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Transition Cow Metabolites and Physical Traits Influence Days to First Postpartum Ovulation in Dairy Cows

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Abstract

The objective of this study was to assess key metabolites and patterns of prepartum and postpartum physical activity as they relate to the onset of first postpartum ovulation in lactating dairy cows. Close-up dry Holstein cows (n = 82) and late gestation heifers (n = 78) were enrolled beginning 3 weeks before expected calving date (day 0). Cows were fit with Cow SensOor ear tags to assess transitional changes in eating, resting, rumination, activity, and ear-surface temperatures. Rectal temperatures were assessed and blood samples were collected on days 0, 3, 7, and 14 to measure concentrations of glucose, free fatty acids (FFA), β-hydroxybutyrate (BHB), calcium, and haptoglobin (measure of inflammation). Body condition scores (BCS) and body weights (BW) were measured weekly, and blood samples were collected weekly from 3 through 10 weeks after calving to quantify changes in progesterone to detect luteal function after ovulation. Cows first ovulating before median day 33 were classified as early (n = 76), whereas those first ovulating after day 33 were classified as late (n = 84). Early ovulating cows first ovulated earlier than the late ovulation cows. Compared with late ovulating cows, early ovulating cows had lesser concentrations of FFA, BHB, and haptoglobin on days 0, 3, 7, and 14 in addition to having lower rectal temperatures and ear-surface temperatures. Ear-surface temperatures began to decrease 4 days before parturition and remained less after calving than cows that subsequently ovulated late. Early ovulating cows tended to spend more time eating, and less time resting and being active during the first 3 weeks after calving, and lost less BW and BCS during the first 9 weeks compared with late ovulating cows. Although no differences were detected in yields of milk or energy-corrected milk during the first 9 weeks after calving, early compared with late ovulating cows produced more milk protein. We concluded that metabolic measures during the first 2 weeks after calving, and physical and behavioral traits are associated with the onset of postpartum ovarian activity.

Keywords

activity, behavior, dairy cow, postpartum ovulation, transition period

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S. Banuelos and J.S. Stevenson

Summary

The objective of this study was to assess key metabolites and patterns of prepartum and postpartum physical activity as they relate to the onset of first postpartum ovulation in lactating dairy cows. Close-up dry Holstein cows (n = 82) and late gestation heifers (n = 78) were enrolled beginning 3 weeks before expected calving date (day 0). Cows were fit with Cow SensOor ear tags to assess transitional changes in eating, resting, rumination, activity, and ear-surface temperatures. Rectal temperatures were assessed and blood samples were collected on days 0, 3, 7, and 14 to measure concentrations of glucose, free fatty acids (FFA), β-hydroxybutyrate (BHB), calcium, and haptoglobin (measure of inflammation). Body condition scores (BCS) and body weights (BW) were measured weekly, and blood samples were collected weekly from 3 through 10 weeks after calving to quantify changes in progesterone to detect luteal function after ovulation. Cows first ovulating before median day 33 were classified as early (n = 76), whereas those first ovulating after day 33 were classified as late (n = 84). Early ovulating cows first ovulated earlier than the late ovulation cows. Compared with late ovulating cows, early ovulating cows had lesser concentrations of FFA, BHB, and haptoglobin on days 0, 3, 7, and 14 in addition to having lower rectal temperatures and ear-surface temperatures. Ear-surface temperatures began to decrease 4 days before parturition and remained less after calving than cows that subsequently ovulated late. Early ovulating cows tended to spend more time eating, and less time resting and being active during the first 3 weeks after calving, and lost less BW and BCS during the first 9 weeks compared with late ovulating cows. Although no differences were detected in yields of milk or energy-corrected milk during the first 9 weeks after calving, early compared with late ovulating cows produced more milk protein. We concluded that metabolic measures during the first 2 weeks after calving, and physical and behavioral traits are associated with the onset of postpartum ovarian activity.

Introduction

Resumption of postpartum ovarian cycles before first insemination is positively associated with first-service pregnancy outcomes. Formation of a corpus luteum during postpartum weeks 3 to 5 was related to subsequent improved reproductive performance when compared with herd mates without luteal function in 18 Holstein herds. Pregnancy outcomes in dairy cows also rely heavily on how well cows adapt to the physiological transition from a non-lactating pregnant status to lactation. Failure to adapt during the transition period increases the risk for periparturient diseases and hinders future reproductive performance and milk yield.

