The relation between first oestrus postpartum and reproductive disorders 4 weeks postpartum in dairy cattle

Research project 'Sense of Sensors in Transition Management'

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Abstract

Fertility problems are one of the main culling reasons on dairy farms and therefore, there seems to be room for improvement. Generally, the first heat postpartum (FH) is not important to a farmer, due to the voluntary waiting period a farmer chooses. But in addition to focussing on the first heat after this waiting period, it might also be worthy to put more emphasis on the resumption of cyclicity after calving much earlier. Diseases such as reproductive disorders (RDs) can be the cause for a delayed resumption of cyclicity and therefore, a relatively late first ovulation postpartum. At the same time, more and more sensors are developed to detect oestrus in dairy cattle, for example the pedometer. Perhaps, the pedometer could be used to detect cows with a RD in an early stage. Early diagnoses can lead to a fast and adequate intervention and therefore less costs due to decreased calving to insemination intervals and thus shorter calving intervals for example. The objective of this study is to investigate if there is a relationship between the moment of FH, measured with a pedometer, and various RDs (such as clinical endometritis, metritis, decreased tone and involution of the uterus and cystic ovaria). Furthermore, to investigate if it is possible to use the calving to first heat interval (CFHI) to predict the manifestation of RDs. In this experiment 605 cows (138 primiparous and 467 multiparous), of eight different farms were equipped with a Nedap Smarttag Leg pedometer. The average CFHI is defined for cows with the various RDs. A significant relation was detected between cystic ovaria and the CFHI (P=0.005). Contrary to what was expected, cows with a cystic ovarium had a 1.49 times shorter CFHI (mean 17,62 days) compared to cows without a RD (mean 30,88 days). Furthermore, the difference in the CFHI between the participating farms, was significant (P=0.000). Further research is needed to determine if the FH, detected with a pedometer, is an accurate predictor of RDs. However, the outcome of this study is a promising result, regarding the use of the pedometer in this area.

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Introduction

Reproduction problems are one of the main culling reasons on dairy farms (Ahlman *et al.* 2011, Ansari-Lari *et al.* 2012), with a percentage of 20,9% in the Netherlands (Zijlstra *et al.* 2016). Thus, there seems to be room for improvement regarding fertility. Generally, the first heat postpartum (FH) is not important to a farmer, due to the voluntary waiting period a farmer chooses. But it may be beneficial to pay attention to the resumption of cyclicity after calving much earlier. An early start of cyclicity increases the chances of early insemination after calving, shortens the interval from calving to conception and increases the conception rate (Petersson *et al.* 2006). Reproductive disorders (RDs) may negatively affect the resumption of cyclicity (as described below) and therefore, RDs should be diagnosed and treated as soon as possible.

Nowadays, more and more sensors are developed and used in livestock farming, for example the pedometer for heat detection. Perhaps, the pedometer can be used to visualize the resumption of cyclicity postpartum (pp) and can be a useful tool for early diagnosis of RDs. Early diagnoses of RDs can lead to a fast and adequate intervention and therefore less costs due to decreased calving to insemination intervals and thus shorter calving intervals for example.

Resumption of cyclicity and reproductive disorders

The calving to first ovulation interval has been investigated in several experiments. In these studies, different results are reported, with a calving to first ovulation interval varying from 16.1 to 30.9 days (Beam and Butler 1998, El-Din Zain *et al.* 1995, Sakaguchi *et al.* 2004). Galvao *et al.* (2010) reported that in their experiment only 25,6% of all cows (N=445) started cycling within 21 days in milk. These cows had better insemination and pregnancy rates, and lower odds of getting subclinical endometritis, compared to cows which started cycling after 21 days in milk.

