INTERPRETIVE SUMMARY. Heat detection attentions and LH surge. Adriaens.

Both detection of heat and timely insemination are important factors in optimizing fertility management. The latter is dependent on ovulation time, which is preceded by the LH surge. In this study, the performance of 4 heat detection systems at predicting the LH surge was evaluated. Visual observation of standing heat correlates best with the LH surge, but is the least sensitive, while activity attentions are the least reliable. Using milk progesterone, one can reliably detect luteolysis, which in healthy cows is followed by the LH surge about 62 hours later.

Linking heat attentions and the moment of LH surge in dairy cows – how close can we get?

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ABSTRACT

Both heat detection and timely insemination contribute to the optimization of fertility management on farm. In this study, 4 systems were compared for their ability to accurately detect heat and relate to the LH surge preceding ovulation in dairy cows. As the moment of LH surge has been reported to correlate strongly with time of ovulation, the potential to predict the optimal insemination can in this way be evaluated. The systems included were the traditional visual observation of heat, activity attentions and 2 progesterone-based methods. For the latter, it was also investigated whether the prediction of the LH surge could be improved by fitting a longitudinal model to the progesterone data. First, the systems were compared in terms of sensitivity and positive predictive value for heat detection. Then, the time interval between the attentions and the LH surge was investigated. The range on this interval was used as main criterion to evaluate the time-relation between the heat attention and the LH surge. The smaller this range, the better the correlation with the LH surge, and accordingly, ovulation. Heat attentions based on visual observations were noted from 4 hours before until 5 hours after the LH surge (range of 9 hours), indicating a high correlation. However, they also had the lowest sensitivity to detect heat (40%), making it less useful on-farm. Using activity-attentions proved more sensitive (80%), but was less accurate. Moreover, these attentions had the least accurate correlation with the moment of the LH surge and were observed from 39 hours before until 8 hours after it (range of 47 hours). Attentions based on milk progesterone measurements through the detection of luteolysis preceding a follicular phase correctly identified all estrous periods. Alarms generated when the smoothed progesterone level crossed a 5 ng/mL threshold, were followed by the LH surge after 21.6 to 66.4 hours (range of 44.8 hours). The model-based

approach performed slightly better with attentions generated 48.8 to 81.2 hours (range 32.9 hours) before the LH surge.

Key words; preovulatory LH surge, heat detection systems, milk progesterone, visual estrus detection, cow activity

INTRODUCTION

Both maximal detection of heat and timely insemination are important to improve on-farm fertility performance (Roelofs, 2005; Hockey et al., 2010). Traditionally, heat is detected through visual observation of external symptoms and insemination based on the detection of cows standing to be mounted. Here, the generally applied rule-of-thumb is to inseminate in the evening when a cow is showing standing heat in the morning and vice versa, which requires the farmer to observe his herd at least twice a day (Roelofs et al., 2015). However, visual detection is time-consuming and external heat symptoms are short-lived in high-producing dairy cows (Van Eerdenburg et al., 1996a; Lopez et al., 2004; Wiltbank et al., 2006; Løvendahl and Chagunda, 2010). Therefore, automated systems are increasingly used for heat detection. For example, activity measurements are widely used to identify increased restlessness during estrus and were shown to be profitable (Rutten et al., 2014). The main advantages are that they do not consume any of the herdman's valuable time to make observations and within-cow comparisons can be made to generate an alert. However, behavior-based heat detection systems are still unable to identify silent heats, which occur in 10 to 55% of the estrous periods (Ranasinghe et al., 2010). The recent developments in on-line milk progesterone (**P4**) measurement systems allow to overcome this problem. P4 levels are widely used as a gold standard for estrus and can indicate the timing of luteolysis preceding ovulation. It was shown to be able to identify up to 99.2% of the estrous periods with a specificity of 93.7% (Friggens et al., 2008). However, luteolysis is not the ultimate trigger to ovulation despite it being a necessary condition for it, and as such, is not completely conclusive (Dobson et al., 2008).

Although it is essential to optimize the insemination window, only a limited number of studies investigated the link between heat attentions and ovulation (Van Eerdenburg et al., 2002; Roelofs et al., 2005a; Hockey et al., 2010). Moreover, these studies had variable success and their outcome depended strongly on the used gold standard for detection of ovulation. Three approaches have been proposed to verify if ovulation took place: (1) using insemination success and gestation which are strongly dependent on other factors, (2) using ultrasonography for the detection of the disappearance of a preovulatory follicle and (3) monitoring the preovulatory LH surge (Roelofs et al., 2004; Fisher et al., 2008). Monitoring the LH surge is useful because of the small window (21.5-27.5 hours) after this peak in which ovulation occurs (Roelofs et al., 2004).

