Short communication: Validation of a novel milk progesterone-based tool to monitor luteolysis in dairy cows using cost-effective, on-farm measured data

Ines Adriaens,^{1*} Wouter Saeys,¹ Katleen Geerinckx,² Bart De Ketelaere,¹ and Ben Aernouts^{1,3}

¹Department of Biosystems, Mechatronics, Biostatistics and Sensors, KU Leuven, Kasteelpark Arenberg 30, 3001, Heverlee, Belgium

²Province of Antwerp, Hooibeekhoeve, Hooibeeksedijk 1, 2440 Geel, Belgium

³Department of Microbial and Molecular Systems, Cluster for Bioengineering Technology, KU Leuven, Campus Geel, 2440 Geel, Belgium

ABSTRACT

The progesterone (P4) monitoring algorithm using synergistic control (PMASC) uses luteal dynamics to identify fertility events in dairy cows. This algorithm employs a combination of mathematical functions describing the increasing and decreasing P4 concentrations during the development and regression of the corpus luteum and a statistical control chart that allows identification of luteolysis. The mathematical model combines sigmoidal functions from which the cycle characteristics can be calculated. Both the moment at which luteolysis is detected and confirmed by PMASC, as well as the model features themselves, can be used to inform the farmer on the fertility status of the cows. **Key words:** milk progesterone, fertility, dairy cow, monitoring tool

Short Communication

Until now, the progesterone (P4) monitoring algorithm using synergistic control (PMASC) methodology was designed and optimized using milk P4 data obtained through experimental trials, collected at maximum frequency (e.g., every milking) and analyzed post-hoc using the state-of-the-art laboratory ELISA technique. Moreover, this method was not yet challenged on data sampled at reduced frequency to show its possible cost effectiveness, nor was it shown that PMASC can work on data measured with an online device, proving its value as an on-farm monitoring algorithm. The objective of this study was to show the capability of PMASC to work in an on-farm setting, identifying luteolysis using smart-sampled data and analyzed via an on-farm lateral flow immunoassay.

Moreover, we compared the PMASC algorithm with the state of the art for P4 monitoring on farm (i.e., smoothing the raw P4 values), combined with a fixed threshold for detection of luteolysis and alerts.

Data sets of on-farm measured milk P4 were collected on 2 farms in Flanders, Belgium. The first data set (data set 1) was obtained from an experimental research farm between March 2017 and January 2019. On this farm, the herd was split into 2 groups, each consisting of on average 55 lactating dairy cows but not different from each other in terms of treatment or herd composition. Both groups were milked with an automated milking system (VMS, DeLaval, Tumba, Sweden). The second data set (data set 2) was collected on a commercial dairy farm between November 2016 and December 2018. The herd consisted of on average 250 animals, milked by 4 automated milking systems (VMS, DeLaval). On both farms, animals were fed a mixed ration of grass and corn silage, supplemented with concentrates through automated concentrate feeders and in the milking robot.

For the P4 analysis, milk samples were taken automatically by the sampling unit integrated in the Herd Navigator (DeLaval; Lattec, Hillerød, Denmark), and the milk P4 concentration was determined by the built-in lateral flow immunoassay milk analyzer. More details on the working principle of this device can be found in Friggens et al. (2007) and Blom and Ridder (2010). The samples for P4 analysis were taken with a variable sampling frequency, of which the concept has been described by Friggens and Chagunda (2005). Basically, the time between samples is determined by the DIM, the likelihood of luteolysis, the P4 concentration of the previous sample, the performed inseminations and their timing, the likelihood of pregnancy, and the probability of prolonged postpartum anestrus or cysts. As the consumables are an important cost for P4 monitoring on farm, this sampling scheme ensures that the necessary fertility information can be gathered on crucial moments with a minimum number of samples.

Received January 30, 2019.

Accepted June 3, 2019.

