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Technical note: Validation of an automated in-line milk progesterone analysis system to diagnose pregnancy in dairy cattle

T. C. Bruinje¹ and D. J. Ambrose^{1,2*}

¹Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, T6G 2P5

²Livestock Research and Extension Branch, Alberta Agriculture and Forestry, Edmonton, AB, Canada, T6H 5T6

ABSTRACT

The in-line milk analysis system (IMAS) is an automated biosensor technology that samples and quantifies milk progesterone concentrations (P4c) at frequent intervals starting early postpartum until pregnancy. The objective was to validate the use of pregnancy notifications (PregN) generated by an IMAS based on P4c profiles after artificial insemination (AI) to determine pregnancy and nonpregnancy status in dairy cows. Records of 1,821 AI events from 715 Holstein cows that had milk P4c (ng/mL) measured every 2.2 ± 1.9 d (mean $\pm$ standard deviation) between 24.5 ± 8.2 and 173.4 ± 49.3 d in milk through a real-time IMAS (Herd Navigator, DeLaval International, Tumba, Sweden) were evaluated. Based on variations in adjusted milk P4c ($<$ vs. $\geq$ the 5.0 ng/mL threshold), the system determined the sampling frequency, onset and cessation of luteal phases, and pregnancy. If a luteal phase initiated (P4c increased to ≥ 5.0 ng/mL) after AI and remained uninterrupted, a PregN was generated starting at (mean $\pm$ standard deviation) 31.0 ± 4.3 d until 53.4 ± 7.9 d after AI, when sampling stopped, unless a decline in P4c (to < 5.0 ng/mL) occurred indicating nonpregnancy and imminent estrus. The assessment of IMAS PregN at 4 weekly intervals was tested, and a confirmed calving occurrence between 262 and 296 d after AI, with no other subsequent AI recorded, was the gold standard for pregnancy. In total, 14.1 (256/1,821), 41.0 (746/1,821), and 50.7% (924/1,821) of AI events were followed by a decline in P4c before 19, 23, and 30 d after AI, respectively. Frequency of the last 3 sampling events preceding P4c decline was greater if P4c decline occurred between 18 and 25 d after AI (1.4 ± 0.5 samples per day) compared with before 17 or beyond 26 d after AI (1.0 ± 0.5 samples per day). At 30 ± 3 (27 to 33) d after AI, PregN occurred in 46.8% (853/1,821) of AI events, of which 15.2% (130/853) had a decline in

P4c between 30 and 55 d after AI and 17.1% (146/853) was later confirmed nonpregnant based on the gold standard. A total of 40.7% (742/1,821) of AI events was confirmed pregnant by the gold standard, which was no different than the proportion of PregN at 51 ± 3 (48 to 54) d (40.9%; 744/1,821). At any time point between 27 and 54 d after AI, sensitivity and negative predictive values for PregN were greater than 95.0 and 96.0%, respectively, whereas specificity values were less than 90.0% for PregN before 40 d but greater than 94.0% for PregN beyond 41 d after AI. In conclusion, IMAS is able to diagnose pregnancy based on P4c profiles with high precision and determine early nonpregnancy based on the spontaneous cessation of the luteal phase. However, for accuracy greater than 95.0%, pregnancy declaration based on IMAS notifications alone should occur no earlier than 41 d after AI.

Key words: biosensor, dairy cow, pregnancy test, reproductive management

Technical Note

The accurate diagnosis of pregnancy or nonpregnancy at the earliest opportunity after AI is critical for the reproductive efficiency of dairy operations. A direct pregnancy diagnosis method such as transrectal ultrasonography can be accurately performed as early as 29 d after AI (Romano et al., 2006), whereas indirect methods such as pregnancy-associated glycoprotein measured in milk or blood are accurate when performed 28 d after AI (Mayo et al., 2016). Diagnosis of nonpregnancy is possible even earlier by Doppler ultrasonography (20 d; Siqueira et al., 2013) and progesterone measurement (25 d; Wilsdorf et al., 2016). However, such methods have poor true positive rates for confirming pregnancy (Siqueira et al., 2013: 53.7%; Wilsdorf et al., 2016: 76.0%) because of the high incidence of early pregnancy losses (Santos et al., 2004; Ricci et al., 2017), resulting in a high proportion of false positives necessitating a subsequent re-examination to confirm pregnancy. The aforementioned methods require considerable animal handling, labor, time, and technical

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*Corresponding author: divakar.ambrose@gov.ab.ca

inputs. Besides, laboratory tests are often not on farm and pregnancy or nonpregnancy determination may require up to several days. Therefore, for a pregnancy diagnosis to be effective, testing should start early after AI and occur frequently to identify nonpregnant cows in a timely manner until the risk of pregnancy losses wanes (Giordano et al., 2013; Fricke et al., 2016).