Roelofs et al. (2005a) concluded that pedometer activity readings are the most accurate to predict ovulation as in their study, it occurred 22 to 39 hours (range 17 hours) and 12 to 35 hours (range 23 hours) after respectively the onset and the end of increased number of steps. In contrast, the intensity of visually detected estrous behavior did not influence ovulation time, which occurred between 18.5 and 48.5 hours after onset (range of 30 hours) and between 9.5 and 33.5 hours (range of 24 hours) after the visually observed heat (Roelofs et al., 2005b). Until now, milk P4 dropping below a fixed threshold is shown to correlate poorly with actual timing of ovulation. The best results were obtained using a fixed threshold of 5 ng/ml, with ovulation occurring 54 to 98 hours later (range of 44 hours) (Roelofs et al., 2006). As mentioned by Friggens et al., (2008) and Adriaens et al., (2017), milk P4 measurements are subject to a large variability. Accordingly, the methodology used to monitor milk P4 might have a large impact on the accuracy of ovulation prediction (Friggens et al., 2008; von Leesen et al., 2013). To this end, different methodologies have already been developed and one of them is implemented in the on-line Herd Navigator™ (**HN**) system (Friggens and Chagunda, 2005; Friggens et al., 2008; Mazeris, 2010). This commercial instrument uses a smoothing filter to process the measured P4 values and advises to inseminate 30 to 45 hours after the smoothed P4 level drops below a fixed threshold, depending on the parity. Unfortunately, in this way the exact moment of the P4 drop is strongly influenced by the sampling frequency and luteolysis rate, and subsequently, so is the recommended insemination window. To overcome this issue, a novel direct modelling approach was developed and described in Adriaens et al., (2017) and Adriaens et al. (*unpublished data*).

The objective of this study was to compare 4 different heat detection methods, different both in terms of sensitivity and positive predictive value (**PPV**), and in their accuracy to relate to the moment of the LH surge preceding ovulation. We hypothesized that the P4-based systems would outperform the behavior-based systems in terms of sensitivity and that prediction of the spontaneous occurring LH surge could be improved using a mathematical model describing P4 during luteolysis.

MATERIALS AND METHODS

Animals, Feed and Housing

The study was conducted in an experimental dairy farm housing 58 Holstein-Friesian cows in Flanders, Belgium in the spring of 2017. Approval of the ethical committee of KU Leuven was obtained under file ID P010/2017. Housing consisted of a freestall barn with slatted concrete floor. The cows were milked with an automated milking system (VMS, Delaval, Tumba, Sweden) and were fed with a mixed ration of grass and corn silage. Concentrate feeds were provided in the milking robot and through concentrate feeders. In the 2 months preceding the start of the study, all open cows which were more than 30 days post-partum were regularly checked for cyclicity. When it concerned cows with known fertility problems, a treatment was started to induce normal cyclicity. These animals were excluded from the trial if normal cycling did not start before the beginning of the trial. Pregnant cows and cows less than 30 days in lactation at the time of the trial were also excluded. As such, 24 animals were eligible for this study. Because open cows more than 150 days in lactation were allowed to be inseminated by the herdsman to avoid too much disruption in the normal fertility program of the test farm, in the first 10 days of the sampling period, 2 animals conceived successfully and were excluded, leaving 22 animals. These remaining cows were on average 3.4±1.2 years of age (mean±SD), had parities between 1 and 4 and were between 30 and 293 days in lactation at the start of the study. For additional details the reader is referred to Adriaens et al. (*unpublished data*).

Milk Progesterone (P4)

Over a period of 53 days, a mixed milk sample of each milking was taken automatically by the sampling unit (VMX, Delaval, Tumba, Sweden) following the procedure of the dairy herd improvement protocol prescribed by ICAR (ICAR International committee for animal recording, 2014). The fresh milk samples were supplemented with $0.1\%_{vol/vol}$ preservative (Qlip N.V., Leusden, the Netherlands) and stored at 3°C. Three times a week, they were brought to a certified lab (MCC-Vlaanderen, Lier), where 2 mL of each sample was frozen at -20°C. At the end of the trial, the P4 concentration was measured with a Ridgeway ELISA kit, for which more details are described in Adriaens et al., (2017). The P4 concentration of each sample was also determined automatically on-line by the HN system (Lattec, Delaval, Hiller□d, Sweden). This device allowed us to monitor the P4 concentration during the study. As such, the moment at which the raw milk P4 started a consistent drop towards concentrations under 5 ng/mL, staying below it for a period of at least 24 hours is named the **HN-P4-drop**.

Estrus Detection

At first, this study compared different heat detection systems in terms of sensitivity and PPV. Hereto, a ‘reference heat period, **RHP’**, was defined, which starts with a HN-P4-drop followed by a period of low milk P4 (< 5 ng/mL) of minimum 5 and maximum 10 days in which a preovulatory follicle of at least 13 mm was detected as described below (Hopper, 2015). The sensitivity of a method was defined as the number of times an attention was given by the system during the RHP (true attentions) divided by the total number of RHP (true estrus). The PPV was the number of true attentions compared to the total number of attentions for that method. Sensitivity and PPV of the different heat detection systems were determined for the period between day 7 and the end of the study in which visual heat observations were performed.

Visual observations were done by an independent observer twice a day for 30 minutes according to the scoring system proposed by Van Eerdenburg et al., (1996) starting from day 7 of the trial, once in the late morning (11 AM) and once after evening feeding (6 PM). The observer was not aware of the P4 profiles nor of activity attentions. A cow was considered observed in estrus, and thus an ‘attention’ was raised when the sum of 2 successive observation scores exceeded 50. Because a cow standing to be mounted is considered conclusive for estrus, this was noted separately. If a single observation period resulted in a score above 50, the attention was set at that period. Otherwise, the time midway in-between the 2 successive observations was recorded. Observations of estrus (score > 50) not associated with a RHP were considered false alarms.