*Corresponding author: ines.adriaens@kuleuven.be

Data set 1 consisted of 10,791 P4 measurements originating from 144 lactations for 104 unique cows (parity 1 to 6, 2.15 ± 1.16 , average milk production per d 36.47 ± 12.66 kg, mean $\pm$ SD). The second data set consisted of 36,645 P4 measurements, originating from 460 lactations and 342 unique cows, with parities between 1 and 8 (2.3 ± 1.26 , average milk production per d 34.24 ± 10.10 kg).

The state of the art (milk) P4 monitoring in dairy cows builds on a multiprocess Kalman filter (**MPKF**) to smooth the profiles and reduce the effect of potential outliers (Korsgaard and Løvendahl, 2002; Friggens and Chagunda, 2005; Friggens et al., 2007). Luteolysis is considered to have taken place if the smoothed P4 value goes below a 5 ng/mL threshold (**T**) after it was at least twice above this 5 ng/mL threshold. The values above 5 ng/mL are required to confirm luteal activity and to ensure a single measurement was not a measurement error. This algorithm, further referred to as MPKF+T, was implemented and used to identify the reference for luteolysis, which was then used to benchmark the performance of our PMASC algorithm in terms of detecting the luteolysis events.

Progesterone profiles (i.e., the P4 time series of one lactation) with less than 20 measurements in total (e.g., because they just started at the moment of data collection), or less than 5 measurements below or above 5 ng/mL were discarded. Also, P4 data following a gap in the P4 data of at least 20 d were excluded. Accordingly, 719 and 5,322 measurements originating from 47 and 317 lactations were deleted from each data set, respectively, leaving 10,072 and 31,213 measurements from 133 and 409 lactations belonging to 98 and 316 unique cows. The characteristics of the remaining profiles and related P4 data are summarized in Table 1.

Adriaens et al. (2018) describes the original PMASC to process time series of P4 measurements taken each milking, 2 to 4 times a day. To avoid false alerts caused by coincidentally low P4 values (e.g., outliers), a first evidence of luteolysis (low P4) identified by the statisti-

cal control chart needed to be confirmed by 2 additional successive measurements before a luteolysis alert was raised by PMASC. However, in the data sets employed in this study, the P4 concentration was not available for every milking as the number of and moment of samplings depended on the algorithm implemented in the analyzer. For example, when an upcoming estrus was confirmed by this algorithm, no new milk samples were analyzed in the following 3 to 6 d. In case of a steep drop in milk P4 in combination with very low absolute P4 concentrations (<5 ng/mL), this often resulted in less than the 3 consecutive out-of-control measurements available to confirm luteolysis with PMASC. Therefore, the main decision rule of PMASC needed to be adjusted in function of these new data sets. In previous studies on the timing between luteolysis and ovulation time (Roelofs et al., 2006; Adriaens et al., 2019b), it was found that ovulation followed about 60 to 80 h after the onset of luteolysis. To have the largest chance on successful conception, insemination should take place 24 to 12 h before ovulation (Roelofs, 2005). Based on this information, we evaluated all the samples collected within 36 h following an out-of-control measurement to confirm or reject luteolysis. More concretely, the following rule was implemented: if more than half of the measurements within this 36 h window were also out of control, luteolysis was assumed confirmed, an alert was raised, and a new cycle started. In the other case, the out-of-control measurement was considered an outlier. In practice, this would ensure that luteolysis has taken place, while there would still have been sufficient time left to arrange the insemination. Moreover, this rule gives some flexibility to deal with missing samples and outliers. In future studies when the sampling frequency can be defined based on PMASC, other rules can be implemented.

Next, PMASC was applied to the time series of P4 values of each lactation to evaluate its performance when in this cost-effective setting controlled by the device's algorithm. As the analysis was done post-hoc,

Table 1. Summary of the data sets of farm 1 and farm 2: minimum to maximum (mean $\pm$ SD)

Data set	No. of cows	No. of profiles	MY ¹ (kg)	MI ² (h)	P4 ³	
					Concentration (ng/mL)	No. of measurements per profile
Data set 1	98	133	9.98–65.5 (36.47 ± 12.66)	5.6–29.7 (7.9 ± 2.62)	0.03–27.98 (14.71 ± 10.23)	23–168 (75.7 ± 29.97)
Data set 2	321	421	10.1–66.4 (32.0 ± 11.7)	5.0–29.05 (9.36 ± 3.19)	0.01–27.99 (15.36 ± 9.69)	21–232 (80.2 ± 35.39)

¹Daily milk yield in kilograms.