Some blood metabolite profiles are associated with first postpartum ovulation risk, disease risk, and may predict reproductive success. Periparturient disease negatively affects early postpartum ovulation risk and is associated with measurable changes in periparturient physical activity and postpartum metabolic profiles. Cows with reduced non-esterified fatty acids (NEFA) or free fatty acid (FFA) concentrations during the dry period tended to have earlier resumption of ovarian activity.

Automated monitoring of activity provides measurements that correlate with visual observations and provide accurate information for use in management of dairy cattle. These new technologies have capabilities to assess various behaviors including general activity, rumination, resting, and eating, in addition to ear surface or body core temperatures, and provide "health" and "heat" alerts based on proprietary algorithms. Relationships and associations of these automated-monitored activities with onset of postpartum ovulation have not been determined.

The objective of this study was to determine the relationship between key transition metabolites, physical activity indicators (eating, rumination, resting, general activity, and ear-surface temperatures), and onset of first postpartum ovulation in lactating dairy cows.

Experimental Procedures

Housing and Nutrition

Close-up dry Holstein cows (n = 82) and late gestation heifers (n = 78) in the Kansas State University Dairy Teaching and Research Center were enrolled beginning 3 weeks before expected calving date (day 0). Calvings occurred December through August. Until parturition, close-up dry cows and late gestation heifers were housed in an open-front, straw-bedded maternity barn, half of which was covered by roof. After parturition, cows were housed in open lot free-stall barns bedded with sand. Routine daily monitoring of fresh cows by herd personnel included assessing rectal temperature and urine ketones during the first 7 to 10 days after calving.

Cows were fed a total mixed diet twice or thrice (summer) daily that was calculated to meet nutrient requirements for lactating dairy cows producing 110 lb of 3.5% fat-corrected milk. The diet consisted of alfalfa hay, corn silage, triticale-clover silage, soybean meal, whole cottonseed, ground corn grain, corn-gluten feed, vitamins, and minerals. Cows were milked thrice daily and had *ad libitum* access to water. Daily milk yield data were captured electronically to summarize yield during the first 14 weeks of lactation. In addition, daily milk yield and milk components from the third DHI test day between days 51 and 94 for all cows were analyzed. Milk components assessed included yield and concentrations of fat and true protein in addition to somatic cell counts.

Activity Monitoring

Cows in the study were fitted with an ear tag sensor on their left ear when moved from the far-off pen to close-up pen between days 21 and 28 before expected calving date (CowManager SensOor system, Agis Automatisering BV, Harmelen, Netherlands). The sensors include a 3-dimensional accelerometer with proprietary software algorithms that provided hourly measurements recorded in minutes for eating, ruminating, activity, and resting (inactive) statuses.

Estrus alerts generated by the SensOor ear tags produced dates, duration (hour), and peak activity (intensity) of estrus during the postpartum period until first insemination. Duration of estrus represented the number of hours of "high activity" that exceeded the threshold value of 3.5 on a 12-unit scale. Intensity of estrus represented the peak value of high activity. Numbers of "heat alerts" and intensity were recorded for each cow between calving and insemination.

Blood Sampling, Metabolite and Mineral Assays

Blood samples were collected on days 0, 3, 7, and 14 after calving by puncture of the medial caudal coccygeal vein or artery for later determination of concentrations of glucose, free fatty acids (FFA), β-hydroxybutyrate (BHB), calcium, and haptoglobin (Figure 1). Before blood sampling, rectal temperature was measured by using a digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA).

Ovarian Measures

Blood samples also were collected weekly beginning on days 21 through 56 ± 3 in addition to samples collected during ovulation synchronization (days 63 ± 3, 70 ± 3, and 73 ± 3) for later analysis of progesterone (Figure 1). Day of first postpartum ovulation was defined to occur midway between the last weekly sample when progesterone < 1 ng/mL and the next weekly sample when progesterone ≥ 1 ng/mL. Cows first ovulating before the median day 33 were classified as early ovulation (n = 76), whereas those first ovulating after day 33 were classified as late ovulation (n = 84).

Statistical Analyses

Cow was the experimental unit. All analyses were performed in SAS v. 9.4 software (SAS Inst. Inc., Cary, NC). All mean comparisons of ovulation status (early vs. late), parity (primiparous vs. multiparous), and season (winter-spring vs. summer) were by F-tests. When interactions occurred in which more than two means were compared, means were separated by the LSD test in SAS. In all cases, statistical significance of effects was set as $P < 0.05$, with tendencies as $0.05 < P < 0.10$.