Each cow was fitted with a commercial activity meter (ActoFIT, version 2015, Delaval, Tumba, Sweden) on the neckband. Increased activity was monitored automatically via the algorithm included in the DelPro Farm Manager software (Delaval, Tumba, Sweden). This algorithm generates an activity attention in 3 possible levels (+/++/+++) dependent on the actual restlessness of the cow compared to her normal behavior. Most estrous periods are associated with a gradually increasing activity level, returning successive '+', '++' and '+++' activity attentions. In this study, only the highest level of activity within the RHP was considered. Each attention not associated with a RHP was considered a false alarm.

P4-based attentions for heat detection were included in 2 different ways: (1) based on the current HN algorithm on the on-line P4 measurements and (2) based on a newly developed on-line monitoring system named **P**4 **M**onitoring **A**lgorithm using **S**ynergistic **C**ontrol (**PMASC**) applied on ELISA measurements. For the first, HN raw measurements were smoothed according to the model described by Friggens and Chagunda (2005) using a Kalman filter. An attention was defined as the moment that the smoothed P4 value undercut a 5 ng/mL threshold. It should be noted that in our study, the measurement frequency was increased to 1 sample each milking, and all measurements were used to obtain the HN attentions. The second P4-based algorithm, PMASC, makes use of a mathematical model describing the different parts of the P4 profile (Adriaens et al., 2017) and a statistical process control chart to indicate luteolysis (Adriaens et al, *unpublished data*). An attention was raised when the system detected luteolysis followed immediately by a RHP within 24 hours. This is a complete novel approach to manage raw P4 values and detect luteolysis as an indicator for the onset of estrus. The actual moment when luteolysis was detected was taken as the reference moment for calculations of PPV and sensitivity.

Serum LH

One of the main difficulties when investigating the optimal insemination time is to have a good reference measurement for ovulation. Basically, the possibilities are limited to monitoring gestation after insemination, ultrasonography and detection of the LH surge. In contrast to ultrasonography, measuring LH is not dependent on immediate on-spot interpretation. As such, it is more feasible when collecting samples over a longer period of time and different observers can join forces without decreasing the performance of the reference method. Therefore, we used the preovulatory LH surge as a proxy for ovulation in this study. As the LH surge lasts only for 9-9.5 hours on average (Roelofs et al., 2004; Fisher et al., 2008), high-frequent 2-hourly samplings around the expected ovulation time were needed. The availability of on-line P4 measurements allowed for optimizing sampling time without the need for external behavior-based estrus detection or the use of an estrous synchronization protocol.

Starting from day 28 of the trial, an expert veterinarian checked the ovaries of each cow for the presence of a preovulatory follicle 15 to 50 hours after the HN-P4-drop, using a transrectal ultrasound scanner (A6v, Sonoscape Medical Corp., Shenzhen, China). Next, 2 teams of 2 researchers took blood samples from the jugular vein each 2 hours starting 36 hours after the HN-P4-drop over a period of 72 hours, unless the cow showed post-estrous bleeding (in this case, sampling stopped). Disposable 20G needles were used, which were replaced for each sample taken to ensure minimal impact. The cows in the trial were trained to accustom them to human handling, and did not show symptoms of distress in the process. As much attention as possible was paid by the researchers to limit distress and it was assumed that taking the blood samples in this herd influenced the (stress-level of the) cows very little. The blood samples were allowed to clot at room temperature for 2 hours and were centrifuged at 2300 *g* to collect the serum. Next, three aliquots of each serum sample were stored at -20°C. Between 7 and 11 days after the HN-P4-drop, the ovaries of each cow were checked for the disappearance of the follicle and the presence of a *corpus luteum* (**CL**). At the end of the trial, LH concentration in the collected serum samples was measured with a commercially available bovine LH ELISA kit (Abnova, Taipei City, Taiwan) on a BEP2000 system (Siemens Healthcare Diagnostics, Marburg, Germany). This kit is based on a solid phase enzyme-linked immunosorbent assay, which utilizes a polyclonal anti-LH antibody for solid phase immobilization. The antibody-enzyme conjugate solution consists of a mouse monoclonal anti-LH antibody and horseradish peroxidase. The included standards contained 0, 1, 5, 10, 20 and 50 ng/mL LH. The within-run coefficient of variation was 6.3% (average LH 13.2 ng/mL) and 5.5% (average LH 45.2 ng/mL). The limit of quantification of this test was set at 3 ng/mL. All serum samples of the same cow were analyzed in the same analytical run. The LH surge was visually determined on a time versus LH concentration graph. The LH surge height was between 17 ng/mL and 49 ng/mL and was in all cases more than 8-fold the baseline LH concentration.

Heat Attentions and the LH Surge

The second part of the study consists of a comparison of the different heat detection attentions and the LH surge preceding ovulation. As LH was monitored only during the last 25 days of the trial, only a selection of the RHP was used for the comparisons, further referred to as RHP_S. In total, LH samples were taken during 24 RHP from 22 cows. Moreover, to obtain unbiased results, cows with severe health or fertility problems known to affect endocrinology, were excluded (Dobson et al., 2008; Walker et al., 2008) and are discussed in the next paragraph.

A total of 15 RHP_S (9 first parity, 6 multiparous cows, 158±53 days in lactation, body condition score 3.2±0.3, mean±SD) remained for the analysis.