In this regard, a fully automated in-line milk analysis system (**IMAS**; Herd Navigator, DeLaval International, Tumba, Sweden) that samples and immediately quantifies milk progesterone concentrations (**P4c**) at frequent intervals in every eligible cow in the herd, has been in use in some Canadian herds since 2012. For the purpose of monitoring spontaneous ovarian activity, the IMAS biomodel starts sampling milk at approximately 3 wk postpartum and adjusts the sampling frequency according to the stage of the estrous cycle (Friggens and Chagunda, 2005). The main features of the IMAS for reproductive management include monitoring of resumption of postpartum luteal activity and subsequent luteal phases, declaring imminent estrus based on cessation of a luteal phase, and estimating early nonpregnancy or pregnancy status, all based on P4c profiles (DeLaval International, 2011; Saint-Dizier and Chastant-Maillard, 2012; Bruinje et al., 2019). If determined accurate, the IMAS can be used as an automated diagnostic tool for pregnancy that includes identification of nonpregnant cows in a timely manner with no animal handling. Therefore, the objective of the present study was to validate the use of pregnancy notification (**PregN**) generated by the IMAS to determine pregnancy status in dairy cows.

Records of AI ($n = 1,821$) from 811 lactations (365 primiparous and 446 multiparous) of 715 Holstein cows were obtained from 4 dairy herds located in Alberta, Canada, from 2014 to 2016. Cows had a mean ($\pm$ SD) milk yield of 38.0 ± 9.0 kg/d between 10 and 60 DIM. All herds used the IMAS as the primary reproductive management tool, in which AI were performed at spontaneous estrus determined based on milk P4c profiles, as further described. Milk P4c measurement started at (mean $\pm$ SD) 24.5 ± 8.2 DIM and repeated at algorithm-driven intervals every 2.2 ± 1.9 d until 173.4 ± 49.3 DIM, based on a biomodel described in detail by Friggens and Chagunda (2005). The IMAS biomodel used adjusted P4c values and a cut-off of 5.0 ng/mL to identify onset and cessation of luteal phases based on proprietary calculations to identify variations in P4c for each cow. A luteal phase was defined as the number of days of uninterrupted adjusted P4c greater than or equal to 5.0 ng/mL until P4c declined to less than 5.0 ng/mL (referred to as P4c decline).

After the first luteal phase postpartum, the P4c decline was considered as the reference point for each P4c

profile to determine subsequent sampling frequency. The average sampling frequency at each stage of the P4c profiles and demographics of herds evaluated here were reported in detail by Bruinje et al. (2019). In brief, once a P4c decline was determined, estrus notification occurred in the IMAS software and AI was recommended to occur by 24 to 36 h (DeLaval International, 2011). After P4c decline, the sampling frequency was set by the biomodel to trigger the subsequent samples. Then, the first, second, third, and fourth subsequent samples occurred at (mean $\pm$ SD) 6.1 ± 1.1 , 9.0 ± 2.4 , 13.6 ± 2.4 , and 16.8 ± 2.1 d after P4c decline, respectively (Bruinje et al., 2019). The following samples were expected to occur daily between 18 and 25 d, or until the next P4c decline. If P4c remained above the default cut-off (5.0 ng/mL) at d 25, samples were automatically taken every second day until P4c dropped below 10.0 ng/mL, then samples were taken once or twice daily until the next P4c decline. Once the subsequent P4c decline occurred (e.g., if the cow was nonpregnant), the sampling frequency was recalculated for the next cycle.

When an AI event was recorded by 5 d after P4c decline, sampling frequency was standard until 25 d, then repeated every 2 to 3 d between 25 and 30 d. If P4c was still above the default cut-off at 30 d, a PregN was issued by the system and the sampling frequency reduced to every 5 d until 55 d or until a P4c decline, whichever occurred earlier (DeLaval International, 2011). In case of a true pregnancy where P4c was expected to be continuously above the default cut-off, PregN were generated at every sampling event from 30 to 55 d after AI, when sampling automatically stopped (Figure 1). The IMAS software notified the end user whenever a change in status occurred (i.e., first PregN after AI or P4c decline).

In the present data set, sets of filtering criteria were applied to the data as previously described (Bruinje et al., 2019). The filtering criteria excluded lactations that had unexpected periods of no P4c measurements (i.e., gaps in sampling) and excluded AI events that did not occur based on the IMAS P4c profiles because of management decisions. Therefore, we used only AI records that had consistent IMAS data available before and after AI, and had subsequent confirmation of calving data available.