Results and Discussion

Estrus and Ovulation Events

Results are based on 160 cows (82 primiparous and 78 multiparous) completing the study. Median day to first ovulation was 33 days regardless of parity. On average, early ovulating cows first ovulated earlier ($P < 0.001$) than the late ovulation cows (24.3 ± 1.2 days [range: 16 to 32 d] vs. 48.8 ± 1.2 days [range 33 to 74 days]), respectively. Mean days to first ovulation excluded seven cows that failed to ovulate before insemination. Five cows included in the average days to first ovulation first ovulated in response to GnRH-1 (day 63 ± 3), and three cows ovulated before insemination but not in response to GnRH-1. Early ovulating cows ovulated more ($P < 0.001$) often before insemination on day 73 ± 3 than late ovulating cows (2.4 ± 0.1 vs. 1.5 ± 0.1), respectively.

Mean days to first ovulation did not differ between primiparous and multiparous cows (36.1 ± 1.2 vs. 37.1 ± 1.2 d), respectively, and the proportion of primiparous cows in the early (58%) and late ovulation groups (45%) did not differ ($P = 0.11$). Primiparous cows also ovulated more ($P = 0.01$) often before first insemination than multiparous cows (2.1 ± 0.1 vs. 1.9 ± 0.1), respectively.

First postpartum estrus occurred earlier ($P = 0.01$; 38.0 ± 2.4 vs. 52.6 ± 2.5) and more ($P < 0.001$; 1.2 ± 0.1 vs. 0.7 ± 0.1) estrual periods were detected in early than late ovulating cows before first insemination, respectively. Duration of first estrus ($P = 0.54$) and peak intensity of estrus ($P = 0.52$) did not differ between ovulation groups.

Glucose

Plasma glucose concentrations on days 0, 3, 7, and 14 were not related ($P = 0.25$) to ovulation status (Table 1), but were greater ($P < 0.001$) on day of calving and decreased thereafter on days 3, 7, and 14 (data not shown). Primiparous cows had greater ($P < 0.001$) glucose concentrations than multiparous cows (66.2 ± 0.8 vs. 57.4 ± 0.8 mg/dL), respectively, and concentrations decreased at different rates (21 vs. 11%; $P < 0.001$), respectively, from days 0 to 3. Plasma glucose also was greater ($P = 0.03$) in concentration during summer than winter-spring (62.6 ± 0.8 vs. 61.0 ± 0.7 mg/dL), respectively.

Free Fatty Acids

Early ovulating cows had lesser ($P < 0.01$) serum concentrations of FFA than late ovulation cows on days 0, 3, 7, and 14 (Table 1). Multiparous cows had greater ($P = 0.01$) concentrations of FFA than primiparous cows (0.55 ± 0.02 vs. 0.47 ± 0.02 mEq/L), respectively. An increase ($P = 0.01$) in serum FFA from days 0 to 3 occurred in primiparous cows, but an 18% decrease ($P = 0.01$) occurred thereafter to day 14, whereas concentrations in multiparous cows did not differ on days 0 and 3, but decreased by 18% from days 3 to 14 (data not shown).

β-hydroxybutyrate

Concentrations of BHB in serum followed a pattern similar to those of FFA on days 0, 3, 7, and 14. Early ovulating cows had lesser ($P = 0.03$) BHB concentrations than late ovulation cows (Table 1) and concentrations were greater in multiparous than primiparous cows (8.2 ± 0.2 vs. 7.0 ± 0.2 mg/dL). No interactions of day, parity, or ovulation group were detected for concentrations of BHB, but BHB concentrations were greater ($P < 0.001$) during winter-spring than summer (8.2 ± 0.2 vs. 7.1 ± 0.2 mg/dL).

Calcium

Serum calcium did not differ ($P = 0.13$) between early and late ovulation cows on days 0, 3, 7, and 14 (Table 1). Serum calcium decreased ($P < 0.001$) from day 0 to 3 in primiparous cows and then increased by 10% to day 14, whereas in multiparous cows, calcium increased ($P < 0.001$) by 17% after calving to day 14.

Haptoglobin

Concentrations of haptoglobin (measure of inflammation) in serum tended ($P = 0.08$) to be greater in late than early ovulating cows (Table 1), but differed by day as haptoglobin was only greater ($P = 0.02$) on days 3 and 7 in late ovulation cows. Haptoglobin was greater ($P < 0.001$) in primiparous than in multiparous cows from days 0, 7, and 14, peaking at greater ($P < 0.001$) concentrations on day 3 and then decreasing by 52% to day 14, whereas the decrease ($P = 0.01$) was only 4% in multiparous cows from days 3 to 14 (data not shown). Concentrations of haptoglobin were greater ($P = 0.02$) during summer than winter-spring (11.6 ± 0.4 vs. 10.7 ± 0.4 units).