To investigate the link between the heat detection attention and the LH surge, the time interval between the attention as described above and the moment of maximal LH concentration was calculated in hours, further referred to as the time interval (**TI)**, and was calculated as time of LH peak minus time of attention. For visual observations, this was the interval from the moment the observation cumulative score exceeded 50 points to the LH surge ($\mathbf{TI_{VO}}$). Using the activity meter system, the moment of the highest level of activity within that period was used to calculate $\mathbf{TI_{ACT}}$. In the case of P4, both the HN attention ($\mathbf{TI_{HN}}$) and the moment of luteolysis determined with the PMASC system were included in the time interval ($\mathbf{TI_{L}}$). The latter allowed to evaluate if the LH surge could be predicted more accurately based on metrics obtained from the mathematical model fitted on the P4 data at the time of luteolysis. The different model-based indicators from which TI was calculated were (1) the inflection point of the decreasing Gompertz function describing the luteolysis ($\mathbf{TI_{IP}}$); (2) the intercept of the tangent line at the inflection point with the time-axis ($\mathbf{TI_{IC}}$); (3) the moment that the model surpassed a fixed threshold of 3, 5, 7 or 10 ng/mL ($\mathbf{TI_{TMOD\_X}}$); and (4) the moment the model undercut 85, 90 or 95% of the maximum P4 model concentration minus the baseline ($\mathbf{TI_{TB\_X}}$), with *x* representing the respective percentages and thresholds.

LH Surge in Unhealthy Cows

Despite the limited amount of data and the fact that monitoring unhealthy cows was not the main goal of this paper, it is interesting to take a closer look at the LH data for these animals and compare them with the healthy cows. The ‘unhealthy’ cows included 1 animal treated for milk fever, 1 being severely lame and 1 with endometritis, all undergoing spontaneous luteolysis. Additionally, 3 cows were treated for a luteal cyst with *Dinoprostum tromethamini*, triggering an immediate luteolytic reaction. Furthermore, 3 animals developed a follicular cyst or went in anestrus after a normal cycle and spontaneous luteolysis. This was detected with ultrasonography on day 7 – 11 after the HN-P4-drop.

The animals treated for luteal cysts are known to ovulate, but the moment of LH surge and ovulation is dependent on the stage of follicular growth at the moment of treatment. Accordingly, the relation between luteolysis, which happens right after treatment, and LH surge is not consistent in these cows. Therefore, collection of the serum samples was extended up to maximum 98 hours. The available LH results for these cows were compared with those of the healthy ones. Nevertheless, these cows were not included in the comparison between the attention and LH surge which was handled in the previous section, because luteolysis was induced here by exogenous PGF treatment.

RESULTS AND DISCUSSION

Comparison of Heat Detection Methods

In the following paragraphs, the performance of the systems in terms of sensitivity and PPV will be discussed and compared to the values obtained in other studies. This will place the results of the second part into perspective, where the link between heat attentions and the LH surge is investigated.

Between day 7 and 53 of the study, 42 RHP were detected in the 22 cows. These 42 RHP are used as the basis for comparison and comprise all cows, including these with health problems. The number of attentions per system, together with their sensitivity and PPV are summarized in Table 1. For the visual observations, it is indicated how many detections involved a cow standing to be mounted. Similarly, the activity attentions are expressed according to their maximum activity level of respectively '+', '++' or '+++' for each period.

Table 1. Overview of the number of attentions, false alarms, sensitivity and PPV of the heat detection systems over the 46-day monitoring period

	Total # attentions	# false alarms	Sensitivity (RHP_S[2])	PPV
Visual observations	26 (6)	6	47.6% (40.0%)	76.9%
Standing behavior	13 (5)	0	20.9% (33.3%)	100.0%
Activity system	55 (12)	21	80.9% (80.0%)	61.8%
+	55 (12)	18	88.1% (80.0%)	67.3%
++	28 (10)	3	59.5% (66.6%)	89.3%
+++	18 (5)	0	42.9% (33.3%)	100.0%
P4-based systems[1]				
HN	42	0	100% (100%)	100.0%
PMASC	35+7[*]	0	83+17%* (100%)	100.0%

[1]HN: Herd Navigator system; PMASC: Progesterone-based Monitoring Algorithm using Synergistic Control
[2]Between brackets: only considering the estrous periods included in the LH comparisons: RHP_S
[*]Due to a lack of data at the start of the trial, 7 low periods were not identified by the PMASC system. As the P4 pattern for these is identical to the other 35 cases, and we did not want to discard all those data to avoid more reduction of the already small set, we assumed that they would have been detected and reported them separately.

In this part of the study, cows with health or fertility problems were not excluded, because we think it is up to the farmer to decide on the insemination of a cow. For example, cows treated for a luteal cyst do ovulate, although they probably have a lower chance of conception. Typically, the restlessness-based systems (visual observation, activity) will not raise an attention in unhealthy cows. For example, severely lame animals or cows suffering from endometritis are not expected to show external symptoms. Because the number of monitored heat attentions studied, was rather limited, an in-depth comparison of heat detection systems is not feasible nor the primary goal of this manuscript. For this purpose, we recommend the papers of e.g. Cavalieri et al., (2003), Roelofs et al., (2010), Saint-Dizier and Chastant-Maillard, (2012), Rutten et al., (2013), Chanvallon et al., (2014), Michaelis et al., (2014), Firk et al., (2002) and (Roelofs et al., 2015). To be able to make a fair comparison in the second part of this paper, the total number of attentions and sensitivity of the systems for the 15 RHP_S included in the LH comparisons are shown in-between brackets in Table 1.

