Sensitivity of heat detection attentions and their relation with the moment of LH surge in dairy cows

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Abstract

The sensitivity of an estrus detection system and the consistency of alarms relative to ovulation determine its value for a farmer. The objective of this study was to compare four different heat detection systems for their ability to detect heat and predict the moment of the LH surge in a single herd during the same study period. This comparison, in which the moment of the LH surge was used as an indicator for ovulation, allowed for the objective evaluation of each system and the potential for optimizing the fertility management on farm. The four systems were traditional visual observation, an activity-based system and two progesterone-based methods: (1) Herd Navigator™ and (2) a novel algorithm which combines a mathematical model and synergistic control. The latter algorithm also allowed to test whether using the mathematical model could improve the relation with the LH surge. 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 and compared based on their range and SD. Heat attentions based on visual observations had the lowest sensitivity to detect heat (40%), and were noted from 4 hours before until 5 hours after the LH surge (range 9 hours, SD 4 hours), indicating a strong relation. Activity-attentions proved more sensitive (80%). They had the least accurate relation with the moment of the LH surge and were observed from 39 hours before until 8 hours after it (range 47 hours, SD 16 hours). Attentions based on milk progesterone measurements correctly identified all estrous periods. Herd Navigator™ attentions system were followed by the LH surge

after 22 to 66 hours (range of 45 hours, SD 11 hours). The model-based approach generated attentions 49 to 81 hours (range 33 hours, SD 11 hours) before the LH surge. As detection of the LH surge was very labor-intensive, only a limited number of potential heat periods could be studied. For some of the methods (e.g. visual observations), the sensitivity restricted the number of cases even more. Nevertheless, the approach ensured an objective comparison between relevant heat detection systems in a commercially representative setting. Accordingly, this study helps to place larger studies linking heat detection and ovulation into perspective and shows the potential of new P4-interpreting algorithms, thereby highlighting the need for further research.

Keywords

Preovulatory LH surge; heat detection systems comparison; milk progesterone; visual estrus detection; cow activity

1. Introduction

Both maximal detection of heat and timely insemination are important to improve on-farm fertility performance [1,2]. 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 [3]. However, visual detection is time-consuming and external heat symptoms are short-lived in high-producing dairy cows [4–7]. Therefore, automated systems are increasingly used for heat detection. For example, activity measurements are widely applied to identify increased restlessness during estrus and were shown to be profitable [8]. The main advantages are that they

do not consume any of the herdsman'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 [9].

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% [10]. However, luteolysis is not the ultimate trigger to ovulation despite it being a necessary condition for it, and as such, is not completely conclusive [11].

Although it is essential to optimize the insemination window, only a limited number of studies investigated the link between heat attentions and ovulation [2,12,13]. Moreover, these studies had variable success and their outcome depended strongly on the used gold standard for detection of ovulation. Four approaches have been proposed to verify the moment of ovulation: (1) using insemination success and gestation which are strongly influenced by other factors as well; (2) using the P4 dynamics; (3) using ultrasonography for the detection of the disappearance of a preovulatory follicle and (4) monitoring the preovulatory LH surge [14,15]. Monitoring the LH surge is useful because of the small window (21.5-27.5 hours) after this peak in which ovulation occurs [14].

In [13], Roelofs and colleagues 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, SD 3.9 hours)

and 12 to 35 hours (range 23 hours, SD 4.4 hours) after respectively the onset and the end of increased number of steps. In contrast, the intensity of visually detected estrous behavior did not relate to ovulation time, which occurred between 18.5 and 48.5 hours after onset (range of 30 hours, SD 5.1 hours) and between 9.5 and 33.5 hours (range of 24 hours, SD 4.4 hours) after the visually observed heat [16]. Until now, milk P4 dropping below a fixed threshold is shown to relate 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, SD 11.2 hours) [17]. As mentioned by Friggens et al., (2008) and Adriaens et al. (2017), milk P4 measurements are subject to a large variability [10,18]. Accordingly, the methodology used to monitor milk P4 might have a large impact on the accuracy of ovulation prediction [10,19]. To this end, different methodologies have already been developed and one of them is implemented in the on-line Herd Navigator™ (**HN**) system [10,20,21]. This commercial instrument uses a smoothing filter to process the measured P4 values [22,23] and advises to inseminate 30 to 45 hours, depending on the parity, after the smoothed P4 level drops below a fixed threshold,. Unfortunately, in this way the detected 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*. [18,24].