To validate the use of PregN generated by the IMAS to declare pregnancy and to determine the minimum interval after AI for which PregN will be most accurate, PregN at 4 weekly intervals were tested: at 30 ± 3 (27 to 33) d, at 37 ± 3 (34 to 40) d, at 44 ± 3 (41 to 47) d, and at 51 ± 3 (48 to 54) d after AI. Nonpregnancy was determined if no PregN was issued during each interval evaluated and a P4c decline occurred by 55 d after AI. Based on the expected gestation duration (mean $\pm$ 3

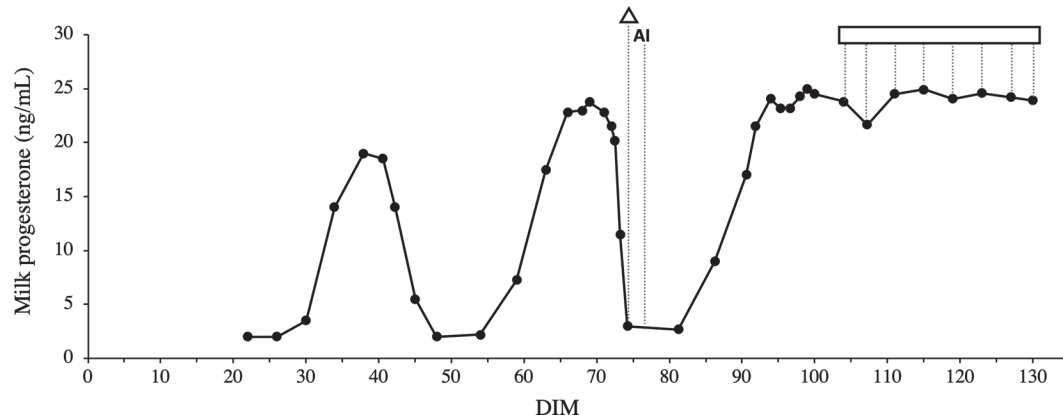


Figure 1. Postpartum milk progesterone profile obtained by an in-line milk analysis system (Herd Navigator, DeLaval International, Tumba, Sweden) of a hypothetical cow that, based on the standard cut-off of 5.0 ng/mL for luteal activity, the first luteal phase occurred between 34 and 48 DIM, the second luteal phase occurred between 59 and 74 DIM, a notification of estrus was generated at 74 DIM (open triangle), an AI was performed at 76 DIM, a new luteal phase initiated at 85 DIM, and pregnancy notifications (open rectangle) occurred between approximately 27 and 54 d after AI.

SD) for Holstein cows of 279 ± 17 d (Norman et al., 2009), a confirmed calving that occurred between 262 and 296 d after AI, with no subsequent AI recorded, was the gold standard for confirming pregnancy status. To assess the precision of the IMAS in determining the day of P4c decline after AI, as an indication of imminent estrus in nonpregnant cows, the average sampling frequency during the last 3 samples before P4c decline was evaluated.

Statistical procedures were performed with SAS 9.4 (Studio 3.71 platform, SAS Institute Inc., Cary, NC). The MEANS procedure was used to obtain descriptive statistics of herd demographics. Comparisons of sampling frequency among categories of interval from AI to subsequent P4c decline were performed using the GLIMMIX procedure, including parity as a covariate and the random effect of herd. The occurrence of PregN at each of the 4 weekly intervals were binary variables (0 = nonpregnant or 1 = pregnant), as well as the confirmation of pregnancy based on the gold standard. The LOGISTIC procedure was used to obtain receiver operator characteristic (ROC) curves and the area under the curve, where PregN at each of the 4 weekly intervals was modeled against the gold standard. The FREQ procedure was used to obtain sensitivity [(true pregnant by IMAS)/(gold standard pregnant) $\times$ 100], specificity [(true nonpregnant by IMAS)/(gold standard nonpregnant) $\times$ 100], positive predictive value [(true pregnant by IMAS)/(IMAS pregnant) $\times$ 100], negative predictive value [(true nonpregnant by IMAS)/(IMAS nonpregnant) $\times$ 100], and accuracy [(true pregnant + true nonpregnant by IMAS)/(all AI evaluated) $\times$ 100] for IMAS PregN assessed at each interval. Furthermore, Cohen's kappa statistics (Viera and Garrett, 2005) and

McNemar's tests were used to measure the agreement between PregN assessed at each weekly interval and the gold standard.

On average (mean $\pm$ SD), AI occurred at 108.2 ± 45.6 DIM. The number of postpartum AI at the time of pregnancy assessment averaged 2.1 ± 1.4 . All AI occurred by 5 d after a P4c decline notified as an estrus event by the IMAS, and the average sampling frequency of the last 3 samples before P4c decline that preceded AI was 1.3 ± 0.6 samples per day. Milk P4c values at the time of P4c decline (last sample preceding AI) averaged 0.6 ± 0.5 ng/mL, and the interval between P4c decline and AI was 1.8 ± 0.6 d. The first, second, and third samples after AI occurred at 4.6 ± 0.6 , 10.0 ± 0.6 , and 14.1 ± 0.6 d after AI, respectively, with corresponding P4c values of 3.1 ± 4.3 , 16.2 ± 7.0 , and 20.8 ± 5.8 ng/mL.

