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Use of rumination time in health risk assessment of prepartum dairy cows

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ABSTRACT

The objectives of this observational cohort study were to evaluate the associations of rumination time (RT) in the last week of pregnancy with transition cow metabolism, inflammation, health, and subsequent milk production, reproduction, and culling. Pregnant nulliparous (n = 199) and parous (n = 337) cows were enrolled 21 d before expected calving. Rumination time and physical activity were monitored automatically by sensors from d −21 to 15 relative to calving. Blood samples were collected on d −14, −5, 4, 8, and 12 ± 1 relative to calving. Diagnoses of clinical health problems were performed by researchers from calving to 15 DIM. In classification 1, cows were ranked based on average daily RT in the last week of pregnancy and classified into terciles as short RT (SRT), moderate RT (MRT), or long RT (LRT) for association analyses. In classification 2, RT deviation from the parity average was used in a receiver operating characteristic curve to identify the best threshold to predict postpartum clinical disease. Cows were then classified as above the threshold (AT) or below the threshold (BT). Compared with cows with LRT, cows with SRT had greater serum concentrations of nonesterified fatty acids (0.47 vs. 0.40 ± 0.01 mmol/L), BHB (0.58 vs. 0.52 ± 0.01 mmol/L), and haptoglobin (0.22 vs. 0.18 ± 0.008 g/L) throughout the transition period, and reduced concentrations of glucose, cholesterol, albumin, and magnesium in a time-dependent manner. Parous cows with SRT had higher odds of postpartum clinical disease (adjusted odds ratio [AOR] = 3.7; 95% CI: 2.1–6.4), lower odds of pregnancy by 210 DIM (AOR = 0.34; 95% CI = 0.15–0.75), and lower milk production (46.9 vs. 48.6 ± 0.5 kg/d) than parous cows with LRT. Deviation in prepartum RT had good predictive value for clinical disease in parous cows (area under the curve = 0.65; 95% CI = 0.60–0.71) but not in nulliparous (area under the curve = 0.51; 95% CI = 0.42–0.59). Separation of parous cows according to the identified threshold (≤−53 min from the parity average) resulted in differences in serum concentrations of nonesterified fatty acids (AT = 0.31 ± 0.006, BT = 0.38 ± 0.014 mmol/L), BHB (AT = 0.49 ± 0.008, BT = 0.53 ± 0.015 mmol/L), and globulin (AT = 32.3 ± 0.3, BT = 34.8 ± 0.5 g/L) throughout the transition period, as well as in serum cholesterol, urea, magnesium, albumin, and haptoglobin in a time-dependent manner. Below threshold parous cows had higher odds of clinical disease (AOR = 3.7; 95% CI = 2.1–6.4), reduced hazard of pregnancy (adjusted hazard ratio = 0.64, 95% CI: 0.47–0.89), greater hazard of culling (adjusted hazard ratio = 2.1, 95% CI: 1.2–3.6), and lower milk production (46.3 ± 0.7 vs. 48.5 ± 0.3 kg/d). External validation using data from 153 parous cows from a different herd and the established threshold in RT deviation (≤−53 min) resulted in similar predictive value, with the odds of postpartum disease 2.4 times greater in BT than AT (37.5% vs. 20.1%). In conclusion, RT in the week preceding calving was a reasonable predictor of postpartum health and future milk production, reproduction, and culling in parous cows but not in nulliparous cows.
Key words: clinical disease, dairy cow, milk yield, pregnancy, rumination

INTRODUCTION

Despite current advances in transition cow management and nutrition, clinical and subclinical health conditions in the early postpartum period are still highly prevalent (Carvalho et al., 2019; Pinedo et al., 2020; Macmillan et al., 2021). The consequences of clinical diseases diagnosed in the first 3 wk after calving might last for the entire lactation, negatively affecting milk production, reproduction, and culling (Carvalho et al., 2019; Pinedo et al., 2020; Macmillan et al., 2021). As a strategy to deal with this challenging period, the use of automatic behavioral sensor systems has been growing in popularity to help manage and diagnose health disorders in postpartum cows (Steeneveld and Hogeveen, 2015).

Wearable sensor technologies provide an alternative approach to the traditional and subjective visual observation to monitor cow behavioral and physiological parameters, such as rumination time (**RT**; Steeneveld and

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Hogeveen, 2015). Rumination time has been proposed as an indicator of rumen function (Simoni et al., 2023), welfare (Maia et al., 2020), and health (Paudyal et al., 2018). Rumination activity is highly influenced by NDF intake and particle size of the diet (Beauchemin, 2018), and cows have been shown to spend more time ruminating after periods of high feeding time and intake (Schirmann et al., 2012).

Prepartum RT has been associated with stillbirth and retained placenta (Liboreiro et al., 2015). In the postpartum period, it has been shown that daily RT starts to decrease before changes in milk production and diagnosis of disease (King et al., 2017). A series of studies have demonstrated that postpartum RT and physical activity data generated by sensor systems are useful in the identification of cows with clinical diseases, including mastitis (Stangaferro et al., 2016b), metritis (Stangaferro et al., 2016c), and metabolic and digestive disorders (Stangaferro et al., 2016a). Low RT in the first week after calving has been linked to impaired metabolism, characterized by decreased liver functionality and increased biomarkers of energy metabolism and inflammation (Calamari et al., 2014). Additionally, early postpartum RT has been positively associated with milk production during the onset of lactation (Kaufman et al., 2018) and peak milk yield (Peiter et al., 2021).

Despite the scientific body of evidence showing the associations of postpartum RT with clinical and subclinical health events, as well as milk production, prepartum RT has not been fully explored as a strategy to identify a group of cows with a greater likelihood to experience health issues after calving. The early identification of cows at risk for clinical diseases and impaired performance would allow the adoption of strategies to prevent or minimize these health problems. Prepartum RT has been suggested as a predictor for timing of calving (Büchel and Sundrum, 2014; Borchers et al., 2017) and metritis (Cocco et al., 2021).

Our objectives were to evaluate the associations of prepartum RT with the transition metabolism, incidence of postpartum clinical and subclinical health problems, and the subsequent lactation and reproductive performances in nulliparous and parous cows. We hypothesized that a short RT in the week preceding calving is associated with impaired transition metabolism, higher incidence of postpartum health problems, and reduced lactation and reproductive performances.

MATERIALS AND METHODS

Research protocols used in this study were approved by the Animal Care Committee of the University of Guelph (Animal Utilization Protocol no. 4479).

Animals and Housing

The observational cohort study was performed on a commercial dairy farm in St. Marys, ON, Canada, from September 2021 to September 2022. The herd was chosen based on willingness to participate in the study and proximity to the University of Guelph. Pregnant nulliparous (n = 199) and parous (n = 337) cows were enrolled 21 ± 1 d before the expected calving date. For consistency, the terms nulliparous and parous will be used in reference to parity groups even for postpartum outcomes, when nulliparous pregnant heifers became primiparous cows.

Dry cows were housed in a freestall barn with wood shavings bedding. Nulliparous and parous cows were moved to the close-up pen at 255 ± 3 d of gestation. Once a week, a list of cows was generated using the farm software (DairyComp305, Vas, Visalia, CA) and used by the farm staff to move cows from the far-off pen to the adjacent close-up pen. Cows were moved to maternity pens with straw bedding 2 d before the expected calving date or when showing signs of calving. After colostrum milking at the maternity pen, cows were moved into 1 of the 2 fresh pens with straw bedding adjacent to the milking parlor, which were also used to house cows diagnosed with clinical diseases that required special care. The decision to move cows out of the fresh pens into the freestall pen housing early lactation cows was made by the producer based on the health status of each cow. When the cow was considered healthy, she was moved to the freestall pen at 4 DIM. If the cow still required special care by 4 DIM, she stayed longer in the fresh pen and was moved when considered healthy. The diet fed in the fresh and early lactation pens was the same. Nulliparous and parous cows were managed together in the same pre- and postpartum pens.

All cows were vaccinated against bovine rotavirus (serotypes G6 and G10), bovine coronavirus, *Escherichia coli* (*E. coli*) having the K99 pili adherence factor, and *Clostridium perfringens* Type C (ScourGuard 4KC, Zoetis) and coliform mastitis (Enviracor J-5, Zoetis) at 215 ± 3 and 240 ± 3 d of gestation. Additionally, cows received a monensin oral bolus (Kexxtone, Elanco) and a 20-mL subcutaneous injection of vitamin E (Selon E, Vetoquinol) approximately 21 and 14 d before the expected calving date, respectively.

Pre- and postpartum diets (Table 1) were delivered once daily at 0700 h and pushed up toward the feed bunk 5 times per day. Refusals were removed daily approximately 30 min before a new feed delivery. Samples of TMR from the far-off, close-up, fresh, and lactating cow diets were collected monthly throughout the study. Feed samples were transported to the Department of Animal Biosciences at the University of Guelph, placed in metal trays, weighed, oven-dried at 60°C for 48 h, and reweighed for determination of DM. Dried TMR samples were ground and sent to A & L Canada Laboratories (London, ON, Canada) for chemical composition analyses by wet chemistry and are presented in Table 1.

Rumination Time and Physical Activity

Cows were fitted with an electronic sensor attached to a collar that measures RT and physical activity (HR-LDn tag; SCR Engineers Ltd., Netanya, Israel) 21 ± 1 d before the expected calving date. This sensor has been previously validated in dairy cows (Merenda et al., 2020) and consists of a 3-axis accelerometer equipped with a processing unit and wireless data communication. The raw data generated by the accelerometer were processed by a proprietary algorithm, which created estimations of rumination and movement activity on a minute-by-minute basis. The behavior data were then transmitted to a computer system through a wireless-based farm antenna, and summarized in 2-h intervals by the monitoring software system (Heatime Pro System, Allflex Livestock Intelligence). The 12 daily measurements of each behavior were retrieved weekly by the research team, and summed to obtain daily rumination time in minutes per day and physical activity in arbitrary units (**AU**) per day. Cows remained with this sensor system until 15 ± 1 DIM.

Blood Sampling and Analyses of Target Metabolites

Blood samples were collected from coccygeal blood vessels into 2 tubes, one containing K2-EDTA and one free of any substance (BD Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ), for plasma and serum, respectively. Blood was sampled on d −14 and −5 ± 1 relative to the expected calving date and at 4, 8, and 12 ± 1 DIM. After collection, samples were immediately placed in a cooler containing ice and then transported to the Department of Animal Biosciences at the University of Guelph. Blood samples were centrifuged at 15,000 × *g* for 15 min at 4°C for extraction of plasma and serum, which were transferred into their respective 1.5 mL tubes and stored at −20°C until further laboratory analyses.

Serum samples were sent to the Animal Health Laboratory at the University of Guelph (Guelph, ON, Canada) for measurement of glucose, cholesterol, urea, nonesterified fatty acids (**NEFA**), BHB, total calcium, phosphorus, magnesium, albumin, globulin, and haptoglobin using an automated biochemistry analyzer (Cobas 6000 c 501, Roche Diagnostics). Concentrations of Ca, P, Mg, glucose, and cholesterol (Roche Diagnostics GmbH, Mannheim, Germany), as well as NEFA and BHB (Randox Laboratories, Mississauga, ON, Canada) had a lower limit of quantification (**LLOQ**) of 0.1 mmol/L. Concentrations of urea (Roche Diagnostics GmbH, Mannheim, Germany) had an LLOQ of 0.5 mmol/L. Albumin and globulin concentrations (Roche Diagnostics GmbH, Mannheim, Germany) had an LLOQ of 2 g/L. Serum haptoglobin was measured using a methemoglobin reduction method previously described by Skinner and Roberts (1994), with an LLOQ of 0.02 g/L. A control sample was included in each batch of samples and the interassay coefficient of variation of all metabolites was ≤8%, except for NEFA (13%) and haptoglobin (14%).

Clinical Diseases and BCS

Cows had their locomotion score (1–5 scale; Shearer et al., 2013) and BCS (1–5 scale, 0.25 increments; Ferguson et al., 1994) visually assessed on d −21, 4, and 15 ± 1 relative to calving. Cows had their locomotion score assessed when standing and when walking in a straight line and were considered lame when the score was ≥3 on a 1–5 scale (Shearer et al., 2013). Cows diagnosed lame on d −21 were retained in the study.

Calving was monitored by farm personnel at least 5 times throughout the day, and information regarding the calving ease, stillbirth, twins, and sex of the calf were recorded. Calving assistance, twins, stillborn, or calving complications that resulted in the death of the cow were grouped as calving problems.

