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Dynamics of pre- and post-insemination progesterone profiles and insemination outcomes determined by an in-line milk analysis system in primiparous and multiparous Canadian Holstein cows

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ABSTRACT

The objective was to evaluate in-line milk progesterone (mP4) data to determine dynamics of pre- and post-insemination mP4 profiles and their associations with parity and outcomes of artificial insemination (AI) in Holstein cows. Milk progesterone (ng/mL) was quantified at pre-determined time points before and after AI through an automated in-line milk analysis system (Herd Navigator™, DeLaval International, Tumba, Sweden). Only AI (~d0; n = 605) preceded by an mP4-decline (at least two samples of mP4 ≥5 ng/mL followed by at least one sample <5 ng/mL; d−2) were evaluated. Maximum mP4 attained between d−15 and d−2 (PrePeak), d−2, d5, d10, d14, maximum mP4 attained within 21d post-AI (PostPeak), and rate-of-daily-change between mP4 time points (ng/mL/d) were analyzed. Primiparous and multiparous cows were classified by AI outcomes based on post-AI mP4 profiles into three groups: (1) non-pregnant (OPEN; mP4-decline ≤ 30d post-AI), (2) presumed-pregnant (PREG; no mP4-decline until 55d post-AI), and (3) presumed-pregnancy loss (P-LOSS; mP4-decline between 31 and 55d post-AI). For profile comparisons, smoothed mP4 data were analyzed using mixed linear models. Primiparous cows had greater ($P < 0.01$) mP4 than multiparous cows at d5 (4.6 ± 0.2 vs. 2.8 ± 0.1), 10 (11.1 ± 0.4 vs. 7.6 ± 0.2), 14 (19.7 ± 0.4 vs. 16.1 ± 0.3) and PostPeak (23.5 ± 0.3 vs. 21.7 ± 0.2). The rate-of-daily-change was greater ($P < 0.01$) in primiparous than in multiparous cows from d−2 to 5 (+0.2 ± 0.03 vs. −0.1 ± 0.02) and from d5 to 10 (+1.2 ± 0.05 vs. +0.9 ± 0.03), but lesser ($P < 0.01$) from d14 to PostPeak (+0.9 ± 0.09 vs. +1.3 ± 0.06). In primiparous cows, mP4 in PREG was greater at d10 and PostPeak than OPEN (11.1 ± 0.5 and 24.2 ± 0.5 vs. 9.6 ± 0.4 and 22.3 ± 0.4, respectively, $P < 0.04$), but lesser at d5 than P-LOSS (4.4 ± 0.3 vs. 5.7 ± 0.4, $P = 0.04$). In multiparous cows, mP4 at d−2 was lesser in PREG than OPEN and P-LOSS (3.2 ± 0.1 vs. 3.4 ± 0.04 and 3.5 ± 0.1, respectively, $P \leq 0.03$), but greater at d10, d14 and PostPeak in PREG than in OPEN (8.2 ± 0.4, 16.8 ± 0.5 and 22.7 ± 0.4 vs. 6.9 ± 0.3, 14.8 ± 0.3 and 19.7 ± 0.2, respectively, $P < 0.01$). Multiparous PREG cows had greater rate-of-daily-change in mP4 than OPEN cows from d5 to 10 and from d10 to 14 (+1.0 ± 0.06 and +2.2 ± 0.11 vs. +0.8 ± 0.04 and +1.9 ± 0.08, respectively, $P < 0.03$). Overall post-AI mP4 increased faster and were greater in primiparous than in multiparous cows. Based on in-line mP4 profiles, greater mP4 levels near time of AI (d−2 in multiparous and d5 in primiparous cows) and lesser mP4 beyond d10 were negatively associated with a successful pregnancy.

1. Introduction

Milk progesterone (mP4) data have been widely used to characterize ovarian activity [1–6], pregnancy status [1,6,7] and to evaluate associations between progesterone (P4) levels around time of artificial insemination (AI) and pregnancy [7,8]. However, characterizing complete P4 profiles from early postpartum period until pregnancy establishment in lactating dairy cows through manual milk sampling is labor-intensive; hence rarely done. The in-line milk analysis system (IMAS; Herd Navigator™, DeLaval International, Tumba, Sweden) is a relatively new herd management

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tool that allows evaluation of postpartum mP4 profiles both in individual cows and in whole herds from about 3 wk postpartum until pregnancy establishment [6]. The IMAS uses a bio-model [9] that drives automatic milk sampling in every cow and measures mP4 at frequent intervals to estimate luteal function, estrus, time to AI and AI outcomes (non-pregnancy, pregnancy, pregnancy loss) [10]. In addition to assisting with reproductive management decisions, the assessment of frequent mP4 data generated by the IMAS gives a new opportunity to evaluate parameters of luteal activity [6,11,12], such as mP4 levels at specific time points, and their associations with fertility. Given the considerable variability in luteal phase length in the modern dairy cow [5,6], evaluating characteristics of luteal activity through mP4 profiles in cows that conceived and maintained a pregnancy would enhance the understanding of the dairy cow fertility.

The IMAS (Herd Navigator™) has been commercially available since 2008 in Europe and since 2011 in Canada. In addition to monitoring reproductive events, it is designed for early detection of ketosis, mastitis, and to monitor milk urea profiles. Although data from European herds using the IMAS have been used to establish benchmarks of luteal activity [11] and endocrine fertility traits [12,13], no report exists on evaluating mP4 profiles in relation to fertility. The IMAS bio-model offers a novel approach to monitor cyclic status (i.e. commencement of postpartum luteal activity) [6,11], abnormal luteal cycles [6], and detection of estrus [14].

