



Relationships among early postpartum luteal activity, parity, and insemination outcomes based on in-line milk progesterone profiles in Canadian Holstein cows

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ABSTRACT

The objectives of this retrospective study were to use in-line milk progesterone (mP4) data to investigate relationships of (1) commencement of luteal activity (CLA), and (2) luteal phase (LP) length and frequency preceding first postpartum AI, with parity and AI outcomes in Canadian Holstein cows. Starting 21 ± 1 days postpartum (DPP), levels of mP4 were assessed every 2.2 ± 2.0 d through an automated in-line milk analysis system (Herd Navigator™, DeLaval International, Tumba, Sweden) until ~55 d after first or second AI in 748 Holstein cows from two herds. The CLA was defined as the DPP of the first of at least two consecutive samples with mP4 ≥ 5 ng/mL, and the period with elevated mP4 (≥ 5 ng/mL) was defined as the LP. Cows were categorized by CLA [earlier (\leq) or later ($>$) than 28, 35, 42, 49, 56, and 63 DPP], and by the pattern of LP frequency preceding first AI as having or not: (1) one or more normal LP (LP length ≥ 7 and ≤ 19 d); (2) one or more abnormal LP (LP length < 7 or > 19 d, or interluteal period ≥ 12 d); and (3) two or more LP (either normal or abnormal). Outcomes of first or second AI were determined by the interval between AI and cessation of the ensuing LP as: non-pregnant (mP4-decline ≤ 30 d), presumed-pregnant (no mP4-decline until 55 d), or presumed-pregnancy loss (mP4-decline between 31 and ≤ 55 d). The odds of pregnancy per AI (P/AI) at 55 d and pregnancy loss were evaluated using generalized linear mixed models. Primiparous cows had lower odds of having CLA ≤ 28 DPP [Odds ratio (OR) = 0.58, $P = 0.002$] and one or more abnormal LP (OR = 0.73, $P = 0.04$) than multiparous cows. In multiparous cows, CLA ≤ 28 DPP decreased pregnancy loss (OR = 0.48, $P = 0.05$) and CLA ≤ 56 DPP increased P/AI (OR = 4.69, $P < 0.01$) compared to a later CLA. Primiparous and multiparous cows that had one or more normal LP before first AI had increased P/AI (OR = 3.85 and 3.45, respectively, $P < 0.01$) and reduced pregnancy loss (OR = 0.26 and 0.27, respectively, $P < 0.01$) than cows without a normal LP. Primiparous cows that had one or more abnormal LP had decreased P/AI (OR = 0.62, $P = 0.04$) and increased pregnancy loss (OR = 1.64, $P = 0.04$) compared to those without an abnormal LP. In summary, AI outcomes were improved in multiparous cows that had early CLA and in cows of both parity groups that had at least one normal LP before first AI. However, primiparous cows that had at least one abnormal LP had reduced AI outcomes. Relationships between early postpartum luteal activity and AI outcomes were inconsistent between primiparous and multiparous cows.

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1. Introduction

The assessment of milk progesterone (mP4) concentrations has

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been widely used to classify luteal phases (i.e. normal vs. abnormal) [1–3], benchmark reproductive status [4], determine estrus [5] and pregnancy [6], and estimate endocrine traits [7]. However, the evaluation of abnormal ovarian activities, such as anestrus and prolonged cycles and their effects on fertility, often rely on manual collection of milk samples [4,8,9]. Manual sampling makes it less practical, often limiting the size of the sampled population and

frequency of sampling. An automated in-line milk analysis system (Herd Navigator™, DeLaval International, Tumba, Sweden) is available in Europe and Canada as a reproductive management tool, which analyzes mP4 at frequent, algorithm-driven intervals, starting approximately 3 wk after parturition. Towards the anticipated end of the luteal phase (LP), samples are taken at least once daily to determine estrus [5] or pregnancy if a previous artificial insemination (AI) was performed. Thus, in-line mP4 data allows the assessment of early postpartum reproductive status at a whole-herd level.

While transitioning from late gestation to early postpartum, high-producing dairy cows experience a number of profound metabolic changes that involve regulation of energy status, liver function, and mammary gland demand for glucose as required for lactation [10]. These metabolic challenges are often linked to abnormal ovarian processes associated with poor reproductive performance, such as prolonged anestrus, short estrus expression, and delayed ovulation [11]. A prolonged anestrus [12,13] and the presence of atypical estrous cycles early postpartum [1] are factors associated with reduced fertility [1,13]. Therefore, the evaluation of ovarian function based on mP4 profiles may enhance the understanding of the declining trend in fertility in the high-producing dairy cow [14].

Metabolic challenges during the early postpartum period related to high milk yield and feed intake, such as negative energy balance [15] and high metabolic clearance rate of steroid hormones [11], are known factors associated with altered ovarian function. For instance, circulating progesterone (P4) and estradiol concentrations are lower in lactating than in non-lactating cows [16,17], likely due to differences in milk yield. In addition, induced-luteal regression occurs at a lower rate in multiparous than in primiparous cows [18,19]. These factors may, at least in part, explain the greater fertility often reported in primiparous than in multiparous cows [20–23]. Nonetheless, the mechanisms by which early postpartum luteal activity may differentially influence the establishment and maintenance of pregnancy in primiparous and multiparous cows are still unclear. The assessment of mP4 profiles using the in-line milk analysis system offers a unique approach to continuously monitor P4 profiles in individual cows over several weeks, and to our knowledge, no such report exists from North American dairy herds.