In the early postpartum period, cows were monitored for health problems at 4, 8, 12, and 15 ± 1 DIM by the research team, and by the farm staff on the days between visits. Farm employees received training regarding clinical diseases definitions and methods of diagnosis before the study commencement. The sensor system was not used for diagnosis of any disease. Rectal temperature was measured using a digital thermometer (GLA M900; GLA Agricultural Electronics, San Luis Obispo, CA). Cows with a temperature ≥39.5°C were considered pyrexic. Cows that had fever and were not diagnosed with any clinical disease were further classified as fever of unknown origin (**FUO**). Cows with fetal membranes retained by 24 h after calving were diagnosed with retained placenta (**RP**) in a combined effort of the research team or the farm staff. Cows that had recumbence and responded to intravenous administration of Ca were considered to have milk fever.

Metritis diagnosis was performed with the aid of a Metricheck device (Simcro, Hamilton, New Zealand) at 4, 8, and 12 ± 1 DIM by the research team. Before each examination, the Metricheck device, and the vulva and vaginal opening of the cow were cleaned using a 2% chlorhexidine solution and paper towel. The retrieved vaginal content was classified according to Merenda et al. (2021), as follows: 1 = normal lochia; 2 = cloudy mucous; 3 = mucopurulent with less than 50% pus content;

Table 1. Feed ingredients and chemical composition (mean ± SD) of the far-off, close-up, fresh, and lactating cow diets

Item	Far-off	Close-up	Fresh	Lactating
Ingredient, % of DM				
Corn silage	18.7 ± 6.2	47.9 ± 2.4	5.2 ± 0	41.2 ± 2.9
Haylage	47.0 ± 11.0	—	—	20.7 ± 4.7
Chopped straw	23.4 ± 1.6	13.9 ± 2.6	—	—
Dry cow supplement 1[1]	0.9 ± 0	—	—	—
Dry cow supplement 2[2]	—	10.8 ± 0.03	—	—
Dry cow supplement 3[3]	—	3.5 ± 0.2	—	—
Transition cow supplement[4]	—	0.8 ± 0	0.25 ± 0	—
Wet brewer grains	—	7.7 ± 0	—	3.3 ± 0.3
Bakery meal	—	4.0 ± 0	2.3 ± 0	1.6 ± 0.3
Soybean meal, 48%	—	11.2 ± 0	—	4.5 ± 0.9
Lactating cow TMR	—	—	84.1 ± 0	—
Fresh cow supplement[5]	—	—	0.5 ± 0	—
Roasted beans, 38%	—	—	2.3 ± 0	2.1 ± 0.9
Canola meal	—	—	2.3 ± 0	4.6 ± 0.9
Grass hay	—	—	3.1 ± 0	—
High-moisture cob meal	—	—	—	8.6 ± 2.5
High-moisture corn	—	—	—	7.8 ± 2.1
Hi-pro corn distillers	—	—	—	5.1 ± 1.1
Tripper silage	—	—	—	8.3 ± 3.7
Lactating cow supplement[6]	—	—	—	3.2 ± 0.04
DM, %	45.5 ± 3.6	48.4 ± 3.0	48.8 ± 2.0	46.8 ± 1.7
Chemical composition, % of DM				
CP	14.49 ± 1.26	14.98 ± 1.26	16.87 ± 0.64	16.86 ± 0.43
NFC	25.72 ± 2.43	33.17 ± 3.05	40.84 ± 1.61	42.46 ± 0.81
Starch	5.91 ± 1.06	18.15 ± 2.57	21.82 ± 1.32	21.42 ± 1.67
ADF	33.93 ± 2.41	24.08 ± 1.68	19.51 ± 1.13	18.95 ± 0.54
NDF	48.53 ± 3.53	39.51 ± 2.75	31.27 ± 1.24	29.62 ± 1.28
TDN	62.47 ± 1.87	70.14 ± 1.31	73.70 ± 0.88	74.14 ± 0.43
Crude fat	2.72 ± 0.38	4.42 ± 0.50	4.15 ± 1.14	3.78 ± 0.57
Ash	8.55 ± 0.62	7.92 ± 0.96	6.86 ± 0.57	7.27 ± 0.58
Ca	0.95 ± 0.13	0.45 ± 0.11	0.83 ± 0.13	0.69 ± 0.13
P	0.46 ± 0.05	0.40 ± 0.03	0.54 ± 0.04	1.18 ± 0.15
Mg	0.29 ± 0.03	0.31 ± 0.09	0.32 ± 0.05	0.31 ± 0.05
K	2.02 ± 0.32	0.77 ± 0.07	1.27 ± 0.15	1.17 ± 0.15
Na	0.15 ± 0.02	0.32 ± 0.08	0.53 ± 0.10	0.51 ± 0.10
S	0.24 ± 0.02	0.26 ± 0.04	0.28 ± 0.03	0.24 ± 0.01
Cl	0.42 ± 0.06	0.26 ± 0.03	0.35 ± 0.06	0.38 ± 0.05
DCAD, mEq/kg	37.4 ± 6.6	16.8 ± 2.4	35.3 ± 5	32.8 ± 8.1
NE_L, Mcal/kg	1.42 ± 0.04	1.60 ± 0.03	1.69 ± 0.02	1.70 ± 0.01

[1]Provided by Grand Valley Fortifiers Ltd. (Cambridge, ON, Canada): DM (97.7%), mineral oil (2.80%), Ca (8.72%), P (3.91%), Salt (21.50%), Na (8.44%), K (2.43%), Cl (13.12%), Mg (8.97%), S (3.74%), F (386.47 mg/kg), Mn (6,927.13 mg/kg), Zn (8,595.83 mg/kg), Fe (2,263.98 mg/kg), Cu (1,002.92 mg/kg), Se (49.98 mg/kg), Co (86.77 mg/kg), I (105.74 mg/kg), monensin sodium (1,500 mg/kg), Vitamin A (920.04 KIU/kg), Vitamin D (217.67 KIU/kg), Vitamin E (8,508.60 IU/kg), Biotin (0.04 mg/kg).

[2]Provided by Grand Valley Fortifiers Ltd.: DM (90.62%), Ca (2.33%), P (0.55%), Salt (1.75%), Na (0.76%), K (0.77%), Cl (1.08%), Mg (2.31%), S (0.88%), F (16.91 mg/kg), Mn (630.90 mg/kg), Zn (841.78 mg/kg), Fe (298.44 mg/kg), Cu (111.89 mg/kg), Se (4.39 mg/kg), Co (12.20 mg/kg), I (18.79 mg/kg), Cr (5.32 mg/kg), organic Mn (162.04 mg/kg), organic Z (349.52 mg/kg), organic Cu (41.36 mg/kg), organic Se (2.92 mg/kg), organic Co (8.47 mg/kg), monensin sodium (134.00 mg/kg), Vitamin A (80.10 KIU/kg), Vitamin D (18.00 KIU/kg), Vitamin E (1,470.10 IU/kg), Niacin (29.42 mg/kg), Biotin (0.17 mg/kg), RUP lysine (3.23%), RUP methionine (1.79%).

[3]Sodium Aluminosilicate (80% of the composition) and Wheat Starch (carrier), X-Zelit (Protekta, Lucknow, ON, Canada).

[4]Blend of B-Complex vitamins, Vicomb (Jefo, St-Hyacinthe, QC, Canada).

[5]Provided by Grand Valley Fortifiers Ltd.: DM (97.54%), Ca (11.91%), P (0.43%), Salt (12.00%), Na (15.67%), K (0.02%), Cl (7.32%), Mg (4.31%), S (0.90%), F (52.26 mg/kg), Mn (1,727.00 mg/kg), Zn (2,643.00 mg/kg), Fe (381.24 mg/kg), Cu (282.62 mg/kg), Se (17.55 mg/kg), Co (33.26 mg/kg), I (21.92 mg/kg), organic Mn (291.96 mg/kg), organic Z (867.78 mg/kg), organic Cu (74.52 mg/kg), organic Se (8.43 mg/kg), organic Co (15.26 mg/kg), monensin sodium (360.00 mg/kg), Vitamin A (197.77 KIU/kg), Vitamin D (47.23 KIU/kg), Vitamin E (1,564.80 IU/kg), Biotin (11.22 mg/kg).

[6]Provided by Grand Valley Fortifiers Ltd.: DM (97.42%), Ca (12.00%), P (0.43%), Salt (12.00%), Na (15.67%), K (0.02%), Cl (7.32%), Mg (3.76%), S (0.90%), F (50.30 mg/kg), Mn (1,722.13 mg/kg), Zn (2,628.94 mg/kg), Fe (381.22 mg/kg), Cu (281.38 mg/kg), Se (17.41 mg/kg), Co (33.00 mg/kg), I (21.92 mg/kg), organic Mn (287.09 mg/kg), organic Z (853.32 mg/kg), organic Cu (73.28 mg/kg), organic Se (8.29 mg/kg), organic Co (15.01 mg/kg), monensin sodium (420.00 mg/kg), Vitamin A (197.77 KIU/kg), Vitamin D (47.23 KIU/kg), Vitamin E (1,560.79 IU/kg), Biotin (11.22 mg/kg).

4 = mucopurulent with more than 50% pus content; and 5 = watery, reddish, or brownish discharge. The vaginal discharge was also evaluated for its odor. Cows with fetid vaginal discharge that scored 5 were diagnosed with metritis. After completion of an examination, the Metricheck device was cleaned again with 2% chlorhexidine solution for use in a subsequent examination of another cow or storage on a dry clean box until next day of exams.

Respiratory problems were characterized by the increased respiratory rate or effort to breathe and detection of abnormal sounds from the lungs upon auscultation using a stethoscope (3M Littmann Classic III, Littmann, Canada). Digestive problems included displaced abomasum and bloating. Displaced abomasum was diagnosed when the cow presented clinical symptoms (e.g., anorexia, dehydration, and scant feces, either firm or diarrheic) associated with a positive metallic sound upon simultaneous auscultation and percussion of the right or left abdominal wall. Mastitis diagnosis was performed by farm personnel by detection of abnormal milk in the stripping procedure before each milking.

Diagnoses of RP, metritis, milk fever, lameness, mastitis, FUO, and digestive or respiratory problems were collectively grouped as clinical disease through 15 DIM (**ClinD15**). The first 2 items (RP and metritis) were classified as uterine disease (**UTD**) and the remaining as nonuterine disease (**NUTD**). Cows diagnosed with more than one ClinD15 were classified as multiple ClinD15.

Subclinical Events

Subclinical conditions evaluated were hypocalcemia, hyperketonemia, and elevated NEFA. Subclinical hypocalcemia was diagnosed when serum concentrations of Ca were ≤2.2 mmol/L at 4 DIM (Seely and McArt, 2023). Hyperketonemia was diagnosed when serum BHB concentrations were ≥1.2 mmol/L in any postpartum sample (McArt et al., 2012). Elevated NEFA was defined as serum NEFA concentrations ≥0.7 mmol/L in any postpartum sample (Ospina et al., 2010). Cows with more than one subclinical condition were further classified as multiple subclinical events.

Milk Yield and Composition

Cows were milked thrice daily at 0400, 1200, and 2000 h in a herringbone parlor (BouMatic), except for cows in the fresh pen, which were milked twice daily at 0400 and 1200h. Individual milk production was recorded automatically at each milking. Daily average milk yield in the first 14 wk, total milk production at 305 DIM (**M305**), and results from the first 4 DHIA milking tests (performed by Lactanet, Guelph, Ontario, Canada) were retrieved from DairyComp 305 (Vas; Visalia, CA). The DHIA data included information of DIM on the test day, daily milk yield, protein (%), fat (%),SCC, somatic cell linear score, and MUN. Energy-corrected milk of the first 4 DHIA tests was calculated according to the following equation: ECM = [(0.327 × kg of milk) + (12.95 × milk fat) + (7.20 × milk protein)] (Carvalho et al., 2019).

Reproductive Management and Culling

Cows were enrolled in a Double-Ovsynch synchronization program at 35 ± 3 DIM and received a timed AI on the day of the last GnRH injection (50 µg/mL; Fertiline, Vetoquinol) injection at 61 ± 3 DIM. The Select Sires Insemination Team (Kemptville, Ontario) performed all timed AI on Thursday mornings. After the first AI, cows that had estrous behavior detected by visual observation or based on an electronic activity logger (BouMatic) placed on the right front leg were re-inseminated by the farm staff.

Pregnancy diagnosis was performed at 40 ± 3 d after AI by ultrasonography, and pregnant cows were re-evaluated 200 ± 3 d after AI. Nonpregnant cows were resynchronized using an Ovsynch protocol. Seventy-five cows (nulliparous = 51, parous = 24) left the herd before the first AI and were excluded from analyses of reproductive performance. Cows had their reproductive performance followed through 210 DIM.

As for culling information, the date and reason for leaving the herd were recorded through 210 DIM. Culled cows included all individuals that either died or were sold for dairy or beef purposes.

Classification 1—Tercile Groups

Within season and parity, cows were ranked based on the average daily RT in the week preceding calving and classified into terciles: short RT (**SRT**), moderate RT (**MRT**), or long RT (**LRT**). The last week of pregnancy was chosen because of previous associations with the level of prepartum feed intake (Santos et al., 2023) and the magnitude of change in prepartum feed intake (Santos et al., 2022), which were more consistent in the week preceding calving but not earlier.