Increased serum haptoglobin is associated with general inflammation and intrauterine infection after calving. The liver synthesizes haptoglobin and greater concentrations are reflective of general immune response and increased susceptibility to postpartum disease. We observed that late compared with early ovulating cows tended to have greater concentrations of haptoglobin on days 3 and 7 after calving, indicating potentially greater inflammation and disease.

Rectal Temperature

Late ovulating cows had greater ($P = 0.03$) rectal temperatures than earlier ovulating cows on days 0, 3, 7, and 14 (Table 1) with steadily increasing ($P < 0.001$) temperatures from days 0 to 7 (data not shown). Rectal temperatures from days 0 through 14 tended ($P = 0.08$) to be greater during summer than winter-spring (38.94 ± 0.03 vs. 38.88 ± 0.03°C).

Milk Characteristics

Average daily milk yield during the first 14 weeks of lactation did not differ ($P = 0.87$) between early and late ovulating cows (46.9 ± 0.7 vs. 46.8 ± 0.6 kg). Test-day milk ($P = 0.12$) and test-day energy-corrected milk ($P = 0.57$) yields did not differ between ovulation groups, whereas milk protein percentage ($P < 0.001$) and total milk protein yield ($P < 0.01$) were greater in early than late ovulating cows (Table 2). Milk fat percentage and yield, and somatic cell count did not differ between ovulation groups (Table 2).

As expected, test-day ECM and test-day milk yields were 12.2 ± 0.6 and 16.9 ± 0.6 kg greater ($P < 0.001$) in multiparous than primiparous cows, respectively. In contrast, milk fat (1.7 ± 0.04 vs. 1.4 ± 0.04 kg, $P < 0.001$), milk fat percentage (4.2 ± 0.1 vs. 2.5 ± 0.1 %, $P < 0.001$), and milk protein percentage (3.1 ± 0.8 vs. 2.2 ± 0.6 %, $P < 0.001$) were greater in primiparous than multiparous cows, respectively. Test-day milk (52.7 ± 0.8 vs. 47.0 ± 0.8 kg) and test-day ECM (50.2 ± 0.8 vs. 44.3 ± 0.8 kg) were greater ($P < 0.001$) for cows calving during the winter-spring than summer months, respectively.

Body Condition and Body Weight

Early ovulating cows lost less ($P = 0.04$) body weight than late ovulating cows between calving and first insemination (-40.5 ± 3.9 vs. -51.6 ± 3.7 kg), respectively. As expected, body weight was greater ($P = 0.03$) in multiparous than primiparous cows at calving (744 ± 7 vs. 588 ± 7 kg, $P < 0.001$) and at insemination (695 ± 7 vs. 546 ± 6 kg, $P < 0.01$), respectively. Body weight at calving (674 ± 6 vs. 658 ± 7 kg, $P < 0.01$) and at insemination (626 ± 5 vs. 615 ± 8 kg, $P < 0.01$) was greater ($P < 0.01$) in cows that calved during winter-spring compared with summer.

Cows ovulating late lost more ($P = 0.03$) body condition than early ovulating cows during the first 9 weeks after calving (Figure 2), with this greater ($P = 0.02$) loss in body condition beginning in week 3. Although primiparous cows tended ($P = 0.08$) to have greater BCS than multiparous cows during the prebreeding postpartum period (2.6 ± 0.03 vs. 2.5 ± 0.03, respectively), the pattern of decrease differed. Body condition score in primiparous cows started at 3.0 ± 0.03 on day 0 and decreased until week 3 and stabilized at 2.5 ± 0.03, whereas multiparous cows started 3.0 ± 0.03 on day 0 and decreased until week 5 and stabilized at 2.4 ± 0.03 until week 9 (parity by week interaction, $P = 0.01$). Body condition at insemination was greater ($P = 0.03$) in primiparous than multiparous cows (2.5 ± 0.04 vs. 2.4 ± 0.04).

Eating Time

Although some daily variation in prepartum eating time was detected ($P < 0.001$), eating time slowly decreased ($P < 0.01$) from days -14 through -1, cows destined to ovulate early or late did not have different prepartum eating patterns (Figure 3A).

