INTERPRETATIVE SUMMARY. Validation of luteolysis monitoring tool Adriaens.

Recently, a novel progesterone-based monitoring algorithm using synergistic control, PMASC, was developed. This algorithm employs the known luteal dynamics of the progesterone profile to estimate the moment of luteolysis with minimal dependence on the absolute measurement values. As its value and implementability strongly dependends on the number of samples it requires to obtain the desired accuracy, its performance on cost-effective, 'smart' sampled and on-farm measured data is demonstrated.

VALIDATION OF LUTEOLYSIS MONITORING TOOL

SHORT COMMUNICATION: Validation of a novel milk progesterone based tool to monitor luteolysis in dairy cows. Performance on cost-effective, on-farm measured data

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[ABSTRACT, max 2500 keystrokes, no statistics, no references, …, only for submission, not for the manuscript in SC]

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The Progesterone (**P4**) monitoring algorithm using synergistic control (**PMASC**) enables to identify fertility events stooled on the underlying physiological basis of the related progesterone dynamics (Adriaens et al., 2017, 2018b). Therefore, PMASC employs a combination of mathematical functions describing the increasing and decreasing P4 concentrations during the development and regression of the corpus luteum (**CL**) and a statistical control chart which allows to identify luteolysis. The mathematical model is a combination of sigmoidal functions, from which the cycle characteristics can be calculated. Both the moment that luteolysis is detected and confirmed by PMASC, and the model features might possibly be used to inform the farmer on the fertility status of the cows.

Until now, the PMASC system was designed and optimized on data obtained through experimental trials and analyzed *post-hoc* via the state-of-the-art laboratory ELISA technique. As a result, it was not yet tested on data sampled according to a reduced frequency to show its possible cost-effectiveness, nor was it shown that PMASC can work on data measured with an on-line 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 (**LFIA**).

To this end, datasets of on-farm measured milk P4 were collected on two farms in Flanders, Belgium. The first dataset (dataset 1) was obtained from an experimental research farm between December 2016 and March 2018. On this farm, the herd was split up in two groups, each consisting of on average 55 lactating dairy cows. Both groups were milked with an automated milking system (VMS, Delaval, Tumba, Sweden). The second dataset (dataset 2) was collected on a commercial dairy farm between November 2016 and December 2018. The herd consisted of on average 250 animals, milked by four automated milking systems of Delaval (VMS, Delaval, Tumba, Sweden). 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, Tumba, Sweden; Lattec, Hillerød, Denmark), and the milk P4 concentration was determined via an on-farm LFIA. More details on the working principle of this device can be found in Blom and Ridder, (2010) and in the patent of Friggens et al., (2007). The samples for P4 analysis were taken with a variable sampling frequency. While the algorithm behind this sampling scheme is confidential, its basis can be found in Friggens and Chagunda, (2005). Basically, the time between samples is dependent on the days in milk, the likelihood of luteolysis, the P4 concentration of the previous sample, the performed inseminations and their timing, the likelihood of pregnancy, prolonged postpartum anestrus or cysts and so on. As the consumables are one of the most important costs for P4 monitoring on farm, this 'intelligent' sampling frequency ensures that as much information can be gathered on crucial moments with as little samples as possible.

Dataset 1 consisted of 10,958 P4 measurements originating from 151 lactations and 102 cows. However, the collection period enclosed two months in which the sampling frequency was increased for several cows to one sample per milking in the context of a research trial, as described in Adriaens et al., (2018a). A total of 3496 samples (31.9%) was taken in the context of this research trial. On the second farm, P4 samples were collected according to the variable 'smart' sampling scheme during the whole period. This dataset consisted of 36,535 P4 measurements, originating from 457 lactations and 342 unique cows, with parities between 1 and 8 (2.3±1.6, mean±standard deviation).

The raw P4 measurements were smoothed using a multiprocess Kalman filter (**MPKF**) as described in Friggens and Chagunda, (2005) and Friggens et al., (2007, 2008), and estrus alerts were generated when this smoothed P4 value undercut a 5 ng/mL threshold (**T**). Due to the absence of a real gold standard for estrus and ovulation, these estrus alerts (MPKF+T) were taken as the reference for luteolysis against which the performance of PMASC was tested.

Progesterone profiles (i.e. the P4 time series of one lactation) with less than 15 measurements in total, 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 days were excluded. Accordingly, 870 and 2780 measurements originating from 52 and 214 lactations were deleted for each dataset respectively, leaving 10,088 and 33,755 measurements from 99 and 421 lactations belonging to 52 and 321 unique cows. The characteristics of the remaining profiles and related P4 data are summarized in Table 1. Here, the P4 measurements taken during the trial period for dataset 1 were not included in the calculation of the number of measurements per profile because this was not the commercial cost-effective setting.