²Average milking interval in hours.

³P4 = milk progesterone.

Table 2. Overview of the detected luteolysis by each algorithm and the corresponding cycle characteristics

Item	Data set 1	Data set 2	Total
No. of profiles	133	409	542
No. of samples/d	0.48	0.5	
No. of days/sample	2.1	1.98	
No. of alerts MPKF+T ²	427	1,416	1,843
No. of alerts PMASC	420	1,411	1,831
No. of unmatched alerts PMASC (% in parentheses)	18 (4.3)	49 (4.3)	72
No. of unmatched alerts MPKF+T (% in parentheses)	25 (5.8)	54 (3.83)	74
Total no. of unmatched alerts	43	103	146
No. of unmatched alerts due to infrequent sampling or low luteal phases	25	43	68
No. of unmatched alerts due to intermediate and noisy P4	18	60	78
Average cycle length (d; mean $\pm$ SD)	27.33 $\pm$ 10.02	26.37 $\pm$ 11.27	26.58 $\pm$ 9.62
% normal cycle length 19 to 25 d	56.33	55.21	56.47
Average baseline concentration (ng/mL; mean $\pm$ SD)	1.09 $\pm$ 0.91	0.93 $\pm$ 0.73	0.97 $\pm$ 0.77
Average luteal concentration (ng/mL; mean $\pm$ SD)	21.89 $\pm$ 4.12	21.42 $\pm$ 3.9	21.53 $\pm$ 3.96
Decreasing slope during luteolysis ¹ (ng/mL per day; mean $\pm$ SD)	34.26 $\pm$ 23.33	37.41 $\pm$ 24.94	36.7 $\pm$ 24.61

¹Calculated using the decreasing Gompertz model implemented in progesterone (P4) monitoring algorithm using synergistic control (PMASC).

²MPKF = multiprocess Kalman filter; T = 5 ng/mL threshold.

the stockmen did not receive any output from these methods. The number and timing of the alerts generated by PMASC were compared with those obtained using the MPKF+T algorithm. Also, the number of measurements per detected cycle was calculated and the cycle characteristics were summarized.

An overview of the results is given in Table 2. Data set 1 consisted of 133 P4 profiles, with an average sampling rate of 0.48 ± 0.07 measurements per day in the period between the first and the last measurement. This corresponds to one measurement each 2.07 d. For data set 2, the 409 profiles had an average sampling rate of 0.50 ± 0.07 measurements per day, corresponding to one sample per 1.98 d. The MPKF+T algorithm gave 427 and 1,416 luteolysis alerts (i.e., smoothed time series undercutting the fixed threshold of 5 ng/mL), respectively, for data set 1 and 2, whereas PMASC gave 420 and 1,411 alerts. Alerts within 5 d of each other were taken as the same luteolysis. Based on this timing, 25 (5.8%) and 54 (3.81%) of the alerts of MPKF+T could not be matched with one of PMASC, and 18 (4.3%) and 49 (3.47%) of the alerts of PMASC could not be matched with one given by MPKF+T. These unmatched alerts could be attributed to the following: (1) A sampling frequency too low to confirm the out-of-control samples, for example, due to quite low luteal phase P4 values, after which only one sample sufficed to generate an alert using MPKF+T. This was the case for, respectively, 58 and 42% of the unmatched alerts for data set 1 and 2. (2) High follicular phase P4 values, resulting in enough evidence for PMASC to generate an alert, whereas MPKF+T did not yet undercut the threshold value. In this case, sometimes the MPKF+T

alert followed later, but outside the time window for matching (i.e., 5 d). This was the case for 42 and 58% of the unmatched alerts in data set 1 and 2, respectively. In the first case, the smart sampling algorithm does not take a new confirmatory sample, while we set PMASC to evaluate at least 2 samples per estrus, and PMASC thus failed to give an alert. When the sampling is based on decision rules implemented in PMASC, outside the scope of this research, these luteolyses can also be detected by PMASC. The intermediate values causing additional PMASC alerts in the second case (2) are mainly due to the calibration technique of the online measurement device, as also discussed in Adriaens et al. (2019a).