After calving, however, early ovulating cows spent more ($P = 0.03$) time eating on days 0, 3, 7, and 14 than late ovulating cows, but the differences ($P = 0.02$) were only observed on days 0, 7, and 14 (data not shown). Pattern of eating time in early-ovulating, primiparous cows decreased by 41% to a nadir on day 7 and then rebounded by 44% to day 14. In contrast, in early-ovulating, multiparous cows, eating time was 13% greater ($P = 0.05$) at calving, reached a nadir on days 3 and 7, and changed very little thereafter to day 14. Late ovulating primiparous and multiparous cows followed similar patterns, decreasing ($P = 0.05$) by 42 to 53% to nadirs on days 3 and 7, respectively, and then increasing from days 7 to 14 by 22%. Multiparous cows spent more ($P = 0.05$) time eating during the first 10 days in milk, and primiparous cows had more ($P = 0.001$) eating time during 8 of the 11 succeeding days to the end of week 3.

During the entire 3-wk postpartum period, early compared with late ovulating cows tended ($P = 0.07$) to spend more time eating (Figure 3A), with daily eating time increasing ($P < 0.001$) gradually during the first 3 weeks. Cows calving during winter-spring spent more ($P = 0.02$) time eating than cows calving during summer (135 ± 7 vs. 114 ± 7 minutes/day), respectively.

Rumination Time

Rumination decreased ($P < 0.001$) gradually during the prepartum period to a nadir on day 0 or 1, but did not differ between future ovulation groups (data not shown). During the last 2 weeks of gestation, multiparous cows spent more ($P = 0.01$) time ruminating than primiparous cows (432 ± 10 vs. 398 ± 10 minutes/day), respectively.

Rumination increased ($P < 0.001$) from postpartum days 0 to 10, and remained unchanged during the remaining 3 weeks and did not differ between ovulation groups. During the first 3 weeks after calving, multiparous cows spent more ($P = 0.004$) time ruminating than primiparous cows (543 ± 11 vs. 499 ± 11 minutes/day), respectively, and rumination time was greater ($P = 0.001$) in cows that calved during summer than during winter-spring (539 ± 9 vs. 503 ± 12 minutes/day), respectively.

Resting Time

Prepartum daily resting time was constant and did not differ between future ovulation groups (Figure 3B). After calving, however, resting time peaked on day 1 or 2 and slowly decreased ($P < 0.001$) thereafter to day 21. In contrast, during the entire 3-wk period after calving, early ovulating cows spent less ($P = 0.02$) time resting than late ovulating cows.

General Activity Time

Prepartum activity increased ($P < 0.001$) during the last 14 days before calving (Figure 3C). Prepartum activity was greater ($P = 0.02$) in primiparous, early-ovulating cows (167 ± 9 minutes/day) than in primiparous, and late ovulating cows (131 ± 11 minutes/day). Primiparous, early-ovulating cows also displayed greater ($P = 0.02$) prepartum activity than multiparous, early ovulating cows (133 ± 10 minutes/day) and multiparous, late ovulating cows (142 ± 9 minutes/day). Prepartum cows were more ($P < 0.001$) active when calving during the summer compared with winter-spring (166 ± 6 vs. 120 ± 8 minutes/day), respectively.

Late ovulating cows were more ($P = 0.06$) active than early ovulating cows during the first 3 weeks after calving and this trend ($P = 0.06$) continued regardless of parity (Figure 3C). Primiparous cows, however, were more ($P < 0.05$) active during the first 3 weeks after calving compared with multiparous cows (185 ± 5 vs. 171 ± 5 minutes/day), respectively. Similar to prepartum cows, postpartum cows calving during summer were more ($P < 0.001$) active than those calving during winter-spring (209 ± 4 vs. 147 ± 6 minutes/day), respectively.

Ear-Surface Temperature

Ear-surface temperature tended to increase beginning 6 days before calving in cows that would ovulate late, whereas cows that would ovulate early, temperature tended ($P < 0.10$) to decrease from day -4 to a nadir on day 1 (Figure 3D). On days 0, 3, 7, and 14, overall ear-surface temperatures averaged 5°C less ($P < 0.001$) in cows that subsequently ovulated early compared with those that ovulated late. The pattern of reduced ($P < 0.05$) ear-surface temperature in early ovulating cows compared with late ovulating cows persisted through the first 3 weeks after calving (Figure 3D).

Although ear-surface temperature did not differ between early and late ovulating primiparous cows (24.9 ± 0.5 vs. 25.1 ± 0.5°C), respectively, early ovulating multiparous cows tended ($P = 0.10$) to have lesser ear-surface temperatures than late ovulating multiparous cows (24.1 ± 0.5 vs. 25.8 ± 0.4°C), respectively. As expected, mean ear-surface temperature was lesser ($P < 0.001$) in cows that calved during the winter-spring than during summer (18.2 ± 0.4 vs. 31.8 ± 0.3°C), respectively.