After ovulation, an optimal P4 environment is required for establishment of pregnancy [15], and increasing concentrations of P4 following AI support embryo development through uterine secretions of proteins and growth factors [16]. To understand the influence of P4 on fertility, recent studies have investigated the effects of P4 supplementation on pregnancy outcomes during or following synchronization protocols [17–19] with inconsistent results in post-AI evaluations. These inconsistencies could be at least partially explained by confounding effects on luteal activity parameters which are still poorly characterized, such as parity, as the levels of feed intake and milk production (expected to be lower in primiparous than in multiparous cows) affect P4 concentrations [20]. While it is frequently reported that pregnancy per AI is greater in primiparous than in multiparous cows [21–24], the underlying factors contributing to the increased fertility in primiparous cows are not fully understood. Recent reports using IMAS in commercial dairy herds [6,11] or continuous manual milk sampling for mP4 determination in a research herd [5] indicate that differences in ovarian function exist between primiparous and multiparous cows, such as in characteristics of luteal cycles early postpartum [5,6,11]. Frequent automated mP4 sampling can help identify characteristics of P4 profiles associated with successful pregnancies, pregnancy losses, and potential differences in P4 dynamics among parity groups, which have not been studied previously.

Therefore, a retrospective study was conducted using data generated through an automated IMAS to determine the dynamics of pre- and post-AI mP4 profiles and their associations with parity (primiparous, multiparous) and AI outcomes (non-pregnant, pregnant, pregnancy loss) in Canadian Holstein cows. As milk yield affects P4 metabolism [20] and elevated P4 post-AI is essential for pregnancy [15], we tested the hypothesis that mP4 levels before and after AI and the rate-of-daily-change in mP4 post-AI are greater (more rapid increase) in primiparous than in multiparous cows and in cows that become pregnant than in cows with other AI outcomes.

2. Materials and methods

2.1. Herds and management

Data relating to 605 AI of 115 primiparous (1st lactation) and 249 multiparous (2nd^{+} lactation) Holstein cows that calved between June 2014 and December 2015, had not been subjected to reproductive hormone interventions during the luteal phases evaluated (see Section 2.3), and had been inseminated beyond 40 d postpartum, were obtained from two dairies located in Alberta, Canada using the IMAS (Herd Navigator™). Records were accessed through a dairy-management software program (AlPro™, DeLaval International). Both herds used the IMAS as their main reproductive management tool and inseminated cows based on mP4 curves (as described in Section 2.2). Artificial inseminations were performed by experienced and trained technicians using frozen-thawed commercial semen. Based on herd records, Herds A and B milked approximately 420 and 350 Holstein cows, and calving intervals averaged 393 (range 324–545) and 372 (range 322–482) d, respectively.

Overall daily milk yield during first 60 d postpartum for Herds A and B averaged 31.4 ± 4.3 and 29.7 ± 5.1 kg for primiparous cows and 45.0 ± 6.6 and 43.8 ± 6.3 kg for multiparous cows, respectively. Cows from both herds were milked thrice daily through a parlor system, housed in free-stall barns, and fed a total mixed ration in accordance with NRC (2001) guidelines [25], using ingredients typical for high-producing herds in Alberta. Major ingredients were silage (alfalfa, barley and/or corn), hay (alfalfa or grass) and concentrates (barley grain, commercial mix, and minerals).

2.2. In-line milk progesterone analysis system

The Herd Navigator™ is programmed to collect milk samples automatically and analyze mP4 in individual cows through a dry-stick biosensor technology and enzyme immunoassay [26], based on a bio-model that establishes frequency and quantification of mP4 samples [9]. To reduce the random variation associated with the testing environment (temperature/humidity) and differences in batches of sticks and reagents, raw (actual) mP4 concentrations are adjusted to smoothed values (i.e. levels) based on a standardized method to control for outliers expected in the serial sampling system, as described by Friggens and Chagunda [9]. The bio-model was validated to detect estrus based on the pattern of sample frequency to detect the mP4-decline at the end of a luteal phase (period of high to low mP4; indicating estrus will occur), with 93.3% of sensitivity and 93.7% of specificity [14].

In brief, milk samples started at 21 d postpartum and were taken, on average, every 2 d until a pregnancy was declared based on mP4 profiles post-AI. Luteal phase was considered to have initiated at the first of two consecutive samples with mP4 ≥5 ng/mL following a sample <5 ng/mL, and the end of a luteal phase was determined when two consecutive mP4 readings of ≥5 ng/mL were followed by at least one reading <5 ng/mL (referred hereinafter as mP4-decline). If the cow was eligible to be inseminated, the mP4-decline was immediately notified in the dairy-management software as a “heat alarm” and AI recommended ~36 h later. After mP4-decline, programmed sampling occurred approximately at 7 ± 1, 12 ± 1, 16 ± 1 and 20 ± 1 days later, followed by samples at least once daily aiming to detect the next mP4-decline. If the luteal phase continued with no mP4-decline until ~30 d post-AI, a potential pregnancy was declared by the system. Then, samples were taken, on average, every 3 d until ~55 d post-AI, when the cow was declared pregnant if the luteal phase remained uninterrupted (no mP4-decline).

2.3. Data description

Six-hundred-five mP4-decline events that were followed by an AI (364 first and 241 s postpartum AI) within 48 h, and a subsequent initiation of luteal phase within 16 d, were assessed. Based on