Visual observation of external estrous symptoms is the oldest and most widely used method for estrus detection. However, several different factors limit its reliability: housing, breed, age, production level, herd size, time of observation, other cows in estrus, etc. (Van Eerdenburg et al., 2002; Roelofs et al., 2005b, 2010; Løvendahl and Chagunda, 2010). In our study, a heat attention based on visual observation was generated 26 times for 14 cows, from which 20 were associated with a RHP (sensitivity 47.6%). Standing to be mounted was noted 13 times (sensitivity 20.9%). Eight of the 22 cows never showed estrous symptoms during the observation periods (36.6%), and 20/42 estruses were silent (52.4%), which is comparable to results reported by other studies (Ranasinghe et al., 2010). No difference was seen between primiparous and multiparous cows (p-value = 0.469), nor was there a relation found with stage of lactation (p-value = 0.153). To verify that blood sampling did not negatively affect the estrus behavior, intensity and frequency of detection were compared for the period in which we sampled (day 28 to 53, 25 days) and the preceding period in which only milk samples for P4 analysis were taken (day 7 to 27, 20 days). Neither the intensity of estrus (score, p-value = 0.824), nor the frequency of detection (10 ‘true’ attentions per period) differed between the 2 periods. Therefore, we can conclude that taking the blood samples did not influence the expression of external estrous symptoms. Visual observations twice a day was found to be the least reliable method for heat detection. For a thorough discussion of heat detection by visual observations, we refer to Van Eerdenburg et al., (1996).

When all 3 activity levels were considered, the activity-based system performed slightly better than the visual observations in terms of sensitivity, and was able to detect nearly 81% of the estrous periods. However, estrus detection solely based on the '+' activity level is not reliable, as 18 of the 55 '+' attentions (32.7%) were false alarms. These moderate increases in activity are most likely to be caused by dominance fighting or environment related factors. Using only the '++' and '+++' attentions drastically decreased the number of false alarms (5.4% and 0% respectively), but the sensitivity decreased accordingly to 59.5% and 42.9%. The main advantage of activity meters over visual observations is the automated nature of the system which limits the time required for estrus detection by the farmer. However, insemination based solely on activity attentions is not recommended. The decision to inseminate should be based on additional evidence such as uterus tension or mucous vulvular discharge. Moreover, the current results only include the pre-selected cycling cows, and the false alarm rate for '++' and '+++' attentions might increase when including 'bulling' animals with follicular cysts which did not respond to pre-trial treatments. Additionally, activity scoring does not identify silent ovulations in which the cow does not show the estrus-associated restlessness (36% in our study).

The comparison period started 7 days after the start of the trial. The PMASC system in its current form needs to start from a period of low P4, as it is designed to start in the postpartum anestrous period. In this way, luteolysis not preceded by the 18-21 day training period, could not be detected by the system. This was the case for 7 out of the 42 RHP in the trial. Accordingly, if these 7 cases would be included in the sensitivity calculation for PMASC, the sensitivity would drop to 83%. However, as these would most likely have been detected if a larger reference sampling period for training before these events had been available, this is an underestimation of PMASC's performance. Therefore, these 7 RHP were excluded from the analysis for PMASC, resulting in a sensitivity of 100%. We marked the calculations for these separately with a "*" in Table 1. In a large study conducted by Friggens et al., (2008), a sensitivity of 93.3% to 99.2%

was reached, using the HN system for milk P4 measurements in combination with the model described by Friggens and Chagunda (2005). It should be noted that the obtained sensitivity results are influenced by the way 'estrus' is defined.

The value of P4-based systems only indicating luteolysis might be over-estimated in terms of sensitivity as long as there is no better standard for ovulation. Basically, the absence of P4 is a necessary condition for the secretion of the GnRH impulse, which stimulates the pituitary gland to produce the preovulatory LH surge. The complete hormone mechanism for ovulation is complex, and depends on a cascade of inhibition and stimulating hormones, all influenced by several physiological factors (Hopper, 2015). For instance, decreasing body condition score and negative energy balance result in reduced oocyte quality and inadequate CL function (Leroy et al., 2008). Future research will determine when it is recommended to inseminate after a detected luteolysis, and which factors (such as BCS, diseases, insufficient uterus tonus, …) should be taken into account.

In the following section, a comparison is given of the heat detection attentions and their correlation with the LH surge preceding ovulation. As such, the timing of optimal insemination based on these attentions could be evaluated.

Predicting the LH Surge

The second part of this study focused on the time interval (TI) between heat detection attentions and the LH surge (TI_{VO}, TI_{ACT}, TI_{HN}, TI_{PMASC}).

The baseline concentration of serum LH was on average 1.46±0.84 ng/mL and was in 393 out of the 491 measurements (80%) below the limit of quantification of the assay (i.e. < 3 ng/mL). The LH surge (36.3±11.3 ng/mL) (mean±SD) developed and faded for all cows within 8 hours (= 4 measurements). An overview of the LH data and the ELISA P4 profiles, centered around the LH surge is shown in Figure 1. A clear peak can be observed for each of the included cows despite the afore-mentioned variability in serum LH concentrations measured with ELISA. The P4 profiles also show a large variability, both before and after the LH surge, but all cows had an active CL producing P4 concentrations of more than 5 ng/mL within 7 days after the LH surge, which is an additional indicator that these cows had ovulated.