General Discussion

Failure of cows to adapt during the transition period increases the risk for periparturient diseases and hinders future reproductive performance and milk yield. We previously reported that periparturient disease delays first postpartum ovulation and is associated with measurable changes in periparturient physical activity and postpartum metabolic profiles.

Our first objective was to characterize the association of metabolic measures with the onset of first postpartum ovulation. We observed no relationship of plasma glucose concentrations with days to first ovulation. In contrast, both BHB and FFA concentrations were greater during the first 2 weeks after parturition in late than early ovulating cows. Elevated concentrations of FFA and BHB are not only associated with delayed first ovulation, but also are related to incidence of displaced abomasum. Elevated serum BHB concentrations from days 3 to 14 postpartum are associated with increased risk for displaced abomasum, retained placenta, and clinical ketosis. Although we did not detect relationships of serum calcium with the interval to first ovulation, peripartum hypocalcemia is associated with postpartum health disorders and decreased reproductive efficiency. General increases in the incidence of transition disease delays postpartum ovulation and onset of estrous cycles. This is consistent with greater rectal and ear-surface temperatures during the first 2 weeks after calving, as observed in the present experiment. The rather large 5°C mean separation of ear-surface temperatures between early and late ovulating cows during the first 2 weeks after calving indicates its potential as a predictor of delayed ovulation.

The second objective of the present study was to characterize the association of various physical traits with onset of postpartum ovulation. Cows that ovulated earlier were more active and spent more time eating on days 0, 3, 7, and 14, while daily resting and rumination did not differ from late ovulating cows. Eating time decreased during the last 2 weeks before calving in all cows regardless of future ovulation status, but late ovulating cows had a greater nadir in eating time after calving and tended to spend less time eating during the first 3 weeks. Limiting early postpartum losses in body condition and body weight is important because earlier ovulating cows in the present study lost less body condition and had profiles of metabolites that were consistent with greater health status.

In summary, compared with cows that first ovulated after 33 days in milk, earlier ovulating cows had more estrual events and earlier first postpartum estrus before 63 days in milk. Compared with late ovulating cows, early ovulating cows: 1) had lower rectal temperatures and reduced concentrations of FFA, BHB, and haptoglobin on days 0, 3, 7, and 14; 2) spent more time eating, less time resting, and had greater general activity during the first 3 weeks after calving; 3) had ear-surface temperatures that averaged 5 °C less during the first 2 weeks after calving; 4) lost less BW and BCS during the first 9 weeks; and 5) produced similar amounts of milk but more milk protein.

Conclusions

Metabolic measures during the first 2 weeks after calving, and physical and behavioral traits are associated with the onset of postpartum ovarian activity. Further studies are warranted to determine the sensitivity and specificity of such assessments as predictors of early postpartum ovulation status of lactating dairy cows. In particular, using ear-surface temperatures and other data collected from automated monitoring systems to develop algorithms capable of predicting early first postpartum ovulation is warranted, given the positive effects of early postpartum ovulation on subsequent pregnancy outcomes.

Table 1. Blood metabolites and rectal temperature in cows first ovulating by day 33 postpartum (least squares mean ± SEM)

	First postpartum ovulation (Ov)[1]		*P*-value		
Item[2]	Early (n = 76)	Late (n = 84)	Ov	Day	Ov × day
Plasma glucose (mg/dL)	62.4 ± 0.9	61.1 ± 0.8	0.25	<0.001	0.64
Free fatty acids (mEq/L)	0.47 ± 0.02	0.55 ± 0.02	0.01	<0.001	0.28
β-hydroxybutyrate (mg/dL)	7.3 ± 0.2	8.0 ± 0.2	0.03	<0.001	0.87
Serum calcium (mg/dL)	9.8 ± 0.2	10.2 ± 0.2	0.13	<0.001	0.38
Serum haptoglobin (units)	10.5 ± 0.5	11.7 ± 0.5	0.08	<0.001	0.02
Rectal temperature (°C)	38.9 ± 0.03	39.0 ± 0.03	0.03	<0.001	0.51

[1] Cows had their first increase in weekly progesterone ≥ 1 ng/mL or ultrasound-detected corpus luteum by day 33 (median). Early = < day 33 and late = > day 33.
[2] Assessed at calving (day 0), and days 3, 7, and 14.

