulation were 27.4 ± 5.3 d (range 9 to 85 d) including the cows that developed cysts. Based on plasma progesterone, the interval from calving to first postpartum ovulation was 24.1 ± 1.5 d without including 4 anovulatory cows ($n = 17$; Zurek et al., 1995) and 24.4 ± 2.3 d ($n = 11$; Spicer et al., 1990). In a recent study, Sakaguchi et al. (2004) reported that days to first ovulation were 30.9 ± 2.4 d in 50 high milk-producing dairy cows based on thrice-weekly ultrasound evaluations. Thus, N cows ovulated earlier than cows in the other 2 treatments and apparently earlier than cows in previously reported research studies.

A follicle of the first follicular wave ovulated in more ($P < 0.01$) N (89%) than T (42%) cows, with S (62%) cows being intermediate (Table 2). The other 2 cows in

N ovulated a follicle from the second follicular wave. However, incidence of ovulation was distributed between first, second, and third follicular wave in S (62, 28, and 10%, respectively) and T (42, 21, and 37%, respectively) cows (Table 2). Savio et al. (1990) reported that most cows (74%) ovulated the first dominant follicle. However, Beam and Butler (1999) found that 46% of cows ovulated the first dominant follicle postpartum, whereas 31% developed at least 2 waves of dominant follicles before the first ovulation. Sakaguchi et al. (2004) reported first postpartum ovulations of 46% from first wave, 22% from second waves, and 12% from third or fourth waves. These ovulation results are similar to T cows in the present study.

Based on previous associations between postpartum EB and time to first ovulation (Butler and Smith, 1989)

Figure 3. Scatter plots between actual days dry and days from calving to first postpartum ovulation for cows having traditional (T) 56-d dry periods, shortened (S) 28-d dry periods, or no (N) planned dry period. Each symbol represents an individual cow with different symbol type for each of the 3 treatments; T ($\blacktriangle$), S ($\blacksquare$), and N ($\bullet$). Average days from calving to first ovulation also are shown for each treatment (mean $\pm$ SEM).

Table 2. Effect of varying dry period and prepartum diet on first postpartum ovulation in lactating dairy cows and ovarian abnormalities.

	Treatments ¹		
	No DP	Shortened	Traditional
Cows, no.	18	21	19
Ovulation from first follicle wave, %	89 ^a (16/18)	62 ^{ab} (13/21)	42 ^b (8/19)
Ovulation from second follicle wave, %	11 (2/18)	28 (6/21)	21 (4/19)
Ovulation from third follicle wave, %	—	10 (2/21) ²	37 (7/19) ²
Double ovulation at first ovulation, %	17 ^a (3/18)	35 ^{ab} (7/20) ²	61 ^b (11/18) ²
Double ovulation at second ovulation, %	11 (2/18)	35 (7/20) ²	22 (4/18) ²
Persistent corpus luteum, %	28 (5/18)	25 (5/20) ²	28 (5/18) ²
Short luteal phases, %	28 ^a (5/18)	0 ^b (0/20) ²	11 ^{ab} (2/18) ²

^{a,b}Means within row having different superscript letter differ ($P < 0.05$).

¹No DP = no planned dry period; shortened = 28-d dry period; and traditional = 56-d dry period.

²One cow from these treatments did not ovulate during the first 70 DIM.

we expected a somewhat earlier time to first ovulation in cows with no planned DP, but the consistency of early ovulation in individual cows in this group was still somewhat surprising. A complete analysis of EB, milk production, and DMI for these cows is reported elsewhere (Rastani et al., 2005), but Table 3 shows EB differences among treatments during the first few weeks after parturition. Cows with no planned DP had,

on average, a 1.7 ± 1.2 Mcal/d positive EB during the first 3 wk postpartum in which 12 of 18 cows were in positive EB during the early postpartum period (Figure 4). Positive EB seemed to be primarily due to greater DMI, and consequently, a greater net energy intake during this period in N cows, combined with somewhat less energy output in milk production (Rastani et al., 2005).

Table 3. Effect of varying dry period and prepartum diet on energy balance (EB), milk production, and body composition variables.