Classification 2—ROC Curve Threshold

To identify a herd-level threshold of prepartum RT with predictive ability to classify cows according to the risk of postpartum clinical disease, receiver operating characteristics (**ROC**) curves were performed using ClinD15 as the classifier. A total of 3 predictive variables were tested: (1) individual deviation from the parity average in the last 2 wk of pregnancy, (2) individual deviation from the parity average in wk −2 relative to calving, and

(3) individual deviation from the parity average in the wk −1. The parity averages were calculated retrospectively using all cows enrolled in the study. The deviation was calculated by subtracting the herd parity average of the respective period from the individual average of the same period. Thus, a negative deviation represented a shorter RT than the average for the specific parity, and a positive deviation represented a longer RT than the average for the specific parity.

The prepartum period (last 2 wk, wk −2, or wk −1) that resulted in the largest area under the curve (**AUC**) with a significant difference from the reference curve was used to calculate the sensitivity, specificity, and Youden Index (Ruopp et al., 2008). The cut-off value that resulted in the greatest Youden Index was then used to classify cows as either above the threshold (**AT**) or below the threshold (**BT**) and explore the associations of predictive risk with transition metabolism, postpartum health, and subsequent milk production, reproduction, and culling.

External Validation of RT Deviation Threshold

Data on prepartum RT collected in a previous study (Mion et al., 2022) using an older version of the same sensor system (HR tags; SCR Engineers Ltd.) were used to test the ability of the established cut off in RT deviation to predict the incidence of postpartum clinical disease in the first 21 DIM. The older version of the sensor tags had built-in microphones to capture the sounds of regurgitation and rumination, and was also validated for estimations of RT in dairy cows (Schirmann et al., 2009). Diagnoses of clinical diseases were performed by researchers using the same criteria of this study, and described in detail by Mion et al. (2023). Data from 153 parous cows were used, and the effects of dietary treatment and season were considered in the analyses.

Statistical Analyses

Sample size calculations were performed in the WinPepi program version 11.65 (Abramson, 2011). We expected that approximately a third of the population would be classified as high risk for postpartum clinical disease, and the proportion of cows affected with clinical problems would be 45% and 25% for cows classified as high versus low risk. To obtain a statistical power of 80% at 5% significance level, a sample size of 130 in A and 65 in B was deemed necessary. A decision to enroll cows for an entire year was made to represent all seasons of the year in the study and to allow statistical inferences within a parity group.

Continuous data were analyzed by ANOVA using the GLIMMIX procedure of SAS OnDemand (SAS Institute Inc., Cary, NC). Models included the fixed effects of group (classification 1 or 2), parity, time, season, the interactions between group and parity, group and time, and the random effect of the cow nested within the experimental group. Seasons represent 3-mo intervals of calving dates (Season 1: October, November, and December; Season 2: January, February, and March; Season 3: April, May, and June; Season 4: July, August, and September). Pre- and postpartum RT and physical activity were analyzed separately, and the day of calving (d 0) was included in the postpartum period. Milk production during the first 14 wk and M305 were analyzed separately for each parity group. The GEBV of the respective trait or total milk production at 305 DIM from the previous lactation was added as a covariate for nulliparous and parous cows, respectively. Residuals were evaluated for their normality and homogeneity of variance using the UNIVARIATE procedure and ODS graphics of SDS (SAS Institute Inc.). Variables that did not meet the model assumptions were log-transformed and subsequently back-transformed to the original scale returning the value of the exponential function for reporting the results. Results are reported as the LSM and SEM.

The incidence of clinical and subclinical health events, pregnancy at first AI, pregnancy by 210 DIM, culling before first AI, and culling by 210 DIM were analyzed by logistic regression using the GLIMMIX procedure of SAS. Models included the fixed effects of group, parity, season, and the interaction between group and parity. For pregnancy at first AI, the fixed effects of AI technician and sire were added to the statistical model. The results are presented as adjusted odds ratio (**AOR**) followed by the lower and upper 95% CI limits.

Time to pregnancy and time to culling were analyzed by Cox's proportional hazard model using the PHREG procedure of SAS. Models included the effects of group, parity, season, and the interaction between group and parity. Results are reported as adjusted hazard ratio (**AHR**) and 95% CI.

For all analyses above, when they were performed using data from only one parity, then the effects of parity and interactions with parity were removed from the statistical models.

Receiver operating characteristic curve analyses were performed using the LOGISTIC procedure of SAS to identify the optimal threshold of prepartum RT relative to the herd parity average with predictive ability to classify cows according to the risk of ClinD15.

RESULTS

Of 536 cows initially enrolled in this study, 2 cows were excluded. One cow was excluded because of early calving (one day after enrollment) that precluded the collection of prepartum data, and one cow had an accident in the prepartum pen that resulted in a broken leg.

Classification 1

As per the experimental design, the average daily RT in the last week of pregnancy differed ($P < 0.01$) among tercile groups. The averages for the SRT, MRT, and LRT groups, respectively, were 388, 458, and 521 ± 4 min/d in nulliparous cows and 374, 461, and 531 ± 3 min/d in parous cows.

Close-up Duration and Lameness. The time spent in the close-up period (including days in the maternity pen) was shorter ($P < 0.01$) in the LRT group compared with the other 2 groups (SRT = 25.8 vs. MRT = 24.3 vs. LRT = 21.6 ± 0.7). In addition, LRT cows had shorter ($P = 0.01$) gestation length than SRT cows, and both did not differ from MRT cows (SRT = 278.7 vs. MRT = 278.0 vs. LRT = 276.9 ± 0.4).

The overall incidence of lameness on d −21 was 16.1% (87 out of 534). Of all cases, 74% were score 3 and 26% were score 4. When separated by parity, the incidence was 1.5% (3 out of 199) in nulliparous and 24.9% (84 out of 337) in multiparous. Within parous cows, those with SRT had a higher ($P < 0.01$) incidence of lameness on d −21 than parous cows with MRT and LRT (SRT = 36.6% vs. MRT = 19.5% vs. LRT = 18.8%). On average, cows diagnosed lame on d −21 ruminated less during the last 3 wk of pregnancy than nonlame cows (445 ± 11 vs. 481 ± 2 min; $P < 0.01$).

BCS. An interaction ($P < 0.01$) between group and day was observed for BCS. Although all groups started with similar BCS, cows with SRT had a lower ($P = 0.01$) BCS than cows with LRT at 15 DIM. Consequently, cows in SRT lost more BCS ($P < 0.01$) than LRT from d −21 to 15 relative to calving (SRT = −0.41 vs. MRT = −0.36 vs. LRT = −0.30 ± 0.02).

Rumination Time and Physical Activity. When daily RT was evaluated from d −21 to d −1, an interaction ($P < 0.01$) between tercile group and parity (Figure 1A) and an interaction ($P < 0.01$) between tercile group and time (Figure 1B) were observed. Although all tercile groups differed regardless of parity, differences in parous cows were larger than in nulliparous cows (Figure 1A). Moreover, as cows approached calving, the differences in RT between groups increased (Figure 1B).

Regarding postpartum RT, group also interacted ($P = 0.02$) with parity (Figure 1C). After calving, nulliparous cows in SRT and MRT ruminated less ($P < 0.01$) than LRT cows, but SRT cows did not differ from MRT cows. In contrast, parous cows in all groups differed ($P < 0.01$) for postpartum RT. Additionally, there was an interaction ($P < 0.01$) between group and time for postpartum RT (Figure 1D). Cows in the SRT group spent less ($P < 0.01$) time ruminating than LRT cows through the first 9 DIM but not after that. Short rumination time cows ruminated less ($P < 0.01$) than MRT cows on d 0 only, and MRT cows had shorter ($P < 0.05$) RT compared with LRT cows from 2 to 8 DIM.

There was an interaction ($P < 0.01$) between group and parity for prepartum physical activity. Although no difference was found among nulliparous cows, parous cows with SRT had lower ($P < 0.01$) activity than parous cows with MRT and LRT, and the latter 2 groups did not differ (SRT = 406 vs. MRT = 420 vs. LRT = 424 ± 3 AU/d). After calving, SRT cows had a lower ($P < 0.05$) activity compared with MRT and LRT cows (SRT = 448 vs. MRT = 459 vs. LRT = 460 ± 3 AU/d), and the latter 2 groups did not differ. No interaction between group and parity or between group and time was observed for postpartum physical activity.

Metabolites in Serum. There was an interaction ($P < 0.01$) between group and day of sampling for the concentration of glucose in serum (Table 2). Cows with SRT had lower glucose serum concentrations on d −5 ($P = 0.06$) and 4 ($P < 0.01$) than cows with LRT, and both groups did not differ from cows with MRT (Figure 2A).

An interaction ($P < 0.01$) between group and day of sampling was detected for serum concentrations of cholesterol (Table 2). SRT cows had lower ($P < 0.05$) cholesterol in serum than LRT cows on d 8 and 12 (Figure 2B). We also observed an interaction ($P = 0.05$) between group and parity for concentrations of urea in serum (Table 2). No differences were observed among nulliparous cows, but parous cows in SRT had a lower ($P < 0.01$) concentration of urea in serum than MRT and LRT parous cows (Figure 2C).

An effect of tercile group ($P < 0.01$) was observed for concentrations of NEFA and BHB in serum (Table 2). Cows with SRT had greater ($P < 0.01$) concentrations of NEFA and BHB in serum than cows with MRT or LRT throughout the transition period, and the latter 2 groups did not differ (Figure 2D and 2E).

There was an interaction between group and time ($P < 0.01$) for serum concentration of albumin (Table 2). The SRT cows had lower ($P < 0.05$) albumin in serum than LRT cows from d 4 to 12, but no differences were detected between MRT and LRT cows (Figure 2F). An interaction between group and parity ($P = 0.08$) was observed for the serum concentration of globulin (Table 2). Nulliparous cows did not differ for globulin in serum, but parous cows with SRT had greater ($P = 0.01$) concentrations of globulin than parous cows with LRT, but the latter 2 groups did not differ from parous cows with MRT (SRT = 33.95 vs. MRT = 32.79 vs. LRT = 32.06 ± 0.40 g/L). Furthermore, concentrations of haptoglobin in serum were associated with group ($P < 0.01$) and with the interaction between group and day ($P = 0.06$; Table