In Figure 2, the TI in hours from the attention to the LH surge is plotted for each of the RHP_S for which an attention was given, together with the average (thick red line). A summary of these results is also given in Table 2. The upper black dots show the data for the first parity, the lower grey dots for parity 2 and higher.

In 9 of the 15 RHP_S, no attention based on visual observation of heat symptoms was generated, and in only 5 cases standing heat was noted. Attentions based on visual observation of heat have a very strong correlation with the LH surge (range of 9 hours, average TI of -1.5 hours, as the LH surge preceded the attention by on average 1.5 hours). So, visual observation of heat is a very conclusive way of determining the LH surge, despite it being less sensitive. Both literature and common practice advise to inseminate cows 12 hours after visual detection and thus, according to the study of Roelofs and colleagues, 12 to 14 hours before ovulation (Roelofs et al., 2004). As the LH surge precedes ovulation with 24 to 26 hours, our study confirms their results. The same applies for the average time of 26.4±5.2 hours from standing heat to ovulation (Roelofs et al., 2004), which also agrees with our findings. There was no difference between the parities.

Although not included in these results, 2 more cows showed standing heat during the night. These cows didn’t show any symptom of estrus during the periods in which they were observed according to the aforementioned protocol (during the day). The LH surge of these cows was 2 and 0 hours apart from the observation, which is an additional confirmation of the higher mentioned agreement between LH surge and cows standing to be mounted.

Table 2. Summary of the time intervals (TI_X) for the different heat detection systems

			TI_X				
	N	**Sensitivity**	**Minimum**	**Maximum**	**Range**	**Mean**	**SD**
Visual observation	6	40%	-5.0	4.0	9.0	-1.5	3.9
Parity 1	2		-4.0	3.0	7.0	-0.5	5.0
Parity >2	4		-5.0	4.0	9.0	-2.00	4.1
Activity attentions	12	80%	-8.0	39.0	47.0	9.42	16.1
Parity 1	7		-8.0	26.0	34.0	2.29	11.2
Parity >2	5		-1.0	39.0	40.0	19.4	17.7
Herd Navigator	15	100%	21.6	66.4	44.8	46.8	11.4
Parity 1	9		21.6	62.2	40.6	44.1	11.7
Parity >2	6		38.6	66.4	27.8	50.9	10.6
PMASC - Luteolysis	15	100%	45.9	81.6	35.7	62.0	12.2
Parity 1	9		45.9	79.8	33.9	61.2	12.7
Parity >2	6		50.0	81.6	31.6	63.2	12.4
PMASC - IP[1]			52.0	87.2	35.2	73.4	11.1
PMASC – IC[1]			38.7	79.3	40.6	55.7	12.4
PMASC - TB_85[1]			41.8	78.4	34.6	56.4	11.2
PMASC - TB_90[1]			21.6	75.5	43.9	46.2	15.5
PMASC - TB_95[1]			4.9	71.3	66.4	43.0	17.2
PMASC - TMOD_10[1]			48.3	81.2	32.9	66.6	11.1
PMASC - TMOD_7[1]			45.6	80.3	34.7	61.6	11.4
PMASC - TMOD_5[1]			35.1	79.3	44.2	56.8	12.6
PMASC - TMOD_3[1]			13.9	77.4	63.5	47.8	18.1

[1]PMASC – Progesterone-based monitoring algorithm using Synergistic Control; IP = inflection point, IC = intercept tangent line at IP and t-axis; TB_X: threshold based on model passing X% of the maximal + baseline P4 value; TMOD_X: model passing a threshold of X ng/mL

Three RHP_S didn't have an activity attention and from the other twelve, two had only a '+' attention, which was reported in part 1 to be very unreliable for heat detection. Furthermore, activity attentions generated by commercialized activity sensors were the least reliable system to relate to the LH surge. Twelve of the 15 RHP_S had at least a '+' attention, but the range from attention to LH surge varied from 39 hours before the LH surge to 8 hours after it. When addressing the different parities, it seemed that the RHP_S of cows in the first parity cows had a more consistent TI from attention to the LH surge than the multiparous cows. The single outlier of the first parity animals is a '+' attention, which is rather unreliable for heat. The 2^{nd} and higher parity cows clearly show a higher variability (range TI = 40 hours). It is possible that these results improve when using different data processing algorithms. For example, Roelofs and colleagues reported decent accuracy in predicting ovulation using activity measurements. They reported that ovulation occurred 29.3±3.9 hours after the onset of an increased number of steps (TI between 22-39 hours) and 19.4±4.4 hours after the end of the increased number of steps (TI between 12-35 hours) (Roelofs et al., 2005a). However, evaluating alternative data processing algorithms for raw activity data was outside the scope of this study.

The P4-based systems generated attentions for all the RHP_S. The HN system gives an attention when the processed P4 data undercuts a fixed 5 ng/mL threshold on the test farm. In our study, this attention preceded the LH surge by 21.6 to 66.4 hours (range 45 hours), with almost no difference between first and higher parity cows. It should be noted that these attentions were based on a maximum sampling frequency of once per milking, which might be reduced in a real farm setting. It is expected, but not proven, that the range of 45 hours between maximum and minimum TI from HN attention to LH surge increases when the system runs in a normal setting, due to an additional time lag caused by the smoothing algorithm when less samples are taken. This time-lag is an inherent characteristic of the Kalman filter. The HN algorithm at the test farm advised to inseminate the cows 30-35 (first parity) to 40-45 (multiparous) hours after the HN attention. As such, in 14 of the 15 RHP_S the cow was inseminated in a window between 24 before and 8 hours after LH surge, depending on when the inseminator was available. From these, 6 conceived successfully while 2 others might have been pregnant, but encountered a P4 fall between day 40 and 50 after insemination. Two cows were inseminated too early (40 and 24 hours before LH surge), and two cows had a rather low P4 concentration during the luteal phase after insemination, possibly insufficient to support pregnancy (Hopper, 2015; Bruinjé et al., 2017). One cow was culled before pregnancy control, due to udder health problems. The last one had a timely insemination and no abnormalities were noted, but did not conceive for unknown reasons.