The objective of this study was to compare four different heat detection methods simultaneously during the same study period and on the same farm. The comparison included both the sensitivity and positive predictive value (**PPV**), as well as the consistency of the time delay between the heat attention and the moment of the LH surge preceding ovulation. The first aspect is the

necessary first condition to be useful on farm, while the latter provides information on how well the attention relates to ovulation time, and ultimately, the optimal time of insemination. Because assessing the LH surge is very labor-intensive, only a limited number of heat periods were taken up in this study. This prevents from making steady claims in this paper, but it enables to place larger studies, which typically only evaluate one heat detection system in absence of a good reference for ovulation, in perspective. Moreover, it might provide valuable data for future meta-analyses on heat detection, P4 and ovulation. In this study, we hypothesized that the P4-based systems would outperform the behavior-based systems in terms of sensitivity and that using a mathematical model describing the P4 drop during luteolysis could improve the relation between the heat attention and preovulatory LH surge.

2. Materials and methods

2.1 Animals, Feed and Housing

The study was conducted in an experimental, but commercially representative 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 two 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 start 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 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 one and four and were between 30 and 293 days in lactation at the start of the study.

2.2 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 [25]. 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**.

2.3 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 in section 2.4 [26]. This methodology allowed us to identify all estrous periods of the cows, including silent heats, while getting around the need of daily ultrasound examinations. 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 seven 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 seven 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 two 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 two 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 two different ways: (1) generated by the HN system 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. The HN system measures the milk P4 with a lateral flow immunoassay method [27] and generates a heat attention when the smoothed P4 value undercuts a 5 ng/mL threshold [20,22,23]. It should be noted that in our study, the measurement frequency of the HN system was increased to one 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 [18] and a statistical process control chart to indicate luteolysis (Adriaens *et al.* [24]). A heat attention based on milk P4 (HN or PMASC) was taken as true positive if followed immediately by a RHP within 24 hours. All other attentions were considered

false positives. This is a complete novel approach to manage raw P4 values and detect luteolysis as an indicator for the onset of estrus.

2.4 Serum LH

One of the main difficulties when investigating the optimal insemination time is to have a good reference measurement for ovulation. Practically, 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 [14,15], high-frequent two-hourly samplings around the expected ovulation time were needed. This high-frequent sampling is not only labor-intensive, but also expensive, limiting the feasibility and extensiveness of these type of studies. Previous studies investigating moment of ovulation applied a synchronization protocol first [14,15,36–43], limiting the time in which frequent monitoring is needed, or based the starting moment for monitoring on visual symptoms of heat [12,13,16,37]. This method is not useful when non-behavioral estruses should be included too. 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. In our study, identification of the right moment to start sampling for

the best chance to detect the LH surge was based on (1) the absence of P4 in the milk and (2) the presence of a preovulatory follicle, similar to the methods followed by Hockey et al. [2,28].

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, two teams of two researchers took blood samples from the jugular vein each two 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 was terminated). A preliminary study showed that this amount of samples was needed to ensure a full image of all LH surges was obtained. Per sample one disposable 20G needle was used 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 two hours and were centrifuged afterwards at 2300 *g* to collect the serum. Next, three aliquots of each serum sample were stored at -20°C. Between seven and eleven 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). The ELISA 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. Presence of an LH surge was visually determined on a time versus LH concentration graph, and the moment of the LH surge taken as the maximal LH concentration, which was between 17 ng/mL and 49 ng/mL. In all cases, this surge concentration was more than 8-fold the baseline LH concentration.

2.5 Heat Attentions and the LH Surge

The second part of the study consists of a comparison of the different heat detection attentions and the moment of 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 these comparisons. 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 [11,29] and are discussed in the next paragraph. A total of 15 RHP (9 first parity, 6 multiparous cows, 158±53 days in lactation, body condition score 3.2±0.3, mean±SD) remained for the analysis, further referred to as RHP_S.

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 (**TI_{VO}**). Using the activity meter system, the moment of the highest level of activity within that period was used to calculate **TI_{ACT}**. In the case of P4, both the HN attention and the moment of luteolysis determined with the PMASC system were used to calculate respectively time intervals **TI_{HN}** and **TI_{L}**. Additionally, as the output from the PMASC system is not only limited to the detected moment of luteolysis, it was also possible to evaluate how well the LH surge related with the metrics obtained from the mathematical model fitted on the P4 data at the time of luteolysis. The different model-based indicators for which TI was calculated were (1) the inflection point of the decreasing Gompertz function describing the luteolysis (**TI_{IP}**); (2) the intercept of the tangent line at the inflection point with the time-axis (**TI_{IC}**); (3) the moment that the model surpassed a fixed threshold of 3, 5, 7 or 10 ng/mL (**TI_{TMOD_X}**); and (4) the moment the model undercut 85, 90 or 95% of the maximum P4 model concentration minus the baseline (**TI_{TB_X}**), with *x* representing the respective percentages and thresholds.