A majority (53.2%; 968/1,821) of AI events were not followed by an IMAS PregN, and 95.5% (924/968) of these events was followed by a P4c decline and a new estrus alert generated by the IMAS by 30 d after AI. The mean ($\pm$ SD) interval between AI and P4c decline, indicating imminent estrus and opportunity for re-insemination, was 21.7 ± 3.1 d in the cycles ($n = 924$) in which a P4c decline occurred by 30 d after AI. Of these, 57.7% (533/924), 22.8% (211/924), and 19.5% (180/924) had a P4c decline by 21 d, between 22 and 23 d, and beyond 24 d after AI, respectively (Figure 2a). Similarly, previous studies that evaluated P4c profiles or luteal dynamics after AI in cows confirmed as nonpregnant at 32 d reported that 43.8% of cows had an extended luteal phase beyond 22 d after AI (Ricci et al., 2017), and 25% of cows retained their luteal structures up to 28 d after AI (Stevenson and Pulley, 2012).

The distribution of the interval between AI and P4c decline in cows that did not receive an IMAS PregN is presented in Figure 2a, which was similar to the distribution of over 114,000 inter-service intervals reported by Remnant et al. (2015). We observed only 1.9% (18/924) of nonpregnant cows with a P4c decline before 15 d after AI, which was similar to what was previously reported (Remnant et al., 2015; Ricci et al., 2017). The monitoring of the day of P4c decline after AI is a unique feature of the IMAS as it provides an opportunity for reinsemination as early as the spontaneous cessation of the luteal phase in nonpregnant cows, shortening the inter-service interval. In the present data set, the mean ($\pm$ SD) inter-service interval (i.e., interval between consecutive AI) in AI that were not followed by a PregN was 25.8 ± 9.0 d, and 25, 50, 75, and 90%

of the inter-service intervals were less than or equal to 22.0, 23.0, 26.0, and 32.0 d, respectively.

The IMAS biomodel reduces sampling frequency if P4c remains high beyond 25 d after P4c decline. Therefore, cows with extended luteal phases (e.g., P4c decline beyond 25 d after AI) could have the day of subsequent P4c decline detected with reduced precision. The average sampling frequency of the last 3 samples before P4c decline following AI of 1.3 ± 0.6 samples per day indicates a relatively high precision of the IMAS in detecting P4c decline, as an indication of imminent estrus for reinsemination. In contrast, among AI events that were followed by a P4c decline by 30 d, the sampling frequency (mean $\pm$ SE) before P4c decline was reduced ($P < 0.001$) when P4c decline occurred either before 17 d or beyond 26 d after AI (1.0 ± 0.1 samples per