The average TI from luteolysis to the LH surge detected with the PMASC system in this study was 62 hours, with a minimum of 46 and a maximum of 82 hours. The resulting range is thus 36 hours, and did not differ between first and higher parity cows. However, this range depends on the sampling frequency, as does the HN attention. Therefore, it would be better to have an indicator which is equally predictive, but less dependent on the sampling.

One of the research questions of this study was whether the estimation of the LH surge could be improved by developing model-dependent rather than data-dependent guidelines through the PMASC system. In that way, it is possible to optimize the insemination advice by (partially) decoupling the generation of the attentions from the sampling frequency and luteolysis rate, which makes the monitoring system more robust. In Figure 3, the correlation between the model-based indicators and the LH surge is shown for the 15 RHP_S. Each dot represents the TI from the model-based indicator to the moment of the LH surge for each individual RHP_S. These results are also summarized in Table 2.

As described above, the moment of luteolysis 'L' detected by the PMASC system has a good correlation with the LH surge and TI_L occurred in a range of 36 hours. The other lines show that both IP, Tb_{85}, $Tmod_{10}$ and $Tmod_7$ have perform similarly (range TI_X of 33 – 35 hours). With maximal sampling frequency, the IP does not diverge more than a few hours from the moment of detected luteolysis, explaining their correlation. Healthy cows typically have a luteal P4 concentration between 20 and 30 ng/mL and a follicular concentration of approximately 2.5 ng/mL. Accordingly, Tb_{85} represents the moment that the model goes below 2.5+0.15*(20-2.5) =

5.1 to 2.5+0.15*(30-2.5)= 6.6 ng/mL. Using this Tb_{85} indicator, and thereby considering the absolute P4 concentration, provided a better result than when a fixed threshold of 5 ng/mL was used on the model (TI_{TMOD5}). However, when a fixed threshold of 10 or 7 on the model was used (respectively TI_{TMOD10} and TI_{TMOD7}), an even smaller range of 33 and 35 hours was noted. These results demonstrate that the actual moment when luteolysis starts, is more indicative for the LH surge than the P4 being fully cleared from the cow's bloodstream. The obtained values for TI_X should be interpreted with any precaution as the number of studied cycles is limited. Nevertheless, this study provides an objective indication of the value of the different heat detection attentions in predicting the LH surge, allowing to compare the different systems. In this way, these results can possibly be extrapolated to small and medium size farms not using synchronization protocols for fertility optimization. Possible advantages of model-based indicators are that they (1) are calculated for each profile, providing flexibility to account for differences in shapes and absolute levels; (2) are disconnected from the exact moment of sampling in contrast to using the exact moment of luteolysis; (3) have the possibility to further optimize sampling rate and model parameters, e.g. by fixing the slope-determining parameters to physiologically relevant ranges when less frequent samples are available (Meier et al., 2009). The latter 3 topics are important subjects for further research.

LH Surge in Unhealthy Cows

In this paragraph, we contrast the unhealthy cases to the normal ones described above. It was hypothesized that unhealthy cows have a larger variation in their moment of LH surge, or even would not have one at all (Dobson et al., 2008; Morris et al., 2009, 2011). This was confirmed in our study. For example, the 3 cows treated for a luteal cyst all showed a clear LH surge within the sampling period. Of those 3, 1 had the LH surge 63 hours after luteolysis which is comparable to the normal cows. The LH surges of the 2 other cows were 45 and 111 hours after luteolysis. For 1 cow, this is far above average, while the other is rather early compared to the normal ones. This can probably be explained by the difference in follicular growth stage of the current follicular wave at the moment of treatment. For the 3 cows treated for other health problems around the period of luteolysis (severe lameness, endometritis, milk fever), neither had an LH surge within the sampled time-period. Unfortunately, it was not possible to check when their P4 concentration increased again and ovulation had taken place with certainty: for 1 cow ovarian activity was boosted using GnRH and $PGF_{2\alpha}$ agonists, the second was culled and did not rise to luteal levels before and the third was treated for a follicular cyst.

CONCLUSIONS

Monitoring P4 allows to identify luteolysis and outperforms classical heat detection systems based on external estrous symptoms in terms of sensitivity and PPV. However, insemination success also depends on correct identification of the moment of ovulation. In this study, we investigated the time interval between heat detection attentions and the moment of the LH surge, which has been reported to occur about 26 hours before ovulation, using different heat detection methods. Although an almost perfect correlation was noted between the moment of the LH surge and the heat attention based on visual observations, its use is strictly limited by the time-consuming nature and low sensitivity of this method. The heat detection system based on increased activity performed better in terms of sensitivity, but was unable to reliably estimate the moment of the LH surge. In this study, activity attentions were noted from 39 hours before until 8 hours after the LH surge (range of 47 hours). P4 based system indicate luteolysis before ovulation, and the P4 based systems are very sensitive. Attentions generated by the HN system were followed by an LH surge after 21.6 – 66.4 (range of 44.8) hours. Accordingly, the correlation with the moment of LH surge was slightly better than for activity attentions. A new methodology using direct modelling of P4 performs as good in predicting luteolysis, generating attentions 48.3 to 81.2 (range 32.9) hours before the LH surge. This model-based approach for milk P4-based heat detection has the potential to be more robust when less samples are available, but further research is required to confirm this. Additionally, further research is also recommended to identify the factors influencing the LH surge, and the time delay between luteolysis and ovulation.