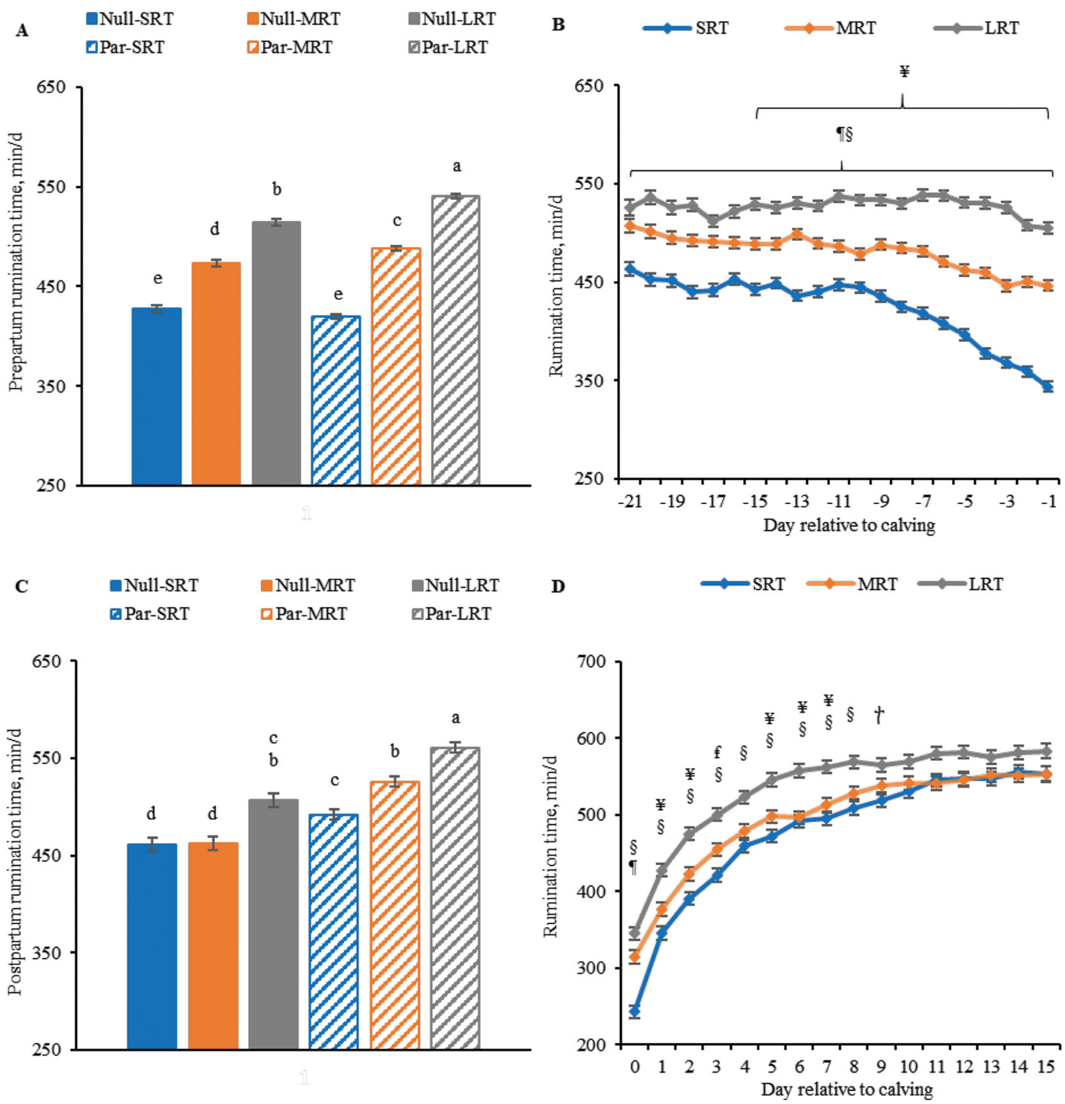

Figure 1. Prepartum (A and B) and postpartum (C and D) RT in cows classified into terciles of average RT in the last week of pregnancy. SRT = short RT; MRT = moderate RT; LRT = long RT; Null = nulliparous cows; Par = parous cows. Data points and error bars represent the LSM and SEM, respectively. Means with different superscripts (a–e) are different at $P \le 0.05$. Within day, pairwise differences ($P \le 0.05$) are represented as follows: ¶ = SRT vs. MRT; § = SRT vs. LRT; ¥ = MRT vs. LRT. Probability values between 0.05 and 0.10 are represented as follows: ψ = SRT vs. MRT; † = SRT vs. LRT; f = MRT vs. LRT.

2). Cows with SRT had greater ($P < 0.01$) concentrations of haptoglobin in serum than cows with LRT throughout the transition period, and both groups did not differ from cows with MRT (Figure 2G).

Concentrations of calcium or phosphorus did not differ between tercile groups (Table 2). However, there was an interaction ($P < 0.01$) between group and day of sampling for the concentrations of magnesium in serum (Table 2). Short RT cows had lower ($P = 0.05$) concentrations of Mg in serum than LRT cows on d 4 and 8, and both groups did not differ from MRT cows (Figure 2H).

Clinical Health Events. The incidences of calving problems, FUO, digestive problems, respiratory problems, mastitis, and milk fever did not differ among groups (Table 3). However, the incidence of lameness was associated ($P = 0.03$) with group (Table 3). Cows with SRT had a greater ($P = 0.01$) incidence of lameness than cows with LRT, and both groups did not differ from cows with MRT (Table 3). Consequently, group was also associated with ($P < 0.01$) the incidence of NUTD (Table 3). Cows with SRT had a greater ($P < 0.01$) proportion of NUTD than the LRT group and did not differ from cows with MRT, which had a greater ($P = 0.08$) incidence of NUTD than cows with LRT (Table 3).

Group and parity interacted ($P = 0.10$) for the odds of RP (Table 3). Although no differences were observed among groups in nulliparous, parous cows with SRT had higher ($P = 0.06$) odds of RP compared with MRT (AOR = 2.23; 95% CI = 0.95–5.24) and both did not differ from parous cows with LRT. An interaction ($P < 0.01$) between group and parity was observed for the incidence of metritis (Table 3). Within nulliparous cows, no differences in the incidence of metritis were detected among groups. However, parous cows with SRT had greater ($P < 0.01$) odds of metritis than MRT (AOR = 2.83; 95% CI = 1.47–5.45) and LRT (AOR = 2.80; 95% CI = 1.45–5.40) cows, but the latter 2 groups did not differ. Likewise, an interaction ($P < 0.01$) between group and parity was observed for the incidence of UTD (Table 3). Although nulliparous cows did not differ in odds for UTD, parous cows with SRT had a greater incidence of UTD than MRT (AOR = 2.37; 95% CI = 1.28–4.41) and LRT (AOR = 2.66; 95% CI = 1.41–5.01) cows, and no difference was found between the latter 2 groups. The proportion of UTD in parous cows was 34.8% (39/112), 18.5% (21/113), and 16.9% (19/112) for SRT, MRT, and LRT, respectively.

An interaction ($P < 0.01$) between group and parity was observed for the incidence of ClinD15 (Table 3). Although no differences were observed among nulliparous cows, parous cows with SRT had higher ($P < 0.01$) odds of ClinD15 than MRT (AOR = 2.17; 95% CI = 1.26–3.74) and LRT (AOR = 3.68; 95% CI = 2.11–6.42) groups. Parous cows with MRT also had greater ($P = 0.05$) odds of ClinD15 compared with LRT (AOR = 1.70; 95% CI = 1.00–2.90) cows. Parous cows with SRT had ClinD15 incidence of 68.7% (77/112), followed by MRT (50.4%; 57/113), and LRT (37.5%; 42/112) groups. An interaction between group and parity was also observed ($P = 0.05$) for the incidence of multiple ClinD15 (Table 3). Although no differences were observed in nulliparous cows, parous cows with SRT had greater ($P \leq 0.01$) odds of multiple ClinD15 than MRT (AOR = 3.09; 95% CI = 1.52–6.30) and LRT (AOR = 2.41; 95% CI = 1.23–4.72) cows. The proportion of multiple ClinD15 for SRT, MRT, and LRT parous cows was 28.6% (32/112), 11.5% (13/113), and 14.3% (16/112), respectively.

Subclinical Health Events. No difference among groups or an interaction between group and parity was observed for the incidence of hypocalcemia, hyperketonemia, elevated NEFA, subclinical disorder, and multiple subclinical disorder (Table 3). In addition, no difference between groups was observed for the proportion of cows with SCC >200,000 in the first DHIA test, which averaged 5.1% for all cows.

Lactation Performance. Group was not associated and did not interact with either parity or time for DIM, milk yield, ECM, fat (%), and SCC of the first 4 DHI tests. However, the percentage of protein differed ($P < 0.01$) among groups. Cows with SRT had lower ($P < 0.01$) protein (%) in milk than both MRT and LRT groups during the first 4 DHIA tests (SRT = 2.95 vs. MRT = 3.02 vs. LRT = 3.02 ± 0.02%). An interaction ($P = 0.01$) between group and parity was detected for MUN. No differences were detected among nulliparous cows, but parous cows with SRT had lower MUN than MRT ($P = 0.05$) and LRT ($P = 0.09$) parous cows (SRT = 7.2 vs. MRT = 8.0 vs. LRT = 7.7 ± 0.18 mg/dL), respectively. Additionally, SRT had greater ($P = 0.02$) SCC linear score than LRT through the first 4 DHIA tests (SRT = 0.65 vs. MRT = 0.57 vs. LRT

Table 2. Concentration of metabolites in serum of cows classified into terciles of average RT in the last week of pregnancy

Item	Group[1]			*P*-value[2]		
	SRT	MRT	LRT	G	G ×P	G × T
Glucose, mmol/L	3.43 ± 0.02	3.47 ± 0.02	3.50 ± 0.02	0.02	0.26	<0.01
Cholesterol, mmol/L	2.37 ± 0.03	2.42 ± 0.03	2.44 ± 0.03	0.23	0.88	<0.01
Urea, mmol/L	3.89 ± 0.04	4.05 ± 0.04	4.07 ± 0.04	0.01	0.05	0.34
NEFA,[3] mmol/L	0.47 ± 0.01	0.43 ± 0.01	0.40 ± 0.01	<0.01	0.93	0.79
BHB, mmol/L	0.58 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	<0.01	0.48	0.23
Calcium, mmol/L	2.34 ± 0.008	2.35 ± 0.008	2.35 ± 0.008	0.95	0.64	0.28
Phosphorus, mmol/L	1.36 ± 0.01	1.34 ± 0.01	1.34 ± 0.01	0.52	0.54	0.96
Magnesium, mmol/L	0.83 ± 0.006	0.85 ± 0.006	0.86 ± 0.006	<0.01	0.26	<0.01
Albumin, g/L	33.53 ± 0.16	33.98 ± 0.16	34.24 ± 0.16	<0.01	0.63	<0.01
Globulin, g/L	31.76 ± 0.33	31.08 ± 0.33	30.89 ± 0.33	0.15	0.08	0.41
Haptoglobin, g/L	0.22 ± 0.008	0.19 ± 0.008	0.18 ± 0.007	<0.01	0.16	0.06

[1]Within parity and season, cows were ranked in ascending order according to the average RT of the last week of pregnancy, and classified into terciles: SRT = short rumination time; MRT = moderate rumination time; LRT = long rumination time.

[2]Probability of values for variables of interest. G, effect of group (SRT vs. MRT vs. LRT); G × P, interaction between group and parity (nulliparous and parous); G × T, interaction between group and day (d −14, −5, 4, 8, and 12).

[3]NEFA = nonesterified fatty acids.

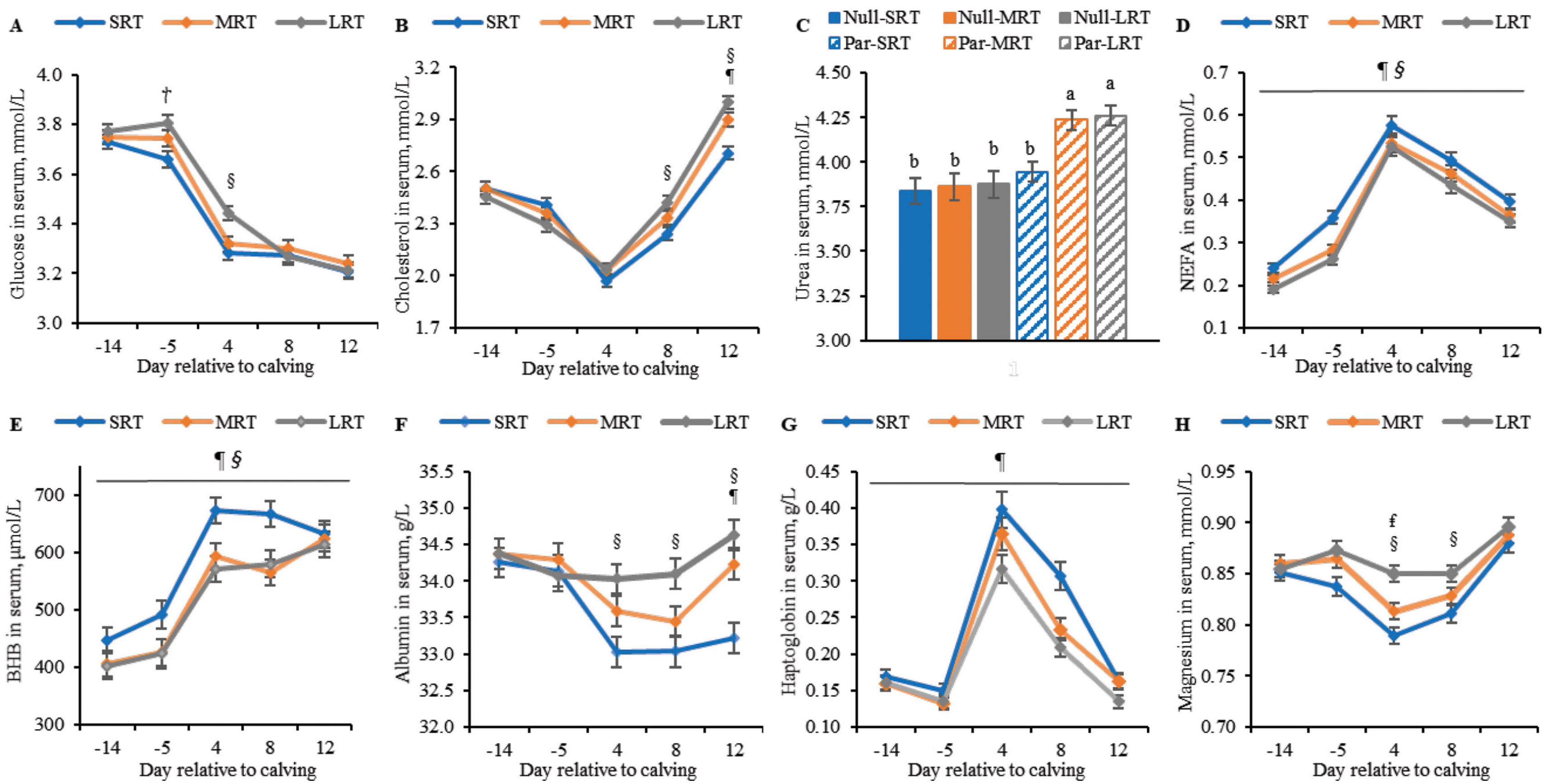

Figure 2. Serum concentration of glucose (A), cholesterol (B), urea (C), NEFA (D), BHB (E), albumin (F), haptoglobin (G), and magnesium (H) in cows classified into terciles of average RT in the last week of pregnancy. SRT = short RT; MRT = moderate RT; LRT = long RT; Null = nulliparous cows; Par = parous cows. Probability values for the effect of group and its interactions with parity and time are presented in Table 2. Data points and error bars represent the LSM and SEM, respectively. Means with different superscripts (a, b) are different at $P \leq 0.05$. Within day, pairwise differences ($P \leq 0.05$) are represented as follows: ¶ SRT vs. MRT; § SRT vs. LRT; ¥ MRT vs. LRT. Probability values between 0.05 and 0.10 are reported as follows: ψ SRT vs. MRT; † SRT vs. LRT; f MRT vs. LRT.