[TABLE 1]

In Adriaens et al., (2018a), PMASC was described for its use on time series of P4 measurements taken each milking. More specifically, a rule was introduced that when a first evidence of luteolysis was obtained via the statistical control chart, two additional measurements should be taken to ensure luteolysis has taken place and to avoid false alerts caused by coincidentally low P4 values (e.g. outliers). However, in the datasets presently employed, the number of samples taken was not dependent on PMASC's detection, but on the smoothed value produced by the MPKF undercutting the threshold of 5 ng/mL. The latter is in its turn dependent on how fast P4 decreases during luteolysis, which may vary from 12 to 72 hours (Blavy et al., 2016), the absolute P4 concentrations measured and the milking interval. Accordingly, when luteolysis went fast and the final raw P4 concentrations were considerably below 5 ng/mL, repeatedly less than the three measurements needed to confirm luteolysis with PMASC were available. As also discussed in Adriaens et al., (2018a), this rule is certainly adjustable, and for example the size of the residual from the fitted mathematical model might be used in practice to decide how many samples should confirm luteolysis. However, for the current validation, we were dependent on the samples taken by the online device. Therefore, an adaption of PMASC was legitimized. In previous studies on the timing between luteolysis and ovulation time (Roelofs et al., 2006; Adriaens et al., 2018c), it

was found that ovulation followed about 60 to 80 hours after the onset of luteolysis. To have the largest chance on successful conception, insemination should take place 24 to 12 hours before ovulation. Based on this information, it was assumed reasonable to take all the samples taken within 48 hours from the first out-of-control measurement into account to confirm or object luteolysis. In practice, this would allow to ensure that luteolysis has taken place, while there is still sufficient time left to arrange the inseminator. More concretely, the following rule was implemented: if a measurement was out of control, all measurements following within a period of 48 hours were evaluated using the current, non-updated model parameters of the increasing Hill function (Adriaens et al., 2017). If in that period another residual was also below the current LCL, we assumed luteolysis had taken place and an alert was raised. Accordingly, this rule gives some flexibility to deal with missing samples and outliers. In practice, the sampling frequency would be dependent on the PMASC itself, and more intelligent rules can be implemented.

Next, PMASC was run on the time series of P4 values of each lactation to evaluate its performance when a reduced sampling scheme, shown cost-effective, was applied. The number of and the timing of the alerts generated by PMASC were compared to those obtained from the currently implemented algorithm (MPKF+T). Also, the number of measurements per detected cycle was calculated and the profile characteristics (length postpartum anestrus, interval calving to pregnancy and cycle properties) were summarized.

In total, the datasets consisted of 520 P4 profiles, with an average sampling rate of 0.51±0.08 measurements per day in the period between the first and the last measurement. This corresponds to one measurement each 1.9 days. The on-farm devices gave 322 and 1664 estrus alerts respectively (i.e. smoothed time series undercutting the fixed threshold of 5 ng/mL). From these, 309 and 1559 were also detected by PMASC, while 13 (4.0%) and 105 (6.3%) were not. These non-detected estruses were all related to infrequent sampling, for example because the smoothed P4 concentration only slightly exceeded the 5 ng/mL threshold, after which only one sample was

sufficient to bring the smoothed value (MPKF) below 5 ng/mL and generate an alert. After this, the smart sampling algorithm does not take a new confirmatory sample, while we set PMASC to evaluate at least two samples per estrus, and PMASC thus failed to give an alert. This mainly occurs during the first estrus after calving, also shown by the fact that 65% of the cases not detected by PMASC in dataset 2 concerned the first alert of that profile, with an average alert time of 47±21 days in lactation. An example is shown in Figure 1. Here, the first luteolysis was not detected by PMASC because of the lack of confirmatory samples out-of-control within 48 hours. 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 days later, providing not enough evidence for PMASC to indicate luteolysis. The model of the detected cycles is plotted in light blue, while the MPKF smoothed data is given in dark blue. The threshold of 5 ng/mL P4 is plotted in green. As seen in the figure, the first samples 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 the milking frequency and absolute P4 values. The time lag between the drop in P4 during luteolysis and the smoothed MPKF value undercutting the threshold is shown to be dependent of sampling rate and the absolute P4 values (yellow box).

[FIGURE 1]

The first out-of-control sample detected by PMASC preceded the MPKF/T alarm on average 20.0±16.1 hours and 2.6±2.1 milkings for dataset 1 and 20.2±15.3 hours and 2.5±1.9 milkings for dataset 2. The time-delay of the MPKF ensures robustness for outliers and measurement errors, and will correct for unexpected variability. However, by combining this MPKF smoothed value with a fixed threshold, this method 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 algorithm fails, the alerts of the MPKF+T are less valuable. However, when these low P4 measurements 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, adjust the decision process accordingly.