	Treatments ¹		
	No DP	Shortened ²	Traditional ²
Cows, no.	18	20	18
Mean EB during first 3 wk, Mcal/d	1.7 ± 1.2^a (10.1 to -12.6) ⁴	-6.3 ± 0.9^b (4.2 to -13.5)	-9.6 ± 0.6^c (-6.0 to -14.6)
EB nadir, Mcal/d	-2.0 ± 1.0^a (0.4 to -16.0)	-9.7 ± 0.3^b (0.1 to -17.7)	-13.4 ± 0.8^c (-8.5 to -22.9)
Time to EB nadir, wk	4.7 ± 0.6^a (1 to 10)	2.6 ± 1.2^b (1 to 6)	2.5 ± 1.1^b (1 to 5)
BW loss from 1 wk to minimum, ³ kg	15.0 ± 6.4^a (-13.6 to 106.8)	39.7 ± 6.1^b (-11.4 to 81.8)	65.7 ± 9.6^c (18.2 to 186.4)
BCS loss from calving to minimum ³	0.53 ± 0.08^a (0.125 to 1.5)	0.76 ± 0.08^b (0.25 to 1.75)	1.31 ± 0.1^c (0.625 to 2.375)
Peak milk production, kg/d	43.2 ± 1.3^a (34.5 to 55.5)	47.4 ± 1.7^b (32.7 to 62.7)	53.6 ± 1.3^c (46.4 to 64.5)
Mean milk production, kg/d	32.4 ± 1.3^a (18.6 to 44.2)	37.1 ± 1.3^b (24.3 to 48.3)	42.3 ± 1.2^c (34.4 to 52.4)

^{a,b,c}Means within row having different superscript letter differ ($P < 0.05$).

¹No DP = no planned dry period; shortened = 28-d dry period; and traditional = 56-d dry period.

²One cow from these treatments did not ovulate during the first 70 DIM. Data from these 2 were excluded from calculations.

³Data from 51 cows were used to estimate BCS loss and BW loss calculations (18, 15, and 18 cows for no DP, shortened, and traditional, respectively).

⁴Data in parentheses represent the range of individual observations.

Figure 4. Scatter plots between average energy balance during first 21 DIM and days to first postpartum ovulation for cows having traditional (T) 56-d dry periods, shortened (S) 28-d dry periods, or no (N) planned dry period. Each symbol represents an individual cow, with a different symbol type for each of the 3 treatments; T (▲), S (■), and N (●). A negative linear relationship was detected between mean energy balance for 21 d and days to first postpartum ovulation: $y = -0.93x + 17.1$; $R^2 = 0.173$.

Figure 4 shows the association between mean EB during the first 21 d after calving and days to first ovulation for each individual cow in the 3 treatments. Although the correlation is significant ($r = -0.42$; $P < 0.01$), it would not be possible to accurately predict the time to first ovulation for an individual cow based on the mean EB during the first 21 d. Interval to first ovulation after calving has been reported to increase with increasing negative EB in some studies (Butler et al., 1981; Ducker et al., 1985; Butler and Smith, 1989; Lucy et al., 1991; Senatore et al., 1996; Beam and Butler, 1998), but not in others (Villa-Godoy et al., 1988; Spicer et al., 1990; Lucy et al., 1992). Similarly, we found that the EB nadir was inversely correlated with days to first ovulation ($r = -0.45$; $P < 0.001$). In contrast, unlike other studies (Canfield and Butler, 1990; Canfield et al., 1990; Beam and Butler, 1997; Beam and Butler, 1998; Senatore et al., 1996), days from calving to negative EB nadir was not related to days to first ovulation in our study, with N having the longest time to EB nadir (Table 3). This discrepancy between studies is probably due to the irrelevant nature of this value in cows that experience almost no negative EB such as N cows in our study. Loss in BW also was not significantly correlated to days to first ovulation, as reported in previous reports (Stevenson and Britt, 1979; Butler et al., 1981; Villa-Godoy et al., 1988; Lucy et al., 1992), but not by Senatore et al. (1996). Similarly, using all cows from the 3 treatments, no significant association was detected between BCS loss and days to first ovula-

tion, which is in contrast to a previous report that used only a traditional DP (Beam and Butler, 1999). Thus, it seems very likely that the very consistent early ovulation in cows with no planned DP was due to the maintenance of a positive EB during the postpartum period in these cows.