Table 3. Incidence of clinical and subclinical health problems of cows classified into terciles of average RT in the last week of pregnancy

Item	Group[1]			AOR (CI)			*P*-value[2]	
	SRT	MRT	LRT	SRT vs. MRT	SRT vs. LRT	MRT vs. LRT	G	G × P
Clinical health problems								
Calving problems[3]	25.1 (45/179)	25.0 (44/176)	20.7 (37/179)	0.96 (0.58–1.57)	1.28 (0.77–2.14)	0.81 (0.79–2.26)	0.50	0.11
Retained placenta	10.6 (19/179)	7.9 (14/176)	8.47 (15/177)	0.62 (0.19–2.02)	0.64 (0.19–2.08)	1.02 (0.45–2.34)	0.71	0.10
Metritis	28.8 (51/177)	21.1 (37/175)	21.0 (37/176)	1.27 (0.75 to 2.13)	1.30 (0.77–2.18)	1.02 (0.60–1.73)	0.55	<0.01
Lameness	34.7 (61/176)	24.7 (43/174)	17.6 (31/176)	1.65 (0.81–3.38)	4.17 (1.37–12.70)	2.52 (0.80–7.98)	0.03	0.65
Fever of unknown origin	2.8 (5/177)	5.7 (10/175)	4.5 (8/176)	0.47 (0.15–1.46)	0.61 (0.19–1.98)	1.29 (0.48–3.46)	0.43	0.59
Digestive problems	1.7 (3/177)	1.1 (2/175)	0.0 (0/176)	—	—	—	0.99	0.99
Respiratory problems	1.1 (2/177)	0.6 (1/175)	0.0 (0/176)	—	—	—	0.96	<0.01
Mastitis	2.8 (5/177)	1.7 (3/175)	0.6 (1/176)	—	—	—	0.77	0.78
Milk fever	1.7 (3/177)	0.1 (2/175)	0.0 (0/176)	—	—	—	0.99	0.99
Uterine disease	29.9 (53/177)	23.4 (41/175)	22.7 (40/176)	1.16 (0.70–1.93)	1.22 (0.73–2.03)	1.05 (0.63–1.75)	0.73	<0.01
Nonuterine disease	40.7 (72/177)	30.3 (53/175)	21.0 (37/176)	1.49 (0.87–2.54)	2.57 (1.42–4.37)	1.73 (0.94–3.18)	<0.01	0.67
Clinical disease	56.5 (100/177)	46.3 (81/75)	37.5 (66/176)	1.37 (0.87–2.15)	1.83 (1.16–2.89)	1.34 (0.85–2.10)	0.03	<0.01
Multiple clinical diseases[4]	20.9 (37/177)	12.0 (21/175)	11.9 (21/176)	1.31 (0.66–2.62)	1.54 (0.74–3.19)	1.17 (0.58–2.38)	0.49	0.05
Subclinical health conditions								
Hypocalcemia[5]	28.4 (50/176)	25.3 (44/174)	21.4 (37/173)	1.12 (0.67–1.86)	1.38 (0.82–2.31)	1.23 (0.73–2.08)	0.47	0.62
Hyperketonemia[6]	11.2 (19/170)	10.5 (18/171)	9.9 (17/171)	1.16 (0.49–2.75)	1.21 (0.51–1.88)	1.05 (0.42–2.61)	0.89	0.95
Elevated NEFA[7]	53.2 (92/173)	51.4 (89/173)	49.4 (86/174)	1.12 (0.71–1.75)	1.33 (0.85–2.09)	1.19 (0.76–1.86)	0.44	0.11
Subclinical disorder	68.6 (120/175)	62.6 (109/174)	63.8 (111/174)	1.35 (0.83–2.18)	1.40 (0.87–2.26)	1.04 (0.65–1.66)	0.32	0.23
Multiple subclinical disorders[8]	21.4 (37/173)	21.4 (37/173)	15.5 (27/174)	0.94 (0.53–1.64)	1.38 (0.76 to 2.51)	1.48 (0.82–2.66)	0.38	0.72

[1]Within parity and season, cows were ranked in ascending order according to the average RT of the last week of pregnancy, and classified into terciles: SRT = short rumination time; MRT = moderate rumination time; LRT = long rumination time.

[2]Probability of values for variables of interest. G, effect of group (SRT vs. MRT vs. LRT); G × P, interaction between group and parity (nulliparous and parous).

[3]Included dystocia, stillbirth, twins, or associated problems that resulted in the death of the cow.

[4]More than one clinical health problem, including RP, metritis, lameness, FUO, digestive problems, respiratory problems, mastitis, or milk fever.

[5]Serum Ca concentration ≤2.20 mmol/L on sample collected at 4 ± 1 DIM.

[6]Serum BHB ≥ 1.2 mmol/L in at least one sample collected at 4, 8, and 12 ± 1 DIM.

[7]Serum nonesterified fatty acids (NEFA) ≥ 0.7 mmol/L in at least one sample collected at 4, 8, and 12 ± 1 DIM.

[8]More than one subclinical health condition, including hypocalcemia, hyperketonemia, or elevated NEFA.

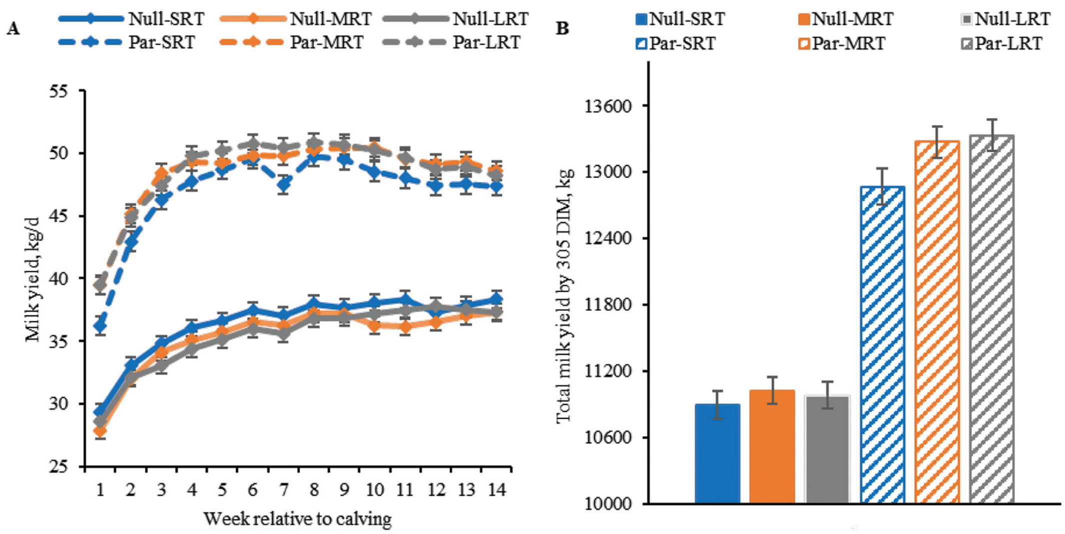

Figure 3. Average daily milk yield in the first 14 wk of lactation (A) and total milk yield by 305 DIM (B) in cows classified into terciles of average RT in the last week of pregnancy. SRT = short RT; MRT = moderate RT; LRT = long RT; Null = nulliparous cows; Par = parous cows. Data points and error bars represent the LSM and SEM, respectively. For both datasets, there were no differences among groups in Null cows, but Par cows with SRT produce less milk ($P = 0.08$) than LRT Par cows.

= 0.50 ± 0.04), but both groups did not differ from cows with MRT.

No differences in milk yield during the first 14 wk of lactation (Figure 3A) and M305 (Figure 3B) were observed among groups in nulliparous cows. However, parous cows with SRT produced less ($P = 0.08$) milk during early lactation (Figure 3A) and by 305 DIM (Figure 3B) than LRT parous cows.

Reproductive Performance and Culling. No differences among groups were observed in the proportion of cows pregnant at first AI, culling before first AI, and culling by 210 DIM. However, an interaction ($P = 0.05$) between group and parity was observed for pregnancy at 210 DIM. Within nulliparous cows, the proportion of pregnancy by 210 DIM did not differ among groups. However, parous cows with SRT had a smaller ($P < 0.01$) proportion of pregnant cows than LRT parous cows (AOR = 0.34; 95% CI = 0.15–0.75) at 210 DIM, and both groups did not differ from the MRT group. The proportion of pregnant parous cows by 210 DIM was 75.5% (74/98), 81.5% (88/108), and 90.1% (91/101) for SRT, MRT, and LRT, respectively. The rate of pregnancy and culling by 210 DIM, measured by Cox's proportional hazard models, did not differ ($P \geq 0.26$) among groups.

Classification 2

The herd average RT for the last 2 wk of pregnancy, during wk −2, and during wk −1 was 470, 485, and 455 ± 4 min, respectively, and did not differ between parity groups. The individual cow variation from the herd average was calculated for all 3 variables and used as predictors of ClinD15 in ROC curve analyses. In nulliparous cows, no differences in AUC were observed between the reference and test curves for any of the variables tested (Table 4). For parous cows, however, the reference and test curves differed ($P < 0.01$) for all 3 variables (Table 4). The RT deviation in the week before calving generated the greatest AUC and Youden Index among the 3 variables tested, and was used to classify parous cows according to the risk of ClinD15 (Table 4). According to the optimal threshold (≤−53 min) generated by the ROC analysis, 78 cows (23.1% of parous cows) were classified as BT (higher risk of postpartum disease) and 259 cows as AT (lower risk of postpartum disease). As a result of our experimental design, there was a difference ($P < 0.01$) between groups for the deviation in RT relative to the herd RT in wk −1. Cows in AT and BT ruminated on average +31 ± 3 and −102 ± 5 min relative to the herd average, respectively.

Close-Up Duration and Lameness. Parous cows in the AT group spent less time in the close-up pen than cows in the BT group (AT = 20.8 ± 0.5 d vs. BT = 25.0 ± 1.0 d; $P < 0.01$). In addition, AT cows had shorter gestation length than BT cows (AT = 278.2 ± 0.4 d vs. BT = 279.7 ± 0.7 d; $P < 0.01$). The incidence of lameness on d 21 was higher ($P < 0.01$) in BT than in AT cows (AT = 20.4% vs. BT = 39.7%).

BCS. An interaction between group and day was detected ($P < 0.01$) for BCS. Although both groups had similar BCS before calving, cows in the BT group had a lower BCS than cows in the AT group on d 15 (AT = 3.05

Table 4. Area under the curve, optimal threshold, sensitivity, specificity, and Youden Index of deviations in prepartum RT to predict postpartum clinical disease

Item[1]	AUC[2] (95% CI)	*P*-value[3]	Threshold (min)	Sensitivity	Specificity	Youden Index[4]
Nulliparous						
RT last 2 wk	0.51 (0.426–0.597)	0.79	≤−23	0.37	0.72	0.09
RT wk −2	0.53 (0.442–0.614)	0.53	≤−16	0.46	0.66	0.12
RT wk −1	0.51 (0.421–0.591)	0.89	≤−72	0.92	0.15	0.07
Parous						
RT last 2 wk	0.64 (0.583–0.700)	<0.01	≤−11	0.65	0.55	0.20
RT wk −2	0.62 (0.564–0.683)	<0.01	≤−32	0.36	0.78	0.14
RT wk −1	0.65 (0.595–0.711)	<0.01	≤−53	0.43	0.81	0.25

[1]RT last 2 wk = deviation in rumination time relative to herd rumination of the last 2 wk of pregnancy; RT wk-2 = deviation in rumination time relative to herd rumination of the week −2 relative to calving; RT wk −1 = deviation in rumination time relative to herd rumination of the last week of pregnancy.

[2]AUC = area under the curve.

[3]Probability of values (reference curve vs. model curve).

[4]Youden Index = sensitivity + specificity − 1.

± 0.02 vs. BT = 2.88 ± 0.03). As a result, BT cows lost more BCS than AT cows throughout the transition period (AT = −0.25 ± 0.02 vs. BT = −0.43 ± 0.03).