2.6 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 one animal treated for milk fever, one being severely lame and one with endometritis, all undergoing spontaneous luteolysis. Additionally, three cows were treated for a luteal cyst with *Dinoprostum tromethamini*, triggering an immediate luteolytic reaction. Furthermore, three 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 (*Dinoprostum tromethamini*).

3. Results and discussion

3.1 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 those 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]

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., Roelofs et al., Saint-Dizier and Chastant-Maillard, Rutten et al., Chanvallon et al., Michaelis et al., Firk et al. and Roelofs et al. [3,8,31–34,36,37]. 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. [4,12,16,31]. 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 22 out of 42 RHP estruses were silent (52.4%), which is comparable to results reported by other studies [9]. 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 estrous 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 two 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) [7].

When all three activity levels were considered, the activity-based system performed 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 only 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 nymphomaniac 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 (11.9% 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. 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. However, the additional check-up of the ovaries using ultrasonography aided to confirm each estrous period, and physiology dictates that estrus cannot occur during luteal phases of the cycle. Therefore, the current method applied is a watertight one, and no better alternative is available today.

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 [26]. For instance, decreasing body condition score and negative energy balance result in reduced oocyte quality and inadequate CL function [38]. 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 relation with the LH surge preceding ovulation. As such, the timing of optimal insemination based on these attentions could be evaluated.

3.2 Relation between heat attentions and 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_{L}).

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.

[FIGURE 1]

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.

[FIGURE 2]

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 relation 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, these results indicate that visual observation of heat is strongly linked to the LH peak, despite it being less sensitive. Both literature and common practice advise to inseminate cows 12 hours after visual detection of cows standing to be mounted and thus, according to the study of Roelofs and colleagues, 12 to 14 hours before ovulation [14]. 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 [14], which also agrees with our findings. We could not observe any differences between parities, possibly because of the low number of observations.

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) and therefore not included in the aforementioned results. The LH surge of these cows was 2 and 0 hours apart from the standing heat observation, which is an additional confirmation of the higher mentioned agreement between LH surge and cows standing to be mounted.

[TABLE 2]

Three RHP_S could not be associated with any activity attention and from the other twelve, two had only a '+' attention, which was reported in the first part of this study to be very unreliable for heat detection. Furthermore, activity attentions generated by commercialized activity sensors were the least accurate 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 than the multiparous cows. The single outlier of the first parity animals is a '+' attention, which is rather unreliable for heat. The 2nd 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) [13]. 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, SD 11.4 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 is typically lower in a real farm setting. However, we needed the maximal sampling frequency to be able to reliably identify the best moment to start LH sampling. 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. Future research should point out consistency of the alarms with different, more economically feasible sampling schemes. However, this was not included in the current study, because we believe a thorough, all-inclusive comparison would be needed to compare the different algorithms, which is outside the scope of this manuscript. 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 and were confirmed pregnant at pregnancy control (palpation/ultrasonography) about six to eight weeks later, while 2 others might have been pregnant, but encountered a P4 fall between day 40 and 50 after insemination, which was before the pregnancy control. 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 [26,39]. 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 detected with the PMASC system to the LH surge 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 heat attention. Therefore, it would be better to have an indicator which is equally predictive, but less dependent on the sampling. The PMASC system is, like the HN system, an on-line system, running automatically each time a new measurement becomes available. Likewise, the comparison of both systems (HN and PMASC) is based on the same sampling frequency and milk samples. The only difference between both is the measurement technique of milk P4, which is for the HN a lateral flow technique, while the samples for PMASC are measured using ELISA.

One of the research questions of this study was whether the relation of P4-based heat attentions with the LH surge (and with that, moment of ovulation) could be improved by developing model-dependent rather than data-dependent guidelines through the PMASC system. In that way, it might be possible to optimize the advised insemination window by (partially) decoupling the generation of the attentions from the sampling frequency and luteolysis rate, which makes the monitoring system more robust. To this end, it was evaluated how well different characteristics of the final mathematical model related to the moment of the LH peak. In Figure 3, this relation is shown for the 15 RHP_S, where 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.