Table 2. Milk yield and milk components of early and late ovulating dairy cows (least squares means ± SEM)

	First postpartum ovulation[1]		
Item	Early (n = 76)	Late (n = 84)	*P*-value
Test-day milk[2] (kg/d)	49.0 ± 0.8	50.7 ± 0.8	0.12
Test-day energy-corrected milk (kg/d)	47.0 ± 0.8	47.6 ± 0.8	0.57
Fat (kg/d)	1.54 ± 0.04	1.51 ± 0.04	0.56
Fat (%)	3.41 ± 0.11	3.22 ± 0.11	0.26
Protein (kg/d)	1.27 ± 0.01	1.21 ± 0.01	<0.001
Protein (%)	2.75 ± 0.06	2.51 ± 0.06	<0.01
Somatic cell count (× 1,000)	139 ± 43	141 ± 41	0.98

[1] Cows had their first increase in weekly progesterone ≥ 1 ng/mL or ultrasound-detected corpus luteum by 33 days in milk (median). Early = < day 33 and late = > day 33.
[2] DHI test-day data between 51 and 94 days in milk.

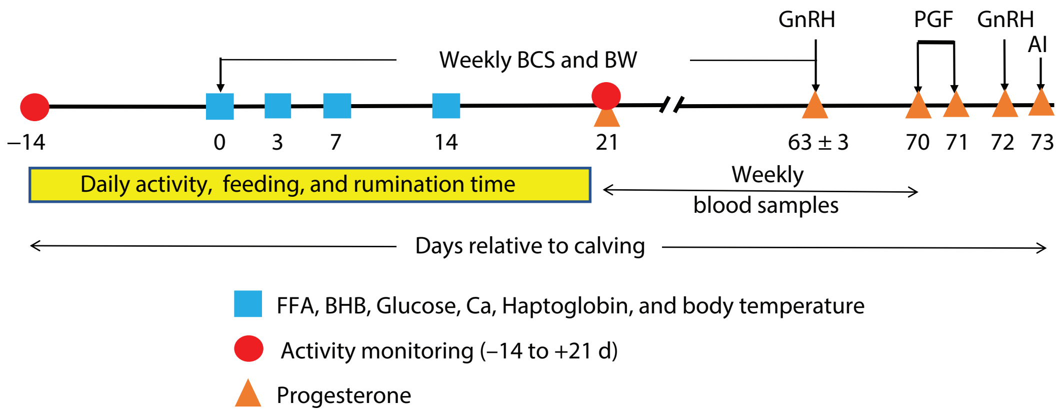

Figure 1. Experimental scheme. Rectal temperatures and blood samples were collected on days 0 (calving), 3, 7, and 14 for later measurement of concentrations of free fatty acids (FFA), β-hydroxybutyrate (BHB), calcium (Ca), haptoglobin (measure of inflammation), and glucose. Blood samples were collected weekly beginning on days 21 through 56 ± 3 in addition to postpartum days 63 ± 3, 70 ± 3, and 73 ± 3 for later analysis of progesterone. Cow SensOor ear tags captured eating, rumination, resting, general activity, and ear-surface temperature during days -14 through +21.