Rumination Time and Physical Activity. An interaction between group and time was observed ($P < 0.01$) for RT in both pre- and postpartum periods. During the prepartum period, cows in the BT group ruminated less ($P < 0.01$) throughout the last 3 wk of pregnancy, but the difference increased as cows approached the day of calving (Figure 4A). During the postpartum period, BT cows continued to have a shorter ($P < 0.01$) RT than AT cows up to 9 DIM (Figure 4B) but not after that. Additionally, BT cows had lower ($P < 0.01$) activity than AT cows in both pre- and postpartum periods (Figure 4C and 4D)

Metabolites in Serum. An interaction between group and time ($P = 0.01$) was observed for concentrations of glucose in serum (Table 5). Although no differences within day were observed between groups, cows in the BT group had numerically higher concentrations of glucose from d −14 to 4, and the opposite on d 8 and 12. An interaction ($P < 0.05$) between group and time was also observed for serum concentrations of urea and cholesterol (Table 5). Compared with the AT group, cows in the BT group had lower ($P < 0.01$) concentrations of urea on d −14 and −5 (Figure 4A) and cholesterol on d 8 and 12 (Figure 4B).

Serum concentrations of NEFA and BHB were associated ($P < 0.05$) with group (Table 5), with greater concentrations observed in BT cows than in AT cows throughout the transition period (Figure 5C and 5D). An interaction between group and day of sampling was observed ($P < 0.01$) for the concentration of albumin in serum (Table 5). Cows in the BT groups had lower ($P < 0.05$) albumin in serum after calving (Figure 5E), and greater ($P < 0.01$) concentrations of globulin in serum (Table 5) than AT cows (Figure 5F). Group and time interacted ($P = 0.10$) for concentrations of haptoglobin in serum (Table 5). The BT cows had greater ($P \leq 0.01$) haptoglobin in serum than AT cows on d 4 and 8 (Figure 5G).

No effect of group or interaction with time was observed for serum concentrations of calcium and phosphorus, but an interaction ($P < 0.01$) between group and time was observed for concentrations of magnesium in serum (Table 5). Cows in the BT group had lower ($P < 0.01$) concentrations of magnesium than AT cows on d 4 and 8 (Figure 5H).

Clinical Health Events. The incidence of digestive and respiratory problems, FUO, mastitis, and milk fever did not differ between groups (Table 6). However, cows in the BT group had greater ($P \leq 0.01$) odds of calving problems, RP, metritis, and lameness than cows in the AT group (Table 6). Consequently, cows in the BT group had a higher ($P < 0.01$) incidence of UTD, NUTD, ClinD15, and multiple ClinD15 than cows in the AT group (Table 6).

Subclinical Health Events. No differences in the incidence of hypocalcemia, hyperketonemia, elevated NEFA, subclinical conditions, or multiple subclinical conditions were observed between groups (Table 6). The proportion of cows with SCC >200,000 was associated ($P = 0.09$) with group, and averaged 10.7% ± 3% and 4.9% ± 1% for BT and AT cows, respectively.

Lactation Performance. Group was not associated with and did not interact with DHIA test number for DIM at the test day, milk yield, ECM, and fat (%). However, there was an interaction ($P < 0.05$) between group and DHIA test number for protein (%) and MUN. Compared with cows in the AT group, BT cows had a lower percentage of protein in milk in the first (AT = 3.22% ± 0.02% vs. BT = 3.12% ± 0.04%; $P = 0.02$) and third (AT = 3.02% ± 0.02% vs. BT = 2.84% ± 0.04%; $P < 0.01$) DHIA tests and lower MUN in the second (AT = 7.75 ± 0.19 vs. BT = 6.33 ± 0.30 mg/dL; $P < 0.01$) DHIA test. Group was also associated with ($P < 0.01$) SCC and SCC

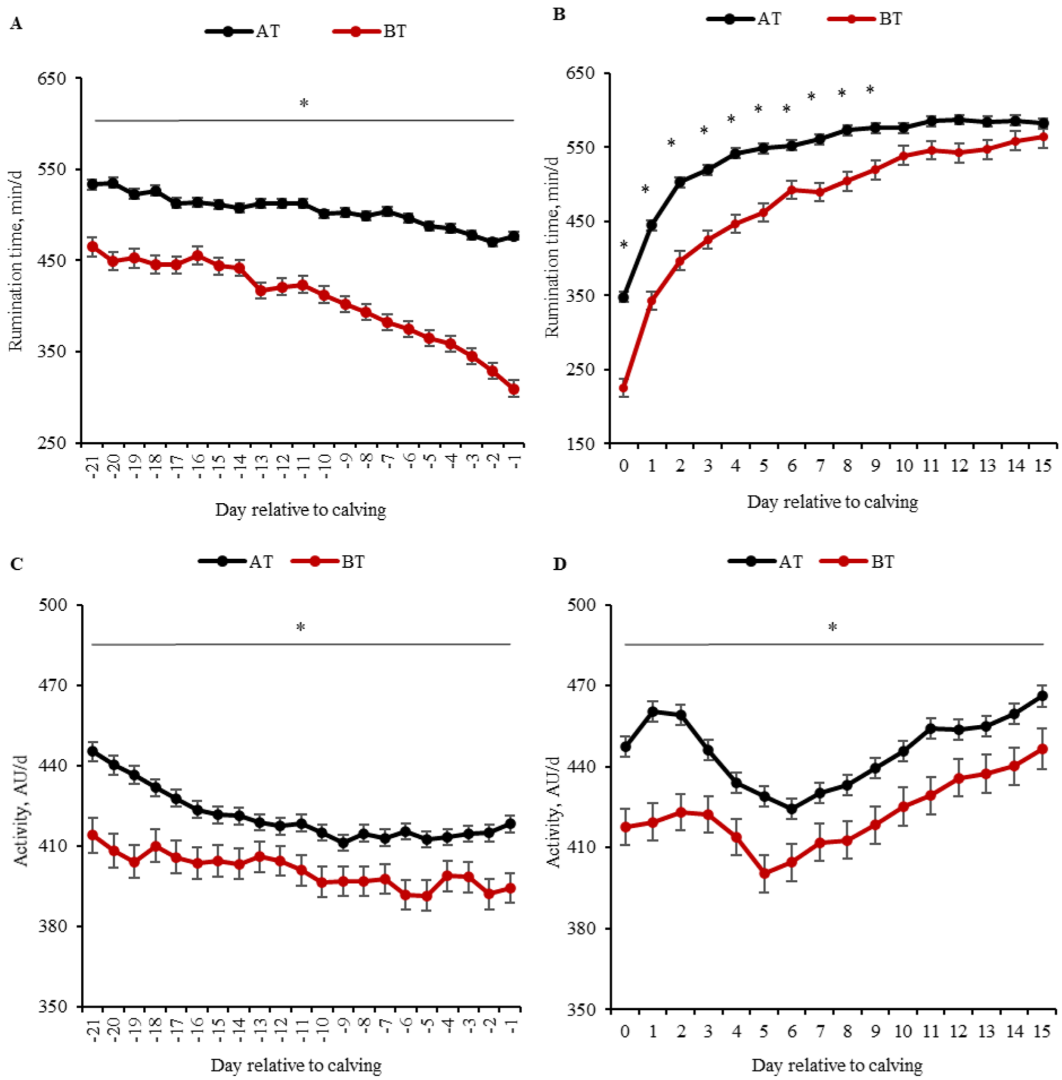

Figure 4. Prepartum rumination time (A), postpartum rumination time (B), prepartum physical activity (C), and postpartum physical activity (D) measured by wearable sensors in cows classified as above the threshold (AT) or below the threshold (BT) of rumination time deviation from the herd average in the last week of pregnancy. The optimal threshold of deviation in rumination time (≤−53 min) was generated through a ROC curve analysis to predict postpartum clinical disease. Data points and error bars represent the LSM and SEM, respectively. Within day, pairwise differences ($P \leq 0.05$) are represented by asterisks (*).

linear score, with higher SCC (AT = 22.4 ± 2.8 vs. BT = 30.4 ± 1.1 × 10^3 cells/mL) and SCC linear score (AT = 0.48 ± 0.03 vs. BT = 0.73 ± 0.08) in BT cows than in AT cows for the first 4 DHIA tests.

Cows in the BT group produced less milk during the first 14 wk of lactation ($P < 0.01$) and by 305 DIM ($P = 0.07$) compared with cows in the AT group (Figure 6A and 6B).

Reproductive Performance and Culling. No difference in pregnancy at first AI was observed. However, cows in the BT group had lower ($P = 0.02$) odds of pregnancy by 210 DIM than AT cows (AOR = 0.44; 95% CI = 0.22–0.87). The proportion of cows pregnant by 210 DIM for AT and BT was 85.1% (206/242) and 72.3% (47/65), respectively. Furthermore, BT cows had a lower ($P < 0.01$) pregnancy rate than cows in the AT group (AHR = 0.64; 95% CI = 0.47–0.89; Figure 7A). The median time to pregnancy was 106 (95% CI = 89 to 110) and 129 (95% CI = 99 to 154) DIM for AT and BT groups, respectively.

There was a difference ($P = 0.02$) between groups for the proportion of cows culled before the first AI, with greater odds of culling for BT cows than AT cows (AOR = 2.94; 95% CI = 1.21–7.14). Before the first breeding, 4.6% (12/259) of AT cows and 12.8% (10/78) of BT cows left the herd. Likewise, BT cows had greater ($P = 0.01$) odds of culling than AT cows by 210 DIM (AOR = 2.24; 95% CI = 1.17–4.28). The proportion of cows that left

Table 5. Concentration of metabolites in blood serum of cows classified as above or below the threshold of RT deviation from herd average in the last week of pregnancy for prepartum health risk assessment

	Risk group [1]		*P*-value[2]	
Item	AT	BT	G	G × T
Glucose, mmol/L	3.23 ± 0.01	3.22 ± 0.03	0.01	0.71
Cholesterol, mmol/L	2.50 ± 0.03	2.41 ± 0.05	0.10	<0.01
Urea, mmol/L	4.22 ± 0.04	3.89 ± 0.07	<0.01	0.04
NEFA,[3] mmol/L	0.31 ± 0.006	0.38 ± 0.014	<0.01	0.53
BHB, mmol/L	0.49 ± 0.01	0.54 ± 0.02	0.02	0.01
Calcium, mmol/L	2.32 ± 0.007	2.31 ± 0.01	0.33	0.11
Phosphorus, mmol/L	1.26 ± 0.02	1.25 ± 0.01	0.67	0.79
Magnesium, mmol/L	0.85 ± 0.005	0.81 ± 0.008	<0.01	<0.01
Albumin, g/L	34.14 ± 0.13	33.32 ± 0.23	<0.01	<0.01
Globulin, g/L	32.30 ± 0.28	34.84 ± 0.51	<0.01	0.69
Haptoglobin, g/L	0.18 ± 0.005	0.24 ± 0.01	<0.01	0.10

[1]Cows were grouped according to the optimal threshold (≤−53 min) of deviation in RT generated in the ROC analysis: AT = above the threshold; BT = below the threshold.

[2]Probability of values for variables of interest. G = effect of group (AT vs. BT); G × T = interaction between group and day.

[3]NEFA = nonesterified fatty acids.

the herd by 210 DIM was 12.4% (32/259) and 24.4% (19/78) for AT and BT groups, respectively. In addition, the rate of culling was also different (P = 0.01) between groups, and the AHR was 2.06 (95% CI = 1.16 to 3.64) in the BT group compared with the AT reference group (Figure 7B).

External Validation of RT Deviation Threshold. Of 153 parous cows used for an external validation, 40 (26%) were diagnosed with a clinical disease in the first 21 DIM. Using the threshold in RT deviation determined above (≤−53 min in the last week of pregnancy), 46 cows (30%) were classified as BT and 107 cows (70%) were classified as AT. The adjusted odds of postpartum disease was 2.4 times greater in BT than AT cows (39.1% vs. 20.6%; P = 0.03). The overall agreement of the test was similar to that observed in the previous study, with 45.0% sensitivity, 75.2% specificity, 39.1% positive predictive value, and 79.4% negative predictive value.

DISCUSSION

The use of automated behavioral data as a strategy to aid on the diagnosis of postpartum health problems has gained popularity in the dairy industry and has been investigated by multiple research groups (Stangaferro et al., 2016a,b,c; King et al., 2017, 2018; Paudyal et al., 2018; Silva et al., 2021). The present study, however, provides evidence that prepartum RT data have the potential to be used as an early indicator of the likelihood of postpartum clinical disease in parous cows. Reduced RT in the week before calving was negatively associated with the overall metabolism of transition cows, the incidence of clinical disease during early lactation and, consequently, the subsequent milk production, reproduction, and culling in parous cows.