[FIGURE 3]

As described above, the moment of luteolysis 'L' detected by the PMASC system relates good to the LH surge, resulting in a range and SD on TI_L of respectively of 36 hours and 12.2 hours. Figure 3 and Table 3 also show that both IP, Tb_{85}, $Tmod_{10}$ and $Tmod_7$ perform similarly (range TI_X of 33 – 35 hours, SD 11.1-11.4 hours). With maximal sampling frequency, the IP does not diverge more than a few hours from the moment of detected luteolysis, explaining their relation. 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 taking into account the drop compared to the absolute maximal P4 concentration, resulted in a lower TI range 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 (SD 11.1 hours). 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; and (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 [40]. The latter 3 topics are important subjects for further research.

In this study, we chose to investigate all systems simultaneously, limiting the number of cases monitored. However, including all these systems is also the strength of this study, as a very fair comparison could be made between them in a commercially representative setting of the modern, medium-sized dairy farm. Of course, the limited number of cases and the fact only one farm was included in the study bring along that no steady claims can be made on e.g. optimal insemination time based on detection attentions, or the variability of these results in genetically different herds. Therefore, more data should be collected, which is unfortunately both time consuming, labor intensive and expensive. The current smaller type of study allows to place larger studies, which typically only evaluate one heat detection system in absence of a good reference for ovulation into perspective, and ultimately, to summarize optimal guidelines for e.g. insemination based on a meta-analysis. We therefore identify the need for additional research including both estrus detection tools and a good reference for the moment of ovulation.

3.3 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 [11,41,42]. This was confirmed in our study. For example, the three cows treated for a luteal cyst all showed a clear LH surge within the sampling period. Of those three, one had the LH surge 63 hours after luteolysis (detected with PMASC) which is comparable to the normal cows. The LH surges of the two other cows were 45 and 111 hours after luteolysis. For one 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 three 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 one 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.

4. 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 also investigated the time interval between heat attentions generated by different detection methods and the moment of the LH surge, which has been reported to occur about 26 hours before ovulation. The strength of this study is the simultaneous analysis of four different heat detection methods on one and the same herd and study period. Although an almost perfect relation 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 did not relate well with 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, SD 16.1 hours). P4 based system indicate luteolysis well before ovulation, and resulted in a very high sensitivity. Attentions generated by the HN system were followed by an LH surge after 21.6 – 66.4 (range of 44.8 hours, SD 11.4 hours). Accordingly, the relation 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 SD 11.1 hours) 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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supplying additional P4 sticks for the HN system and modifying the HN software to take samples at every milking event.

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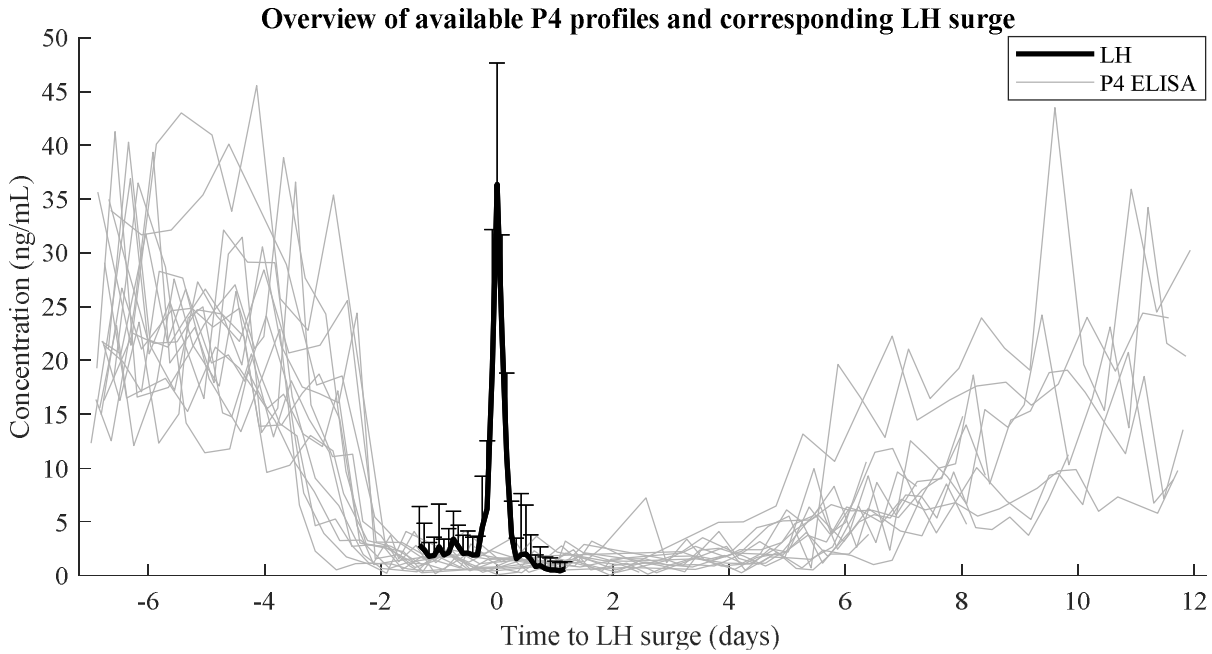
Figure 1. Overview of the P4 profiles centered around the LH surge for the 15 cows selected for this study. A large variability in the P4 profiles is noted both before and after the LH surge, but all cows had an active *corpus luteum*, reflected in a P4 concentration higher than 5 ng/mL within 7 days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