An example of a P4 profile and the corresponding alerts is shown in Figure 1. For the first cycle, no alert was generated with PMASC. However, the sample for which the smoothed value is below the 5 ng/mL threshold was also out of control in PMASC, but the next sample was only taken 6 d later. As such, luteolysis was not detected by PMASC because of the lack of confirmatory samples out of control within 36 h after the first out-of-control measurement. The final PMASC models for which the detected luteolysis matches with the alerts given by the MPKF+T algorithm are given in purple, while the MPKF smoothed data are given in dark blue. The threshold of 5 ng/mL P4 is plotted in green. As seen in the figure, the first sample detected as out of control by PMASC is often not yet below the 5 ng/mL threshold, and the model characteristics can be used to estimate luteolysis reliably and independent of both the milking frequency and the absolute P4 values. The time lag between the drop in P4 during luteoly-

sis and the smoothed MPKF value undercutting the threshold is shown to be dependent on sampling rate and the absolute P4 values (yellow box).

The first out-of-control sample detected by PMASC preceded the MPKF/T alarm on average 20.0 ± 16.1 h and 2.64 milkings for data set 1 and 19.8 ± 23.7 h and 2.2 milkings for data set 2. The time delay of the MPKF ensures robustness for outliers and measurement errors, and will correct for unexpected variability. However, contrasting this MPKF smoothed value against a fixed threshold results in alerts for which the timing strongly depends on the absolute P4 values measured and the sampling rate. In cases where the sampling frequency is insufficiently high for timely detection of luteolysis by the MPKF+T method, for example when the sampling or the analysis fails, the alerts of the MPKF+T are less valuable because they arrive too late for timely insemination. However, when these low P4 values are detected by PMASC, information calculated from the model parameters (e.g., time of the inflection point) can be used to interpret these detections and, eventually, to adjust the decision process accordingly.

Using the model parameters, the P4 cycle characteristics were calculated. The average cycle length calculated as the time between 2 successive luteolysis alerts was 27.33 ± 10.0 d, with 56% of the cycles between 19 and 25 d. The average time between the 2 inflection points of the increasing Hill function and the decreasing

Gompertz function was 14.4 ± 10.0 d, which is slightly higher than the time reported in Adriaens et al. (2017), probably because in this previous work only data of normal cycles and profiles were used. The average baseline and maximal P4 concentrations were 0.97 ± 0.77 and 21.5 ± 3.9 ng/mL, respectively, and the decrease in P4 at luteolysis was typically fast with a slope of 36.17 ± 24.6 ng/mL per day. The latter means that a drop in P4 from 25 to 2 ng/mL during luteolysis on average takes 14.56 h, which is in agreement with the values reported by Blavy et al. (2016) and Adriaens et al. (2019b).

In this study, we investigated the performance of PMASC on on-farm measured data sets using less frequent sampling and the LFAI technique, and compared it with the current state of the art being an MPKF+T algorithm. We showed that PMASC, on the condition of a small adjustment in its decision requirements, was able to deal with fewer samples and the alternative measurement technique, and accordingly, has the potential to work cost effectively in an on-farm setting. More concretely, this study showed that a limited number of samples was sufficient to fit the mathematical model used by PMASC, and did not on average influence the cycle characteristics extracted from it. The nondetected luteolyses were related to cases in which only one sample was taken to confirm estrus, which is rather rare and mainly occurs during the first estrus after calving,

Figure 1. A profile of raw milk progesterone (P4) measurements for one lactation are indicated in black. The light blue crosses are the first out-of-control measurements detected by the P4 monitoring algorithm using synergistic control (PMASC), and the light blue lines give final models fitted by PMASC. The first luteolysis was not detected because of an insufficiently high sampling rate (red box), although the measurements pushing the multiprocess Kalman filter (MPKF; indicated in blue) below the threshold (indicated in green) were also out of control for PMASC (indicated with purple cross). The (inconsistent) time lag between the MPKF dropping below the threshold and actual low measurements of P4 can be seen in the yellow box.

in which the maximum P4 concentration remains low. When the sampling rate would be optimized based on information extracted from PMASC (e.g., the time from the previous luteolysis, out-of-control samples, and so on), these kinds of artifacts could be avoided. Although it would be possible to introduce simple decision rules in the algorithm, the implementation and optimization of the P4 sampling rate based on PMASC was beyond the scope of this study.