Previous research has shown that rumination activity and feed intake follow a similar pattern around the time of calving (Schirmann et al., 2013). We focused our studies on the average RT in the last week of pregnancy because this variable had a stronger correlation with feed intake than the average RT in the weeks −2 and −3 relative to calving (Santos et al., 2023). Reduced prepartum feed intake has been associated with energy and nutrient imbalances (Santos et al., 2022; 2023), impaired neutrophil function (Hammon et al., 2006), greater likelihood of postpartum health problems (Hammon et al., 2006; Pérez-Báez et al., 2019a,b), augmented severity of metritis (Huzzey et al., 2007), and reduced lactation performance (Santos et al., 2023). Given the associations above, we hypothesized that prepartum RT could be useful for health risk assessment of prepartum cows, which could add value to rumination sensor data collected during the prepartum period.

To understand the relationship of prepartum RT with subsequent health, lactation, and reproductive performances, we initially divided cows into terciles of average RT in the last week of pregnancy. Within parous cows, significant associations were observed and they indicated a linear relationship with all outcomes of interest, which motivated us to use ROC curves to identify an optimized threshold deviation from the average prepartum RT of the herd. Among the terciles, parous cows with SRT (first tercile) differed the most from the other groups and were at higher risk of postpartum health problems and subpar performance. The optimized threshold, however, was more stringent and separated 23% of the parous cows with the lowest values of RT, increasing the differences in prepartum RT and postpartum health, milk production, reproduction, and culling between risk categories. In an

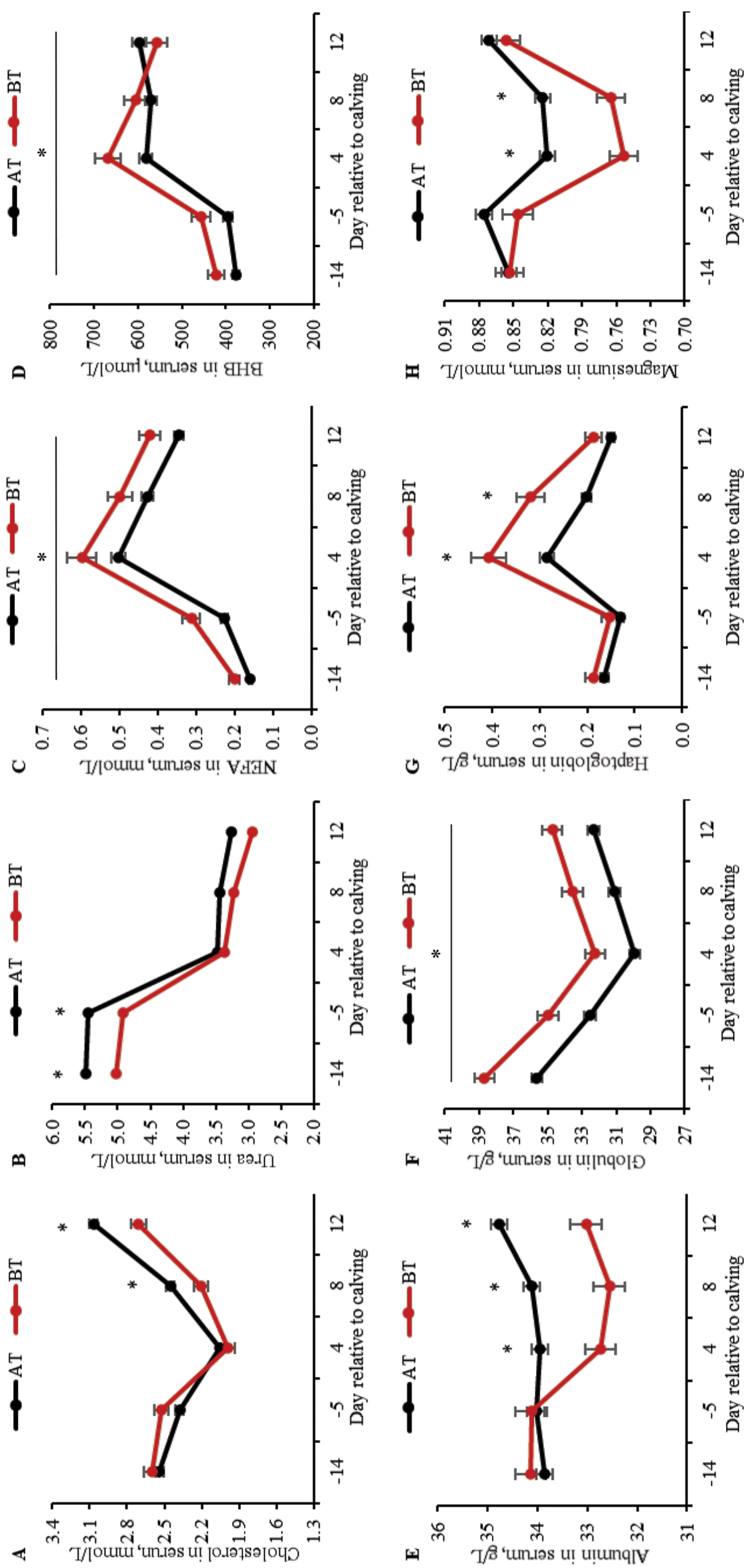

Figure 5. Serum concentration of cholesterol (A), urea (B), NEFA (C), BHB (D), magnesium (E), albumin (F), globulin (G), and haptoglobin (H) in cows classified as AT or BT of RT deviation from herd average in the last week of pregnancy for prepartum health risk assessment. The optimal threshold (≤−53 min) of deviation in RT was generated through a ROC curve analysis to predict postpartum clinical disease. Data points and error bars represent the LSM and SEM, respectively. Means with different superscripts are different at $P \leq 0.05$. Within day, pairwise differences ($P \leq 0.05$) are represented by asterisks (*).

Table 6. Incidence of clinical and subclinical health problems in cows classified as above or below the threshold of RT deviation from herd average in the last week of pregnancy for prepartum health risk assessment

Item	Risk group[1]		AOR (CI)	*P*-value
	AT	BT		
Clinical health problems				
Calving problems[2]	13.9 (36/259)	26.9 (21/78)	1.42 (1.20–4.13)	0.01
Retained placenta	8.9 (23/259)	19.2 (15/78)	2.44 (1.19–5.01)	0.01
Metritis	16.2 (42/259)	37.2 (29/78)	2.96 (1.67–5.25)	<0.01
Lameness	31.4 (81/258)	55.8 (43/77)	2.86 (1.68–4.87)	<0.01
Digestive problems	0.6 (2/259)	2.6 (2/78)	4.01 (0.53–31.43)	0.17
Fever of unknown origin	4.2 (11/259)	1.3 (1/78)	0.31 (0.04–2.49)	0.27
Respiratory problems	0 (0/259)	0 (0/78)	—	—
Mastitis	1.2 (3/259)	2.6 (2/78)	2.20 (0.35–14.05)	0.98
Milk fever	0.8 (2/259)	2.6 (2/78)	3.24 (0.44–24.02)	0.25
Uterine disease	18.5 (48/259)	39.7 (31/78)	2.81 (1.61–4.91)	<0.01
Nonuterine disease	34.4 (89/259)	57.7 (45/78)	2.69 (1.59–4.56)	<0.01
Clinical disease	45.9 (119/259)	73.1 (57/78)	3.19 (1.82–5.59)	<0.01
Multiple clinical diseases[3]	13.1 (34/259)	34.6 (27/78)	3.48 (1.92–6.30)	<0.01
Subclinical health conditions				
Hypocalcemia[4]	29.1 (75/258)	32.9 (25/76)	1.17 (0.67–2.04)	0.58
Hyperketonemia[5]	12.8 (33/258)	14.5 (11/76)	1.16 (0.55–2.45)	0.68
Elevated NEFA[6]	49.2 (127/258)	48.7 (37/76)	0.99 (0.59–1.65)	0.96
Subclinical disorder	65.5 (169/258)	61.8 (47/76)	0.83 (0.49–1.43)	0.51
Multiple subclinical disorders[7]	22.1 (57/258)	28.9 (22/76)	1.45 (0.81–2.60)	0.21

[1]Cows were grouped according to the optimal threshold (≤−53 min) of deviation in RT generated in the ROC analysis: AT = above the threshold; BT = below the threshold.

[2]Included dystocia, stillbirth, twins, or associated problems that resulted in the death of the cow.

[3]More than one clinical health problem, including RP, metritis, lameness, FUO, digestive problems, respiratory problems, mastitis, or milk fever.

[4]Serum Ca concentration ≤2.20 mmol/L on sample collected at 4 ± 1 DIM.

[5]Serum BHB) ≥1.2 mmol/L in at least one sample collected at 4, 8, and 12 ± 1 DIM.

[6]Serum nonesterified fatty acids (NEFA) ≥ 0.7 mmol/L in at least one sample collected at 4, 8, and 12 ± 1 DIM.

[7]More than one subclinical health condition, including hypocalcemia, hyperketonemia, or elevated NEFA.

external validation using data from an independent herd, the optimized threshold separated 30% of prepartum parous cows as high risk. The odds of a postpartum clinical disease in high-risk cows were 3.2 and 2.4 times greater than in low-risk cows in the original and validation data sets, respectively. Future research should expand this approach of health risk assessment in prepartum cows to additional herds and provide recommendations on how to determine the best threshold within or across herds. More importantly, future studies should determine what type of targeted intervention can be used to reduce health problems and their consequences in cows classified as high risk.

The lack of associations between prepartum RT and postpartum health and performance in nulliparous heifers was intriguing and revealing. Compared with prepartum parous cows, prepartum nulliparous heifers are a more homogeneous group and present smaller variability in feed intake, RT, and physical activity (Santos et al., 2022, 2023), different energy and nutrient requirements (NASEM, 2021), different metabolism and physiology (Wathes et al., 2007; Mion et al., 2022), and reduced incidence of postpartum health problems (Santos et al., 2010; Carvalho et al., 2019; Pinedo et al., 2020). Thus, additional factors other than prepartum feed intake and RT are likely more important for determining the postpartum health and performance in nulliparous heifers, and a different approach to health risk assessment is necessary for this subgroup of cows. It is possible that variability in their first-time adaptation responses to the stress of calving, the onset of lactation, and a lactating cow diet overshadow the relevance of prepartum variables to postpartum health and performance in heifers.

The underlying causes of variability in prepartum DMI and RT, and their direct or indirect associations with postpartum health and performance are still not fully understood. High BCS at dry-off has been associated with reduced prepartum DMI (Daros et al., 2021; Santos et al., 2023) and the likelihood of postpartum disease (Chebel et al., 2018); however, we did not observe differences in prepartum BCS between groups of RT in the current study. On average, prepartum cows classified as high risk of postpartum health problems (SRT or BT) spent a few more days in the close-up pen than those classified as low risk of postpartum health problems (MRT and LRT or AT). Previous research have demonstrated that time spent in the close-up has a quadratic relationship with the likelihood of postpartum health problems, with too short

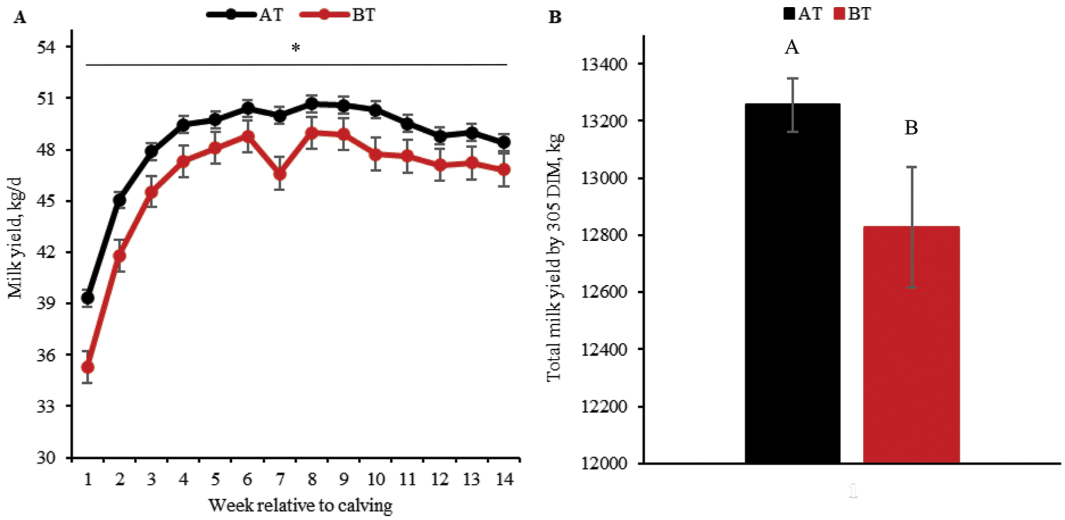

Figure 6. Milk yield during early lactation (A) and total milk yield by 305 DIM (B) in cows classified as AT or BT of RT deviation from herd average in the last week of pregnancy for prepartum health risk assessment. The optimal threshold (≤−53 min) of deviation in RT was generated through a ROC curve analysis to predict postpartum clinical disease. Cows in the BT group produced less milk during the first 14 wk of lactation (*$P < 0.01$, denoted by horizontal line in panel A) and by 305 DIM ($^{A, B}P = 0.07$) compared with cows in the AT group. Data points and error bars represent the LSM and SEM, respectively.

or too long close-up periods resulting in higher incidence of postpartum diseases and impaired lactation performance (Vieira-Neto et al., 2021; Venjakob et al., 2023). Interestingly, Venjakob et al. (2023) described that cows with a short (i.e., 7 d) or long (i.e., 35 d) close-up period had reduced eating behavior and RT (both measured by a wearable sensor) during the last week of pregnancy compared with cows that had an intermediate duration of the close-up period (i.e., 14, 21 or 28 d). An important difference in our study from these 2 references (Vieira-Neto et al., 2021; Venjakob et al., 2023) is that zeolite supplementation, and not acidogenic diets, was used as a strategy to prevent hypocalcemia. Time spent in close-up also depends on gestation length (Olagaray et al., 2020; Cook et al., 2021), which was also longer in cows classified as high risk and seemed to partially explain the differences in duration of the close-up period. Combined with the duration of close-up period, gestation length is also an important predictor of postpartum health, with short and long gestation lengths generally increasing the likelihood of postpartum problems (Vieira-Neto et al., 2017; Olagaray et al., 2020; Cook et al., 2021).