Double Ovulation Rate

Incidence of double ovulation at first postpartum ovulation was greater ($P < 0.05$) in T (61%) than N (17%) cows, with S (35%) cows being intermediate. No difference in double ovulation rate, however, occurred at second ovulation (13/56; Table 2). First follicular wave double ovulation rate was greater ($P < 0.01$) in T (6/8; 75%) than N (3/16; 19%) cows, with S (5/13; 39%) cows being intermediate. Two of 6 cows in S and 1 of 4 cows in T ovulated 2 follicles originating from the second follicular wave and 4 of 7 cows in T ovulated 2 follicles originating from the third follicular wave. In our study, average milk yield during the first 70 DIM was greater in T (42.4 kg/d) than N (33.9 kg/d) cows (Rastani et al., 2005). In addition, the later DIM at first ovulation led to an even greater discrepancy in milk production at the time of first ovulation (43.1 ± 0.9 kg/d for T vs. 28.8 ± 1.9 kg/d for N) between T and N treatments. In previous studies, the incidence of double ovulation was nearly 3-fold greater for cows with high (50.7 ± 0.7 kg; 20.2%) vs. low (31.1 ± 0.7 kg; 6.9%) milk production (Fricke and Wiltbank, 1999; Lopez et al., 2005), although this milk production effect was not detectable at first postpartum ovulation (Lopez et al., 2005). Therefore, greater double ovulation in T cows could be due to greater postpartum milk production than in N cows or other factors such as later DIM at first ovulation.

Luteal Phase Length and Progesterone Concentrations

Prolonged luteal phase, short luteal phase, and an-ovulation have been reported to be important ovarian dysfunctions (Royal et al., 2000). Number of cows (27%; 15/56) with persistent CL (>30 d based on criteria of Bulman and Lamming, 1977) was not different among treatments. In contrast to the present study, a low incidence (1.5 to 12.5%) of persistent CL was reported after the first postpartum ovulation in previous studies (Bulman and Lamming, 1977; Bulman and Wood, 1980; Senatore et al., 1996; Lamming and Darwash, 1998). Short luteal phases were greater ($P < 0.05$) in N (28%; 5/18) than S (0%; 0/20) with T (11%; 2/18) cows being intermediate. In previous studies, variable rates (11 to 54%) of short luteal phases were reported after the first postpartum ovulation in lactating dairy cows (Savio et

Table 4. Effect of varying dry period length and prepartum diet on fertility in lactating dairy cows.

	Treatments ¹		
	No DP	Shortened	Traditional
Cows, no.	20	23	20
Days to first AI	69.4 ± 2.2	68.0 ± 2.2	75.0 ± 3.0
First-service conception rate, %	55 (11/20) ^a	26 (6/23) ^{ab}	20 (4/20) ^b
Overall conception rate, %	31 (16/52)	26 (18/69)	31 (18/59)
Pregnant cows only			
Services per conception, no.	28/16 (1.75) ^a	44/18 (2.44) ^{ab}	54/18 (3.00) ^b
Days open	93.8 ± 11.3 ^a	121.2 ± 11.7 ^{ab}	145.4 ± 18.0 ^b

^{a,b,c}Means within row having different superscript letter differ ($P < 0.05$).

¹No DP = no planned dry period; shortened = 28-d dry period; and traditional = 56-d dry period.

al., 1990; Senatore et al., 1996; Royal et al., 2000). Only 2 cows (1 cow from S and 1 from T) were anovulatory during the 70-d experimental period. Unlike previous studies, the incidence of anovulation was very low in our study (3%; 2 of 58 cows). Previously, we used weekly ultrasound evaluations combined with circulating progesterone concentration to analyze and classify anovulation at the start of the breeding period in a commercial dairy herd and found that 20% of cows were anovulatory (Gümen et al., 2003).

Serum progesterone concentrations were similar among treatments after calving (Figure 2). Progesterone concentration was low (<0.6 ng/mL) in all cows from d 6 to 13 (Figure 2). Postpartum serum estradiol concentration increased ($P < 0.001$) in all cows from d 6 to 13 (Figure 2), but no differences were detected among treatments.