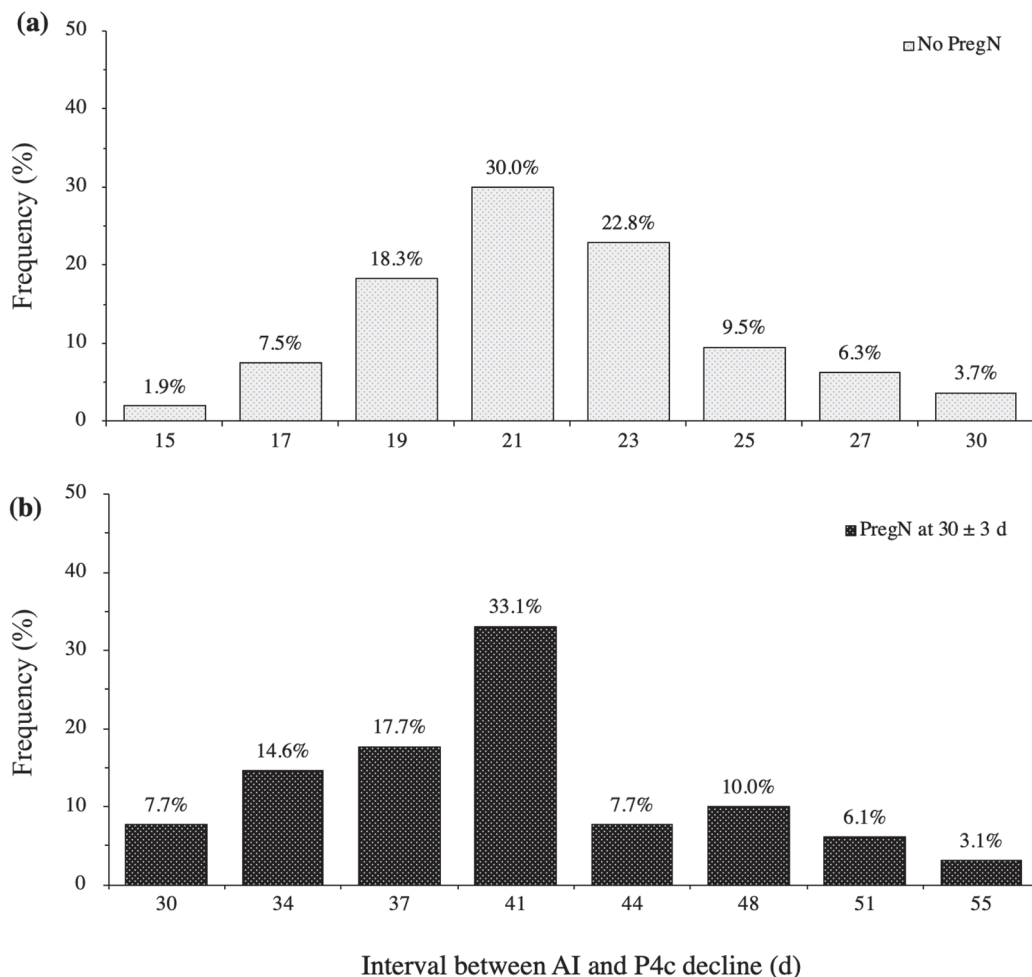


Figure 2. Distribution of the interval between AI and decline in progesterone concentration (P4c decline) of subsequent luteal phase in (a) 924 AI events that were not followed by a pregnancy notification (PregN) and had a P4c decline before 30 d after AI, and (b) 130 AI events of which the in-line milk analysis system (Herd Navigator, DeLaval International, Tumba, Sweden) PregN occurred at 30 ± 3 d after AI and a subsequent P4c decline occurred by 55 d after AI, and no calving occurred between 262 and 296 d after AI. Among the latter, 40.0% (52/130), 80.8% (105/130), and 96.9% (126/130) of the luteal phases after AI had a P4c decline by 37, 44, and 51 d after AI, respectively.

day for both categories) compared with when P4c decline occurred between 18 and 25 d after AI (1.4 ± 0.1 samples per day). As previously suggested (Bruinje et al., 2019), continuing the greater frequency of sampling to beyond 25 d after AI should increase precision of estrus detection and reinsemination in cycles with an extended luteal phase.

Among AI events that were followed by an IMAS PregN, the interval (mean $\pm$ SD) between AI and first day of PregN was 30.6 ± 2.8 d. The overall proportions of AI events followed by a PregN at 30 ± 3 , 37 ± 3 , 44 ± 3 , and 51 ± 3 d were 46.8% (853/1,821), 45.3% (825/1,821), 42.7% (778/1,821), and 40.9% (744/1,821), respectively. Notably, 14 of the AI events that had a PregN at 51 ± 3 d did not have a PregN at 30 ± 3 d after AI, possibly due to delayed entry of the AI event, resulting in 730 AI events with PregN both at 30 ± 3 and 51 ± 3 d after AI.

Among those 123 AI events that had a PregN at 30 ± 3 but not at 51 ± 3 d after AI, 121 had P4c decline by 55 d, 1 had P4c decline at 57 d, and 1 did not have a P4c decline, with a calving recorded. In addition, 9 AI events had PregN both at 30 ± 3 and 51 ± 3 d, but also had a P4c decline recorded by 55 d after AI. Therefore, 15.4% (130/853) AI events had a PregN at 30 ± 3 but were subsequently followed by a P4c decline between 30 and 55 d (regardless of PregN at 51 ± 3 d) after AI, which occurred on average (mean $\pm$ SD) at 39.7 ± 6.0 d after AI. Of those, 40.0% (52/130), 80.8% (105/130), and 96.9% (126/130) of P4c decline events occurred by 37, 44, and 51 d after AI, respectively. The distribution of the interval between AI and subsequent P4c decline in those 15.4% AI events that were followed by a PregN at 30 ± 3 d but had a subsequent P4c decline before 55 d after AI is presented in Figure 2b. A total of 40.7% (742/1,821) of AI events was followed by a confirmed calving (i.e., gold standard). Of those, 3.1% (23/742) was not previously followed by an IMAS PregN. The interval (mean $\pm$ SD) between AI and the last P4c record measured in cows confirmed pregnant by the gold standard was 55.3 ± 1.7 d.

The 15.4% of AI events that had an IMAS PregN at 30 ± 3 but were later followed by a P4c decline by 55 d may have resulted from either embryonic losses during that period (Santos et al., 2004), prolonged luteal phases (Ricci et al., 2017), or both. For instance, approximately 12.8% of pregnancy losses occur from 27–31 to 38–50 d after AI in lactating dairy cows (Santos et al., 2004). Though based exclusively on the IMAS PregN and P4c decline events, our findings indicate a similar prevalence (15.4%; 130/853) of pregnancy losses between approximately 30 and 55 d after AI. Furthermore, 3.4% (25/744) of AI events that had a PregN at 51 ± 3 d were not followed by a confirmed

calving, indicating a relatively small proportion of late pregnancy losses.