Prepartum cows classified as high risk were more likely to be lame when moved to the close-up pen, a characteristic that was associated with reduced rumination. Lameness negatively affects feeding behavior and is normally associated with local and possibly systemic inflammation, and both these factors normally reduce feed intake (Norring et al., 2014; Bradford et al., 2015). It is noteworthy, however, that we did not observe changes in inflammatory markers between risk groups during the prepartum period, which does not support the idea that variability in prepartum RT could be attributed to variability in the levels of prepartum inflammation. Prepartum lame cows were more likely to be diagnosed lame after calving, but had similar incidence of other postpartum clinical diseases. Thus, the association of prepartum RT with postpartum diseases other than lameness, and the fact that inclusion of prepartum lameness as a covariable in our statistical models did not change the associations between prepartum RT and postpartum health suggest that hoof health does not fully explain the observed relationships. Future research should explore what factors influence prepartum feed intake and rumination activity, and how multiple variables (e.g., sensor data, locomotion score, BCS, previous lactation performance) could be combined into a single health risk assessment.

In addition to differences in the incidence of postpartum clinical diseases, health risk groups also had important differences in the concentrations of blood metabolites and changes in BCS. Cows with the lowest prepartum RT had lower glucose concentrations on the days around calving. This is likely a response to reduced DMI summed to the increased glucose requirements of the fetus and mammary gland around parturition (Bell and Bauman, 1997; Grummer et al., 2004). Reduced prepartum RT was also associated with a greater loss of BCS through transition and with greater concentrations of NEFA and BHB, which might be a response to a more intense severe negative energy balance and an accelerated rate of lipolysis. Soriani et al. (2012) also observed higher NEFA and BHB, and reduced cholesterol in cows

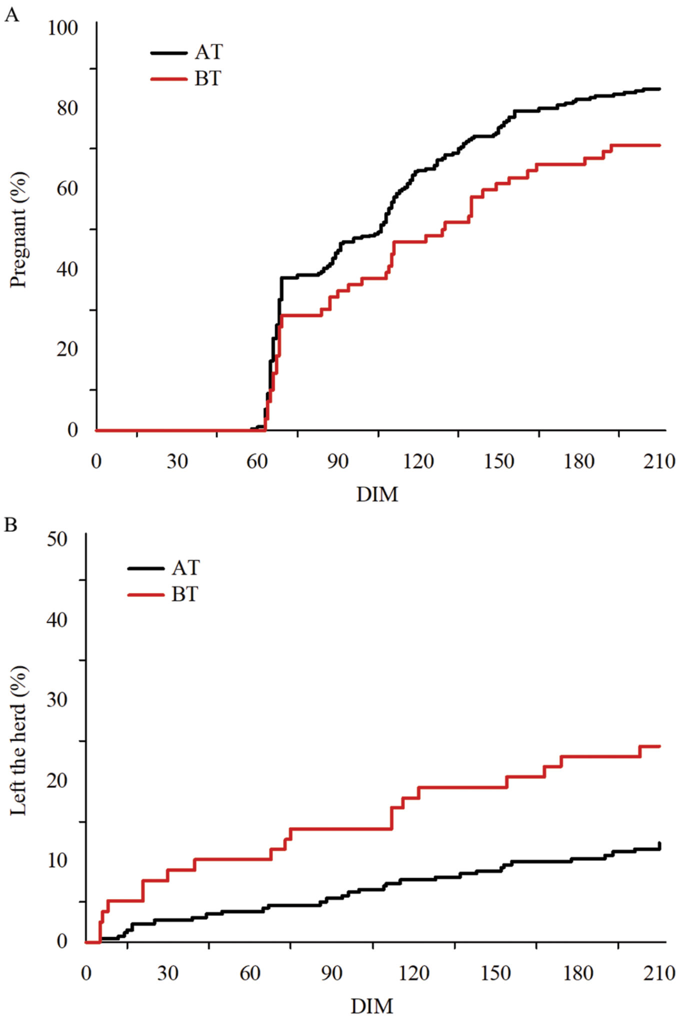

Figure 7. Survival analyses for time to pregnancy (A) and culling (B) in cows classified as AT or BT of RT deviation from herd average in the last week of pregnancy for prepartum health risk assessment. The optimal threshold (≤−53 min) of deviation in RT was generated through a ROC curve analysis to predict postpartum clinical disease. The rate of pregnancy (AHR = 0.64; 95% CI = 0.47–0.89; $P < 0.01$) and the rate of culling (AHR = 2.06; 95% CI = 1.16–3.64; $P = 0.01$) differ between risk groups.

that ruminated less during the prepartum period. Excessive lipolysis and its metabolic changes, in turn, have been linked to immune dysfunction (Contreras et al., 2018) and increased risk of disease (Ospina et al., 2013).

In addition to differences in energy metabolism, a low RT before calving was associated with increased serum concentrations of haptoglobin and reduced concentrations of serum cholesterol, urea, albumin, and magnesium during the early postpartum period. Using postpartum data, Calamari et al. (2014) also found negative associations between a low RT from 3 to 6 DIM and several biomarkers related to transition metabolic adaptations. In their study, cows with the lowest RT had greater concentrations of NEFA, BHB, haptoglobin, and total bilirubin in plasma than cows with the highest RT. Additionally, lower concentrations of albumin, cholesterol, paraoxonase activity, and liver functionality were observed in cows that spent less time ruminating after calving. Although the authors observed a greater proportion of clinical diseases in cows that ruminated less, they did not report the proportion of cows that already had health problems at the time of classification, and did not control for this possible confounding effect in their statistical models.

The greater concentrations of positive acute-phase proteins (i.e., haptoglobin and globulin) and the lower concentrations of a negative-phase protein (i.e., albumin) in postpartum samples of SRT and BT cows suggest that a reduced RT in the week before parturition is associated with an increased inflammatory state during the early postpartum period. In addition, we observed that prepartum RT was related to a lower postpartum concentration of Mg, which has also been associated with postpartum inflammation (Fébel et al., 2023) and higher incidence of dystocia, RP, endometritis, lameness, and impaired reproductive performance (Jeong et al., 2018). Nonetheless, this inflammatory condition could be associated with the development of inflammatory clinical diseases and not a direct consequence of decreased prepartum RT. Previous studies have demonstrated changes in concentrations of acute-phase proteins associated with clinical health problems (Smith et al., 2010; Menta et al., 2023). Although effective inflammatory responses are necessary to maintain health, exacerbated inflammation in periparturient cows may impair their immune responses and increase susceptibility to diseases (Abuelo et al., 2023). In fact, recent research has shown that elevated haptoglobin concentration is associated with a greater incidence of disease, reduced milk yield, and reproductive performance (Kerwin et al., 2022). The observed differences in postpartum inflammation between groups in the current study likely explain the differences in postpartum RT. Cows with reduced RT in the last week before calving required on average 9 DIM to achieve similar levels of rumination to their counterparts that had superior prepartum RT. The differences in early postpartum RT between healthy and diseased cows are well established (Schirmann et al., 2016; Stevenson et al., 2020). Stevenson et al. (2020) also reported that diseased cows take longer to reach peak RT and level off compared with healthy cows.

Despite the observed association between prepartum RT and blood metabolites described above, no associations between prepartum RT and incidence of subclinical health problems were observed. The subclinical conditions considered were hypocalcemia, hyperketonemia, and elevated NEFA, which were determined based on preestablished cut offs for serum concentration of Ca, BHB, and NEFA, respectively, that were previously associated with negative health and performance outcomes. Thus, prepartum RT was associated with continuous values of metabolite concentrations in serum (BHB and NEFA) but not with the proportion of cows that surpassed their critical thresholds. This lack of association could be particular to the herd or an indication that other physiological and metabolic factors such as inflammation could affect the level of fatty mobilization and ketogenesis (Chirivi et al., 2024). Liboreiro et al. (2015) reported no association between hyperketonemia or hypocalcemia with prepartum and postpartum RT in both nulliparous and parous cows. Recently, the average daily change in RT from d −3 to calving had no predictor potential for hypocalcemia and hypomagnesemia after calving (Emam et al., 2023). Nonetheless, previous research described an association between subclinical hyperketonemia diagnosed during early lactation with prepartum RT (Schirmann et al., 2016). In our study, cows received an oral bolus of monensin in the prepartum period, which might be partially responsible for the low incidence of hyperketonemia due to its benefits on prevention of ketosis (Duffield et al., 2008).

Our results also showed that parous cows with reduced prepartum RT might be associated with impaired lactation performance and greater likelihood of culling. In a recent study, Cook (2023) observed that parous cows that received a prepartum alert generated by an automated sensor system, which combines monitoring of eating, activity, and rumination behavior, had greater odds of disease treatment during the early postpartum period. Additionally, those same cows were negatively associated with the predicted 305 d milk production. Our findings are likely a response to the incidence of clinical disease during early lactation, which is in accordance with previous research by Carvalho et al. (2019). In their study, cows that had a single clinical disease or multiple clinical disease during the first 21 DIM also had, respectively, 1.79 and 3.06 greater odds of leaving the herd than healthy cows. The authors also observed that cows diagnosed with clinical disease produced less milk, fat, and protein by 305 DIM compared with cows that remained healthy throughout the transition period.

Parous cows with a decreased RT were associated with impaired reproductive performance in the subsequent lactation, characterized by a reduced pregnancy rate and a smaller proportion of pregnant cows by 210 DIM. Interestingly, a recent study found no association between prepartum RT and the incidence of postpartum diseases, but a greater RT in the week preceding calving was linked to a shorter time to the subsequent pregnancy (Cook et al., 2021). Nonetheless, the differences in reproductive performance in our study are probably a consequence of the higher incidence of postpartum clinical diseases in cows with reduced RT, which is supported by previous studies (Ribeiro et al., 2016; Carvalho et al., 2019). In a series of studies, Ribeiro et al. (2016) investigated the carryover effects of early lactation health on reproductive biology and found negative associations between the occurrence of inflammatory diseases and reproductive outcomes. Their results showed that regardless of the site of inflammation, clinical diseases diagnosed during the early postpartum period decreased both pregnancy and calving per breeding, and increased pregnancy losses. In addition, authors reported long-term consequences of postpartum disease on morula development and quality, and on preimplantation conceptus elongation and secretion of interferon-tau.

CONCLUSIONS

The level of RT in the week preceding parturition was associated with important changes in transition metabolism, including differences in energy metabolism and inflammation. In parous cows, decreased prepartum RT was associated with greater likelihood of postpartum clinical disease, as well as reduced milk production and pregnancy rate, and increased rate of culling. Altogether, these results suggest that prepartum rumination data could be useful in health risk assessment aiming to identify prepartum parous cows at high risk for postpartum clinical diseases and subpar performance in the subsequent lactation.

NOTES

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Nonstandard abbreviations used: AHR = adjusted hazard ratio; AOR = adjusted odds ratio; AT = above threshold; AU = arbitrary unit; AUC = area under the curve; BT = below threshold; ClinD15 = clinical disease diagnosis in postpartum cows through 15 DIM; FUO = fever of unknown origin; LLOQ = lower limit of quantification; LRT = long rumination time; M305 = total milk production at 305 DIM; MRT = moderate rumination time; NEFA = nonesterified fatty acids; Null = nulliparous; NUTD = nonuterine disease; Par = parous; ROC = receiver operating characteristics; RP = retained placenta; RT = rumination time; SRT = short rumination time; UTD = uterine disease.

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