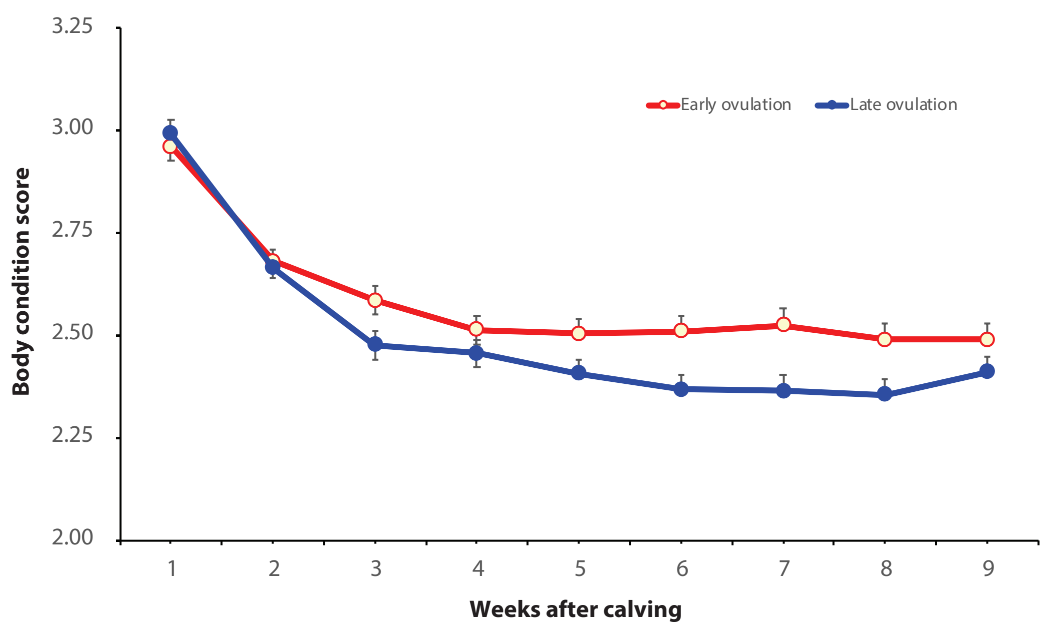

Figure 2. Change in weekly body condition score (BCS; least squares means ± SEM) of cows that subsequently ovulated (Ov) before (early ovulation) or after median postpartum day 33 (late ovulation).

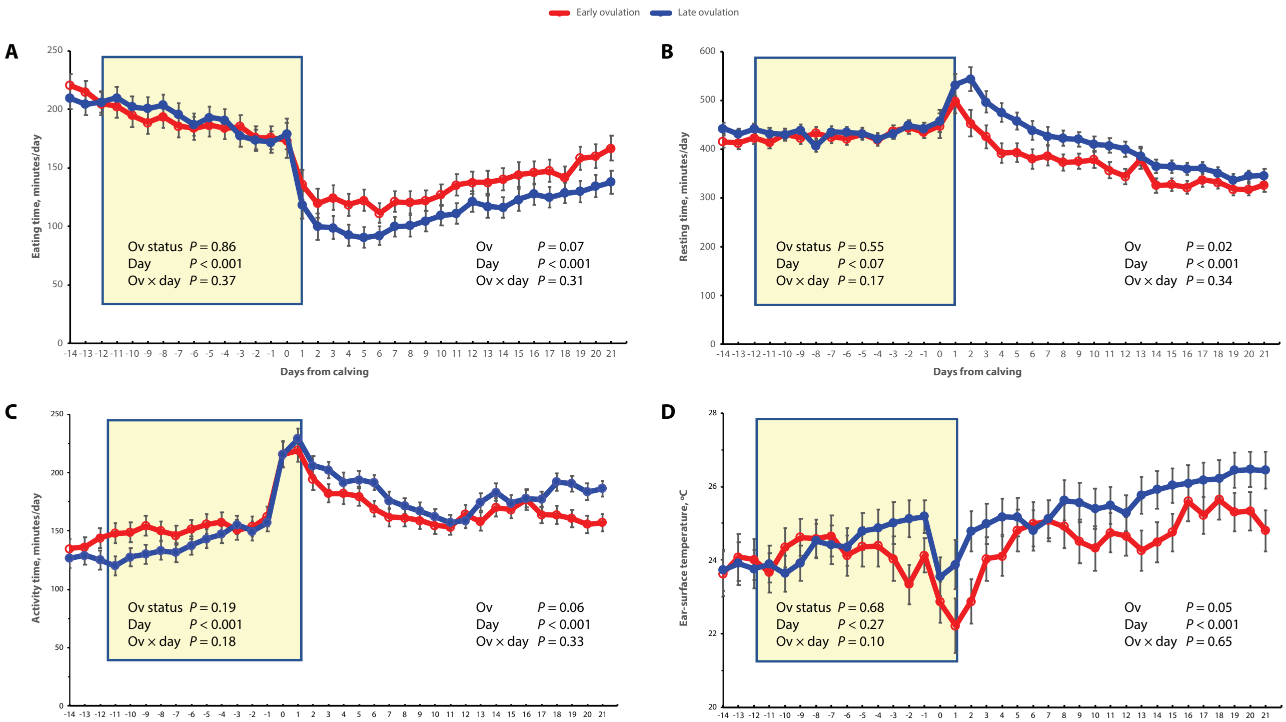

Figure 3. Least squares means (± SEM) of daily (A) eating time; (B) resting time; (C) activity time; and (D) ear-surface temperature assessed on days -14 through -1 (prepartum) and from days 0 (calving) through 21 (postpartum) in cows that subsequently ovulated (Ov) before (early ovulation) or after median day 33 (late ovulation). Ear-surface temperature tended to decrease from day -4 to a nadir on day 1 (ovulation status by day interaction, $P < 0.10$) in early ovulating cows, whereas the temperature increased in late ovulating cows during the same period.