The high prevalence of extended luteal phases might be a limitation of diagnosing pregnancy too early based on P4c profiles alone. If the retention of luteal structures extends beyond when an early pregnancy loss (e.g., at 30 d after AI) occurs, it could result in elevated P4c resulting in false PregN at 30 ± 3 d after AI. In this regard, Stevenson and Pulley (2012) reported that 19.4% of nonpregnant cows that had retained luteal structures until 28 d after AI had elevated pregnancy-specific protein B at d 32. Furthermore, Ricci et al. (2017) reported that 41 and 58% of nonpregnant cows with extended luteal phases beyond 32 d after AI had pregnancy-associated glycoprotein levels indicative of pregnancy at 25 and at 32 d, respectively. These findings indicate that P4c can stay elevated for at least up to 7 d after an early pregnancy loss occurs.

The area under the curve (and 95% CI) for diagnosing pregnancy based on IMAS PregN at 30 ± 3 d, 37 ± 3 , 44 ± 3 , and at 51 ± 3 d after AI were 0.91 (CI 0.90 to 0.92), 0.93 (CI 0.91 to 0.94), 0.95 (CI 0.95 to 0.96), and 0.97 (CI 0.97 to 0.98), respectively (Figure 3; $P < 0.001$). For all intervals tested, sensitivity values were greater than 95.0%, indicating a very high ability for the IMAS PregN at any time point to correctly identify pregnant cows. Specificity values were below 90.0% for PregN at 30 ± 3 and at 37 ± 3 d after AI, 94.4% for PregN at 44 ± 3 , and 97.7% for PregN at 51 ± 3 d after AI, indicating a very high precision ($\sim 95.0\%$) of the IMAS in correctly identifying nonpregnant cows beyond 41 d after AI. The accuracy of the IMAS PregN in correctly diagnosing pregnancy or nonpregnancy was high ($>90.0\%$) between 27 and 40 d after AI, and very high ($>95.0\%$) when assessed beyond 41 d after AI. The level of agreement between PregN and the gold standard was high ($k \geq 0.80$) for PregN assessed at all intervals. The positive and negative predictive values, sensitivity, specificity, accuracy, and Cohen's kappa statistics values for diagnosing pregnancy based on IMAS PregN at each weekly interval are presented in Table 1. McNemar's test revealed that proportions of the outcomes (i.e., sensitivity and specificity values) in the 2×2 contingency tables among all tests were different ($P < 0.001$). However, the proportion of pregnancy and nonpregnancy as determined by PregN of IMAS at 51 ± 3 versus the gold standard did not differ ($P = 0.77$), indicating accordance between the proportion of pregnancies determined by the IMAS beyond 48 d and the gold standard.

According to a simulation model, for an early chemical pregnancy test (at 31 d after AI) to be economically comparable with pregnancy diagnosis by palpation per rectum at 39 d after AI, it had to have sensitiv-