ACKNOWLEDGMENTS

This work was supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders, Belgium (IWT; IWT-LA project 110770). Ines Adriaens is supported by the Fund for Scientific Research (FWO) Flanders, grant number 11ZG916N. We thank Lattec (Hillerød, Denmark) and the commercial dairy farmer who provided us with the second P4 dataset.

REFERENCES

- Adriaens, I., T. Huybrechts, K. Geerinckx, D. Daems, J. Lammertyn, B. De Ketelaere, W. Saeys, and B. Aernouts. 2017. Mathematical characterization of the milk progesterone profile as a leg up to individualised monitoring of reproduction status in dairy cows. *Theriogenology* 103:44–51. <https://doi.org/10.1016/j.theriogenology.2017.07.040>.
- Adriaens, I., O. Martin, W. Saeys, B. De Ketelaere, N. C. Friggens, and B. Aernouts. 2019a. Validation of a novel milk progesterone-based tool to monitor luteolysis in dairy cows. Timing of the alerts and robustness against missing values. *J. Dairy Sci.* <https://doi.org/10.3168/jds.2019-16405>.
- Adriaens, I., W. Saeys, T. Huybrechts, C. Lamberigts, K. Geerinckx, J. Leroy, B. De Ketelaere, B. Aernouts, and K. U. Leuven. 2018. A novel system for online fertility monitoring based on milk progesterone. *J. Dairy Sci.* 101:8369–8382. <https://doi.org/10.3168/jds.2017-13827>.
- Adriaens, I., W. Saeys, C. Lamberigts, M. Berth, K. Geerinckx, J. Leroy, B. De Ketelaere, and B. Aernouts. 2019b. Short communication: Sensitivity of estrus alerts and their relation with the timing of the LH surge. *J. Dairy Sci.* 102:1775–1779. <https://doi.org/10.3168/jds.2018-15514>.
- Blavy, P., M. Derks, O. Martin, J. K. Hoglund, and N. C. Friggens. 2016. Overview of progesterone profiles in dairy cows. *Theriogenology* 86:1061–1071. <https://doi.org/10.1016/j.theriogenology.2016.03.037>.
- Blom, J. Y., and C. Ridder. 2010. Reproductive management and performance can be improved by use of Delaval Herd Navigator. In *Proceedings of First North American Conference on Precision Dairy Management*. Toronto, Canada. <http://precisiondairy.com/proceedings/s5blom.pdf>.
- 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>.
- Friggens, N. C., K. L. Ingvarsen, I. R. Korsgaard, T. Larsen, P. Løvendahl, C. Ridder, and N. I. Nielsen. 2007. System and a method for observing and predicting a physiological state of an animal. US Pat. No. US7302349 B2.
- Korsgaard, I. R., and P. Løvendahl. 2002. An introduction to multi-process class II mixture models. Pages 185–188 in *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*. Département de Génétique Animale INRA, Castanet-Tolosan, France.
- Roelofs, J. B. 2005. When to inseminate the cow? Insemination, ovulation and fertilization in dairy cattle. PhD Thesis, Wageningen Institute of Animal Sciences, Wageningen, the Netherlands.
- Roelofs, J. B., F. J. C. M. Van Eerdenburg, W. Hazeleger, N. M. Soede, and B. Kemp. 2006. Short communication: Relationship between progesterone concentrations in milk and blood and time of ovulation in dairy cattle. *Anim. Reprod. Sci.* 91:337–343. <https://doi.org/10.1016/j.anireprosci.2005.04.015>.