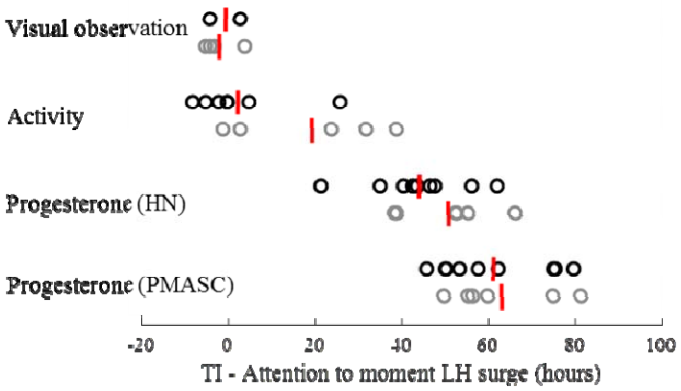
Figure 2. Visual observations (VO) have the smallest range (9 hours) in time interval (TI) from attention to the moment of the LH surge, but are very insensitive (6 out of 15 detected). Activity attentions have the largest range (47 hours) and thus perform the poorest in relating to the LH surge. The 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



ADRIAENS, FIGURE 2



ADRIAENS, FIGURE 3

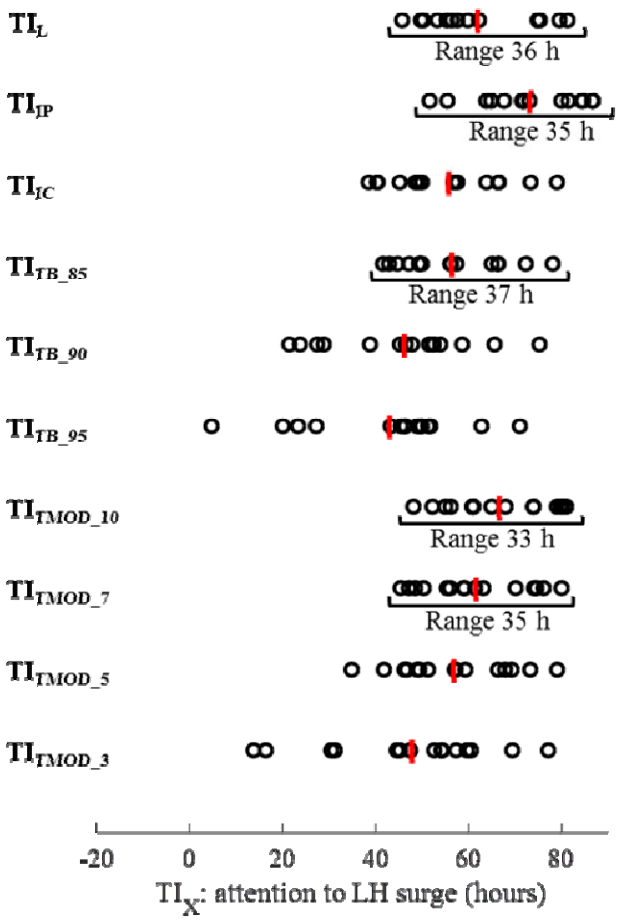


Table 1. Overview of the number of attentions, false alarms, sensitivity and PPV of the heat detection systems over the 46-day monitoring period

	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 (15)	0	100% (100%)	100.0%
PMASC	35+7* (15)	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.

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