Using the model parameters, the P4 cycle characteristics were calculated. The average cycle length calculated as the time between two successive luteolysis alerts was 27.3±10.4 days, with 21% of the cycles between 22 and 25 days, and 11% between 19 and 22 days. The average time between the two inflection points of the increasing Hill function and the decreasing Gompertz function was 14.9±10.5 days, which is slightly higher than the time reported in Adriaens et al., (2017), probably because here only data of normal cycles and profiles were used. The average baseline and maximal P4 concentrations were 0.98±0.9 and 21.6±4 ng/mL respectively, and the decrease in P4 at luteolysis was typically fast with a slope of 37.1±23.9 ng/mL per day. The latter means that a drop in P4 from 25 to 2 ng/mL during luteolysis on average lasts 14.6 hours, which agrees with the results reported by Blavy et al., (2016) and Adriaens et al., (2018b).

In this study, we investigated the performance of PMASC on on-farm measured datasets 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 less samples and the alternative measurement technique, and accordingly, has indeed the potential to work cost-effective in an on-farm setting. More concretely, this study showed that a limited number of samples sufficed to fit the mathematical model used by PMASC, and did on average not influence the cycle characteristics extracted from it. The non-detected 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, in which the maximum P4 concentration remains low. In practice, the sampling rate would be optimized based on information extracted from PMASC (e.g. the time from the previous luteolysis, out-of-control samples etc.), and these kinds of artifacts can be avoided. Although it is possible by

introducing simple decision rules in the algorithm, the implementation and optimization of the P4 sampling rate based on PMASC was considered out of the scope of this research.

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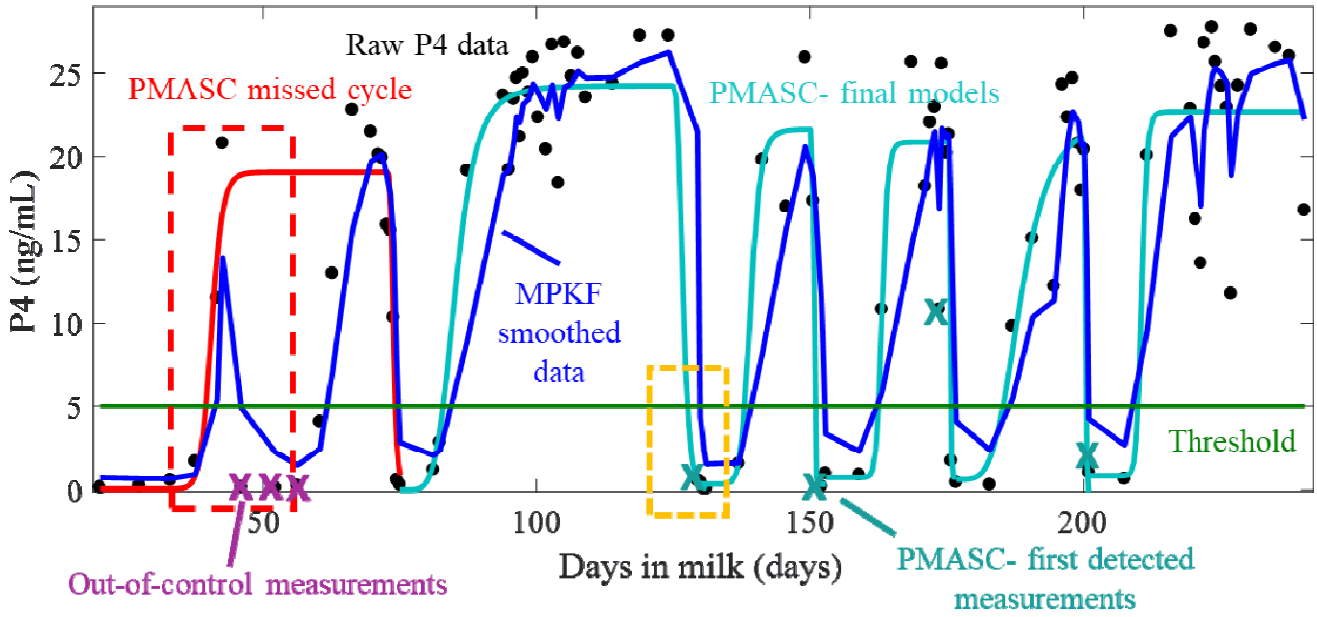
Figure 1. An example profiles of real progesterone (P4) data indicated in black. The light blue crosses are the first out-of-control measurements detected by PMASC, and the light blue lines give final models fitted by PMASC. The first cycle was not detected because an insufficiently high sampling rate, although the measurements pushing the 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.

Table 1. Summary of the datasets of farmer 1 and farmer 2

Adriaens, Figure 1



Adriaens, Table 1

Dataset	No. of cows	No. of profiles	MY[1] (kg)	MI[2] (h)	P4[3]		Estrus alerts
					Concentration (ng/mL)	No. of meas. per profile	
Dataset 1	52	99	20.5-58.2 (39.0±9.2)	5.6-11.8 (8.4±1.3)	0.1-28.0 (14.5±10.4)	24 -325 (137.1±56.8)[4]	322
Dataset 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)	1656

[1] Daily milk yield in kg; [2] Average milking interval in hours; [3]P4 milk progesterone; [4] excluding the measurements taken during the research trial in spring 2017 as described in Adriaens et al., (2018a)