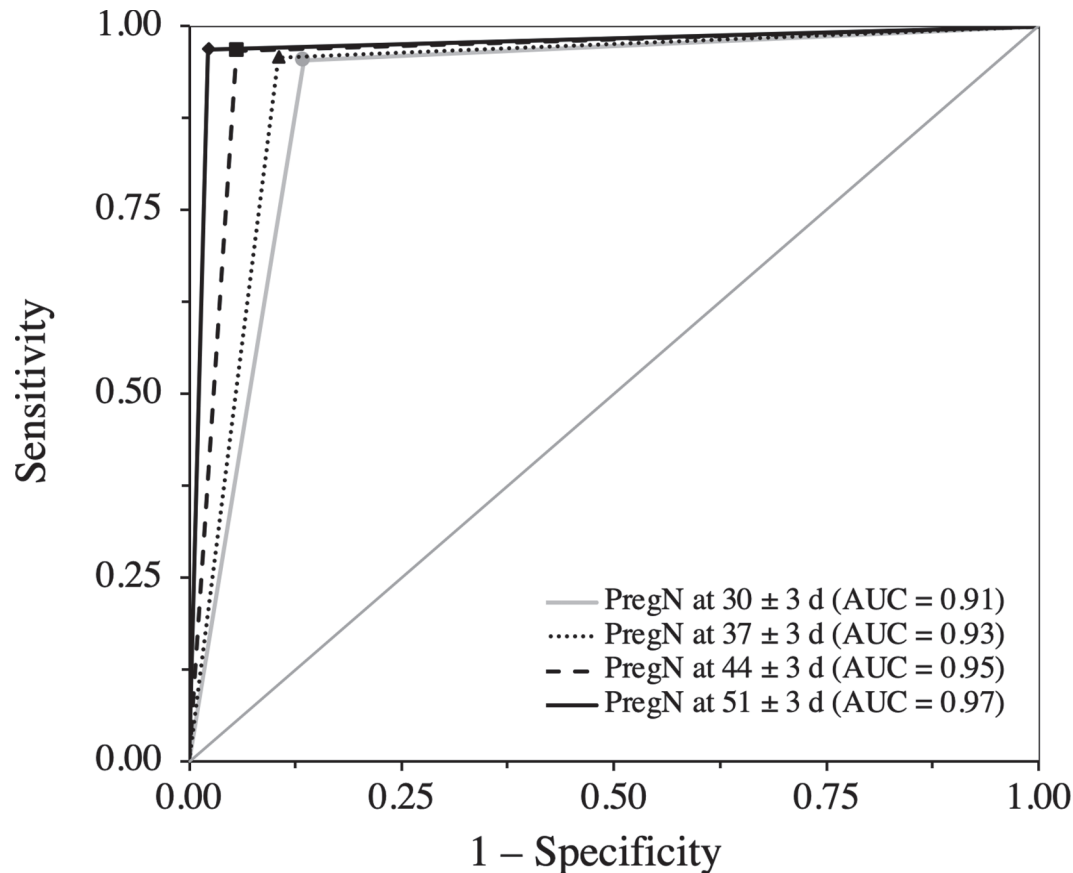


Figure 3. Receiver operating characteristic (ROC) curves for pregnancy notifications (PregN) generated by an in-line milk analysis system (Herd Navigator, DeLaval International, Tumba, Sweden) assessed at different intervals after AI: at 30 ± 3 d, resulting in an area under the curve (AUC) of 0.91 (95% CI 0.90 to 0.92; $P < 0.001$); at 37 ± 3 d, resulting in an AUC of 0.93 (95% CI 0.91 to 0.94; $P < 0.001$); at 44 ± 3 d, resulting in an AUC of 0.95 (95% CI 0.95 to 0.96; $P < 0.001$); and at 51 ± 3 d, resulting in an AUC of 0.97 (95% CI 0.97 to 0.98; $P < 0.001$). Confirmation of calving that occurred between 262 and 296 d after AI was considered the gold standard for pregnancy.

ity and specificity values of at least 96.4 and 95.1%, respectively (Giordano et al., 2013). Based on that, the assessment of IMAS PregN before 40 d after AI had sub-optimal specificity values ($<90.0\%$). However, it is important to note that direct comparisons between

the IMAS for pregnancy determination and a chemical pregnancy test performed at a fixed day, such as pregnancy-associated glycoprotein, or transrectal palpation, might not be appropriate. In contrast to other pregnancy diagnosis methods, the IMAS is dynamic in

Table 1. Positive (PPV) and negative (NPV) predictive values, sensitivity (Se), specificity (Sp), accuracy, and Cohen's kappa statistics (k) for inter-test agreement of IMAS¹ pregnancy notification (PregN) assessed at different intervals after AI in 1,821 AI events

Days after AI (interval) ²	PPV (%)	NPV (%)	Se (%)	Sp (%)	Accuracy (%)	k (95% CI)
30 (27 to 33) d	82.88	96.38	95.28	86.47	90.06 ^a	0.80 (0.77 to 0.83)
37 (34 to 40) d	86.18	96.89	95.82	89.43	92.04 ^b	0.84 (0.81 to 0.86)
44 (41 to 47) d	92.16	97.60	96.63	94.35	95.28 ^c	0.90 (0.88 to 0.92)
51 (48 to 54) d	96.64	97.86	96.90	97.68	97.36 ^{d, x}	0.95 (0.93 to 0.96)

^{a-d}McNemar's test revealed that the proportions of sensitivities and specificities between tests were different ($P < 0.001$).

^xThe proportion of pregnancy as determined by PregN at 51 ± 3 d (40.9%) and the gold standard (40.7%) did not differ ($P = 0.77$).

¹In-line milk analysis system (Herd Navigator, DeLaval International, Tumba, Sweden).

²Categories of different intervals between AI and assessment of the IMAS pregnancy notification. The proportion of AI events followed by PregN at 30 (27 to 33), 37 (34 to 40), 44 (41 to 47), and 51 (48 to 54) d were 46.8% (853/1,821), 45.3% (825/1,821), 42.7% (778/1,821), and 40.9% (744/1,821), respectively. In all, 40.7% (742/1,821) of AI were confirmed pregnant by the gold standard (i.e., calving occurring between 262 and 296 d after AI).

identifying nonpregnant cows on a real-time-basis by monitoring the spontaneous cessation of luteal phase after AI, which indicates an imminent estrus and opportunity for an early reinsemination.

In conclusion, pregnancy notifications generated by the IMAS had high sensitivity (>95.0%) when assessed at any time point, starting at 27 d after AI. Specificity values were less than 90.0% for pregnancy notifications before 40 d after AI, likely resulting from the early pregnancy losses, extended luteal phases, or both. From 41 d after AI, the IMAS was a highly accurate (>95.0%) diagnostic tool for automated determination of pregnancy status. Besides, the system is capable of identifying nonpregnant cows as soon as spontaneous cessation of the luteal phase occurs after AI. However, increasing the period of high sampling frequency beyond the expected luteal phase length might improve precision of reinsemination in cows with an extended luteal phase after AI.