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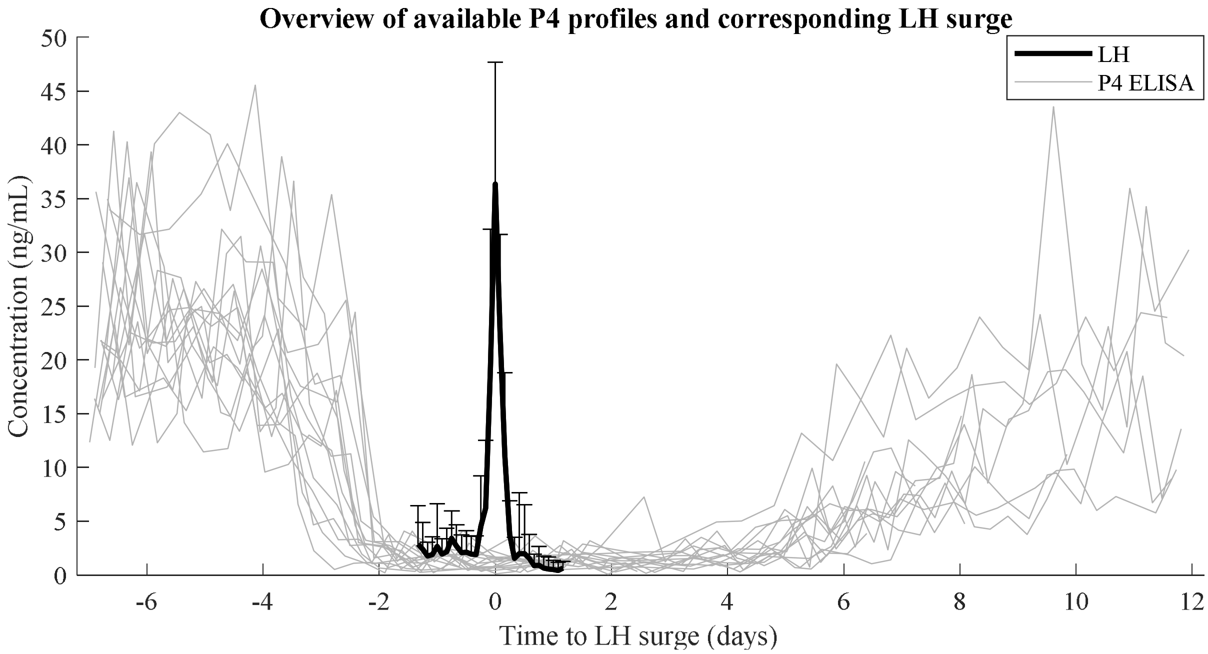
Figure 1. Overview of the P4 profiles centered around the LH surge for the 15 cows selected for this study. A large variability in the P4 profiles is noted both before and after the LH surge, but all cows had an active *corpus luteum*, reflected in a P4 concentration higher than 5 ng/mL within 7 days after the LH surge.

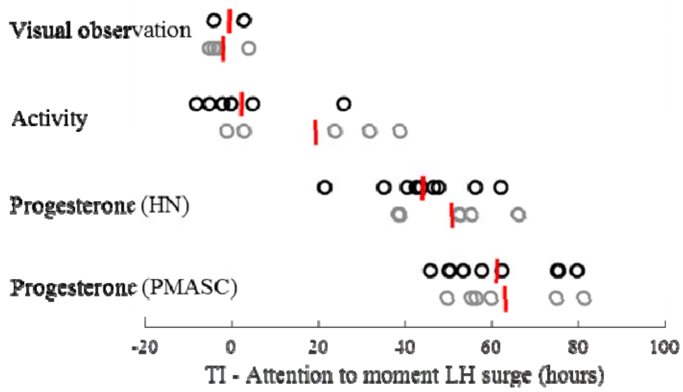
Figure 2. Visual observations (VO) have the smallest range (9 hours) in time interval (TI) from attention to the moment of the LH surge, but are very insensitive (6 out of 15 detected). Activity attentions have the largest range (47 hours) and thus perform the poorest in correlating with the LH surge. The progesterone-based systems (Herd Navigator, HN and Progesterone based Monitoring Algorithm using Synergistic control, PMASC) show ranges of 45 and 36 hours. Further details are given in Table 2. Black circles indicate first parity cows, grey circles parity 2 and higher.

Figure 3. Representation of the link between model-based indicators and the LH surge. Both TI ranges of the inflection point (TI_{IP}), the moment the model reaches 85% of the maximum + baseline model value (TI_{TB85}), and the fixed thresholds on the model (TI_{TMOD10} and TI_{TMOD7}) perform similarly as the luteolysis itself (TI_L).

Adriaens, Figure 1



648 Adriaens, Figure 2



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Adriaens, Figure 3