The objectives of this retrospective study were to use in-line mP4 data to investigate relationships of (1) commencement of luteal activity (CLA), and (2) LP length and frequency preceding first AI, with parity and AI outcomes in Canadian Holstein cows. Considering the greater fertility reported in primiparous cows, and the lower fertility associated with anestrus, and abnormal cycles, we specifically tested the hypotheses that primiparous cows have higher odds of earlier CLA and lower odds of having abnormal LP before first AI than multiparous cows. We also hypothesized that an earlier CLA, the presence of one or more normal LP, the absence of abnormal LP, and the presence of two or more LP (either normal or abnormal) preceding first AI are associated with increased odds of pregnancy and reduced odds of pregnancy loss in both parity groups.

2. Materials and methods

2.1. In-line milk analysis system and records description

Records of mP4 concentrations analyzed through an in-line milk analysis system (Herd Navigator™) were accessed using a herd management software (AIPro™, DeLaval International, Tumba, Sweden). The Herd Navigator™ is an electronic management tool that, based on a bio-model [24], automatically takes milk samples,

quantifies P4 through a dry-stick enzyme immunoassay technique [25], and stores records of both raw (actual) and adjusted (smoothed) mP4 values, at algorithm-driven intervals after parturition. Smoothed mP4 values are based on a local linear growth model that controls for outlier values in time series analyses to reduce random noise [24] due to surrounding humidity/temperature and differences between batches of dry-sticks [26]. This adjustment allows algorithms to distinguish between presence or absence of luteal activity using an mP4 threshold of 5 ng/mL. Using this bio-model, Friggens et al. [5] reported a 93.3% sensitivity and a 93.7% specificity for detection of estrus.

In the data used, milk sampling started 21 ± 1 DPP (Mean \pm SD) at a frequency of 4.4 ± 2.0 d until CLA was determined (i.e. at least two consecutive mP4 samples ≥ 5 ng/mL); then, samples were collected every 2.2 ± 2.0 d. Once a decline (< 5 ng/mL) in mP4 was determined (hereafter referred to as mP4-decline) after CLA, four subsequent samples were taken approximately 7, 12, 16 and 20 d later, and thereafter, samples were taken at least once daily in order to detect the next mP4-decline. If a cow was eligible for AI (after the elective waiting period), the mP4-decline event was immediately flagged as a “heat alarm”, and AI recommended about 36 h later. Following AI, if the mP4 increased to ≥ 5 ng/mL and remained ≥ 5 ng/mL for approximately 30 d post-AI, a potential pregnancy was declared by the system and sampling continued every 3 d until either mP4-decline or until approximately 55 d when sampling stopped and pregnancy was declared.

2.2. Demographics and management of herds

In-line mP4 records from the years 2014–2016 were obtained from two commercial dairy herds (Herds A and B) located in Alberta, Canada, milking approximately 420 and 350 Holstein cows, respectively. Both herds had been using the Herd Navigator™ system as the main tool for making reproductive decisions and inseminating cows based on mP4 curves (see Section 2.1), for approximately 18 months prior to the evaluation of data. Based on herd records, first AI occurred at 68 ± 13 and 71 ± 20 DPP (Mean \pm SD), and average calving interval was 389 ± 54 and 398 ± 53 d in Herds A and B, respectively. After evaluating individual mP4 profiles (as described in Section 2.3), data from 350 primiparous (154 and 196 from Herds A and B, respectively) and 398 multiparous cows (204 and 194 from Herds A and B, respectively) that calved between June 2014 and December 2015 were used. Overall daily milk yield (kg/d) during first 60 DPP averaged 31.4 ± 4.3 and 29.7 ± 5.1 for primiparous cows and 45.0 ± 6.6 and 43.8 ± 6.3 for multiparous cows in Herds A and B, respectively. Cows in both herds were housed in free-stall barns, milked thrice daily through a parlor system, and fed a total mixed ration prepared in accordance with NRC (2001) guidelines [27]. Rations included alfalfa, barley and/or corn silage, alfalfa or grass hay, and concentrates (barley grain, commercial mix and minerals) as major ingredients. All AI were performed by experienced technicians using frozen-thawed commercial semen.

2.3. Enrolment criteria and classifications of luteal activity from parturition to first AI

To be enrolled in the study, all 748 cows had to meet the following criteria: (1) first mP4 record collected before 28 DPP; (2) CLA occurred before 150 DPP; (3) neither subjected to induced-luteolysis nor induced-ovulation treatments from first mP4 record until evaluated AI; (4) AI occurred later than 40 DPP and after the CLA; (5) AI occurred within 4 d following mP4-decline identified through the in-line milk analysis system; (6) increase in mP4 (≥ 5 ng/mL) occurred within 14 d post-AI; and (7) interval between

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