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REFERENCES

- Bruinje, T. C., M. G. Colazo, E. S. Ribeiro, M. Gobikrushanth, and D. J. Ambrose. 2019. Using in-line milk progesterone data to characterize parameters of luteal activity and their association with fertility in Holstein cows. *J. Dairy Sci.* 102:780–798. <https://doi.org/10.3168/jds.2018-14654>.
- DeLaval International. 2011. Herd Navigator™ and reproduction management. Accessed Jul. 8, 2015. [http://delaval-com-www.gtm.episerverhosting.com/PageFiles/171203/53571175_HN_Reproduktion managementLOWRES.pdf](http://delaval-com-www.gtm.episerverhosting.com/PageFiles/171203/53571175_HN_Reproduktion%20managementLOWRES.pdf).
- Fricke, P. M., A. Ricci, J. O. Giordano, and P. D. Carvalho. 2016. Methods for and implementation of pregnancy diagnosis in dairy cows. *Vet. Clin. North Am. Food Anim. Pract.* 32:165–180. <https://doi.org/10.1016/j.cvfa.2015.09.006>.
- Friggens, N. C., and M. G. G. Chagunda. 2005. Prediction of the reproductive status of cattle on the basis of milk progesterone measures: Model description. *Theriogenology* 64:155–190. <https://doi.org/10.1016/j.theriogenology.2004.11.014>.
- Giordano, J. O., P. M. Fricke, and V. E. Cabrera. 2013. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. *J. Dairy Sci.* 96:949–961. <https://doi.org/10.3168/jds.2012-5704>.
- Mayo, L. M., S. G. Moore, S. E. Pooch, W. J. Silvia, and M. C. Lucy. 2016. Technical note: Validation of a chemical pregnancy test in dairy cows that uses whole blood, shortened incubation times, and visual readout. *J. Dairy Sci.* 99:7634–7641. <https://doi.org/10.3168/jds.2016-11224>.
- Norman, H. D., J. R. Wright, M. T. Kuhn, S. M. Hubbard, J. B. Cole, and P. M. VanRaden. 2009. Genetic and environmental factors that affect gestation length in dairy cattle. *J. Dairy Sci.* 92:2259–2269. <https://doi.org/10.3168/jds.2007-0982>.
- Remnant, J. G., M. J. Green, J. N. Huxley, and C. D. Hudson. 2015. Variation in the interservice intervals of dairy cows in the United Kingdom. *J. Dairy Sci.* 98:889–897. <https://doi.org/10.3168/jds.2014-8366>.
- Ricci, A., P. D. Carvalho, M. C. Amundson, and P. M. Fricke. 2017. Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination. *J. Dairy Sci.* 100:9851–9860. <https://doi.org/10.3168/jds.2017-13293>.
- Romano, J. E., J. A. Thompson, D. W. Forrest, M. E. Westhusin, M. A. Tomaszewski, and D. C. Kraemer. 2006. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology* 66:1034–1041. <https://doi.org/10.1016/j.theriogenology.2006.02.044>.
- Saint-Dizier, M., and S. Chastant-Maillard. 2012. Towards an automated detection of oestrus in dairy cattle. *Reprod. Domest. Anim.* 47:1056–1061. <https://doi.org/10.1111/j.1439-0531.2011.01971.x>.
- Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim. Reprod. Sci.* 82–83:513–535. <https://doi.org/10.1016/j.anireprosci.2004.04.015>.
- Siqueira, L. G. B., V. S. Areas, A. M. Ghatti, J. F. Fonseca, M. P. Palhao, C. A. C. Fernandes, and J. H. M. Viana. 2013. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. *J. Dairy Sci.* 96:6461–6472. <https://doi.org/10.3168/jds.2013-6814>.
- Stevenson, J. S., and S. L. Pulley. 2012. Characteristics and retention of luteal structures, extended postinsemination cycle, progesterone, and pregnancy-specific protein B in serum after human chorionic gonadotropin treatment of dairy cows. *J. Dairy Sci.* 95:4396–4409. <https://doi.org/10.3168/jds.2011-5286>.
- Viera, A. J., and J. M. Garrett. 2005. Understanding interobserver agreement: The Kappa statistic. *Fam. Med.* 37:360–363.
- Wilsdorf, L. J., D. H. Keisler, S. E. Pooch, W. R. Lamberson, R. C. Escalante, and M. C. Lucy. 2016. Short communication: Test for nonpregnancy in dairy cows based on plasma progesterone concentrations before and after timed artificial insemination. *J. Dairy Sci.* 99:5858–5865. <https://doi.org/10.3168/jds.2016-10864>.