Other Reproductive Data

Our study was not designed to evaluate fertility data, and the number of cows in each treatment was limited. Reproductive performance results, however, were interesting and may stimulate further studies. Although our experiment ended at 70 d postpartum, fertility data of cows were monitored until 250 d after calving. The first 5 cows in the study were not evaluated in terms of first postpartum ovulation. However, these 5 cows (2 in N, 2 in S, and 1 in T) were evaluated for a total of 63 cows. Days to first AI were less ($P < 0.06$) in N (69 d) and S (68 d) than T (75 d) cows (Table 4). First-service conception rate was greater ($P < 0.05$) in N (55%) than T (20%), with S (30%) cows being intermediate (Table 4). Overall conception rate, however, was similar (26 to 31%) among treatments (Table 4). Embryonic loss was lim-

ited to only 3 pregnancies lost between d 25 to 31 and d 60 to 66 (2 cows in S and 1 cow in T). In a recent study, cows with shortened or no planned DP had similar conception rates, but an insufficient number of cows were studied to make valid conception rate comparisons (Annen et al., 2004). Services per conception in pregnant cows were less ($P < 0.05$) in N (1.75) than T (3.0), with S (2.44) cows being intermediate (Table 4). Days open in pregnant cows were less ($P < 0.05$) in N than T, with S cows being intermediate (Table 4). These reproductive improvements in N cows could be due to differences in EB or milk yield. Cows with no planned DP had greater EB and produced less milk than cows in the other 2 treatments during the 70-d experimental period (Rastani et al., 2005). Nevertheless, it seems that conception rate after the first AI may have been reduced ($P = 0.055$) for N cows (5 pregnancies in 15 breedings) compared with T cows (14 pregnancies in 39 breedings). Future studies should not neglect a careful analysis of this potential reduction in conception rate in second and later AI due to reduction in DP.

Lotan and Adler (1976) reported numerically similar days open, number of inseminations, and pregnancy rates for cows with DP of 30 vs. 60 d, but no statistical analyses of reproductive data were conducted because of the limited numbers of cows. Several researchers have concluded that an early postpartum return to cyclicity was beneficial to the overall reproductive performance of dairy cows (Stevenson et al., 1983; Staples et al., 1990; Darwash et al., 1997). Delay in first postpartum ovulation is associated with reduced conception rates and increased interval from calving to conception (Darwash et al., 1997). Lucy et al. (1992) reported that cows having first ovulation before d 42 required fewer services per conception than cows having first ovulation

after d 42. Moreover, cows not observed in estrus during the first 60 d after calving are at greater risk of being culled than cows that have shown estrus (Opsomer et al., 2000). Early resumption of normal ovulatory cycles seems to be important for greater reproductive efficiency; an increased number of estrous cycles before the onset of breeding is related to fewer services per conception (Thatcher and Wilcox, 1973; Lucy et al., 1992; Darwash et al., 1997). In contrast, some researchers have reported reduced conception rate in cows having early postpartum ovulation (Ball and McEwan, 1998; Smith and Wallace, 1998) or no detectable relationship between early luteal activity and fertility (Royal et al., 2000).

Practical Implications

Poor reproductive performance in lactating dairy cows is a substantial concern for commercial dairy producers. Results of our study demonstrated that eliminating the dry period resulted in a consistent and shorter postpartum interval to first ovulation and possibly improved reproductive performance. These changes may have been due to the maintenance of a positive energy balance during the early postpartum period when cows did not have a planned dry period. Further studies are warranted to evaluate if these encouraging results can be repeated in studies having sufficient numbers of cows to provide sufficient power to test potential differences in conception rate. Reducing the DP to 28 d while maintaining a high-energy diet seemed to cause some of the same effects, although less dramatically, when compared with a traditional dry period protocol. Our results emphasize the pressing need for further research to produce a dry period protocol that optimizes milk production, reproduction, and health for high-producing dairy herds.

ACKNOWLEDGMENTS

Ahmet Gümen was supported by a fellowship from the Ministry of National Education of Turkey. Robin Rastani was supported by ADM Alliance Nutrition, Inc., Church and Dwight Co., Inc., Degussa Bioactives, Diamond V Mills, Inc., Kemin Industries, Land O' Lakes, Inc., Pioneer Hi-Bred International, Inc., and ZinPro Corporation. Support was also provided by the Wisconsin State Agricultural Experiment Station. The authors thank J. N. Guenther and A. H. Souza for their technical support.

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