During the last 20 to 25 days of gestation there is no follicular growth because of the high progesterone (P4) level which suppresses the follicle stimulating hormone (FSH) rise. In the period around parturition, the oestrogen and P4 levels will reduce to basal levels. Within 3 to 5 days pp the FSH concentration rises and within 7 to 10 days pp the first dominant follicle is formed (Crowe 1998). In addition to the availability of insulin-like growth factor 1 (IGF-1) and the size of the dominant follicle, an increase of the luteinizing hormone (LH) pulse frequency is needed to induce the ovulation of a dominant follicle. The latter is the major driver for the ovulation to take place (Austin 2001). Ovulation of the first dominant follicle occurs in 50% to 80% of the dairy cows (Crowe 2008). In case of the absence of ovulation, the first dominant follicle undergoes atresia or becomes cystic (Galvao *et al.* 2010, Crowe 2008, Beam and Butler 1998). Savio *et al.* (1990) concluded that cows had a longer interval to first ovulation in case they developed cysts (58.2 +/- 23.5 days). Cows which did not develop cysts ovulated at day 12 (+/-2.5) pp (Savio *et al.* 1990).
Dourey *et al.* (2011) investigated the effect on the length of the calving to first ovulation interval in case of a high amount (>8%) of polymorphonuclear (PMN) cells in the uterine lumen (indicating subclinical endometritis), compared to a low amount (<8%) of PMN cells. The calving to first ovulation interval was significantly shorter in case of a low amount of PMN cells. Overall, a higher percentage of ‘low PMN cell cows’ ovulated within 25 days pp (45%), compared to the ‘high PMN cell cows’ (11%). Peter *et al.* (1990) reported that the presence of endotoxins of E.coli (a well-known agent in metritis and endometritis) blocks the synthesis of oestradiol in the first dominant follicle, resulting in a failure to obtain a preovulatory LH surge (Kassé *et al.* 2016). Sheldon *et al.* (2002) support this, they reported a decreased oestradiol secretion and a slower dominant follicle growth in case of a high bacterial contamination.

These results all indicate a relationship between uterine disorders and the first ovulation pp, and that RDs can be the cause of a delayed resumption of cyclicity pp. Therefore, ovulation of the first dominant follicle pp may be a good marker for a successful transition period with good uterine health (Galvao *et al.* 2010, Petersson *et al.* 2006).

Expression of oestrous behaviour during the first ovulation pp

The ovulation of the first dominant follicle is mostly called silent. During a silent ovulation, a low intensity of oestrous behaviour is seen, this makes it more difficult to visually detect this ovulation (Shipka 2000). It is assumed that for a maximal expression of oestrous behaviour, P4 of the first corpus luteum (CL) pp is needed to optimize the sensitivity of the brain to oestradiol (Allrich 1994). The first ovulation after calving occurs after a period with low P4 concentrations, therefore generally less oestrous behaviour is seen during this ovulation. Another theory suggests that GnRH is the key factor in regulating oestrous behaviour (van Eerdenburg 2008). It is important to think about the definition of silent ovulation. It's possible that the oestrous behaviour did occur, while the observer/farmer missed it, or there is less expression of oestrous behaviour. Ranasinghe *et al.* (2010) determined the incidence of silent ovulations occurring within 90 days pp, based on P4 profiles (indicating the day of ovulation) and walking activity measured with a pedometer. Silent ovulation was defined as an ovulation without an increase in walking activity (<80% compared with the mean activity) around the day of ovulation. The incidence of silent ovulations at first ovulation pp was 55,2%. Shipka (2000) reported an incidence of 94,7% silent ovulations at first ovulation pp in case of visual oestrus detection, verified with P4 profiles. Thus, pedometers seem to be more accurate. Therefore, and because of the potential difficulty to visually detect a silent ovulation, an automated oestrus detection method (pedometers) is used to detect the FH in this study. Oestrous behaviour will be detected and not the ovulation itself.

Heat detection with a pedometer

Increased walking activity is one of the behaviours a cow will show during oestrus (Roelofs *et al.* 2005, Schofield *et al.* 1991, Firk *et al.* 2002, Kiddy 1977). In 1991 Shofield *et al.* (1991) already called the activity rate a promising parameter to detect the oestrus. Kennedy and Ingalls (1995) investigated the accuracy of pedometers in tie-stall housed dairy cows. They reported a detection rate of 65%. Roelofs *et al.* (2005) concluded that pedometers can accurately detect the oestrus and can be used to predict the time of ovulation. The sensitivity ranged between 51% and 87%. In case more than one cow was in oestrus at the same time it reached 95%. The oestrus was validated with visual observation and the ovulation was validated with ultrasound examinations (the disappearance of the preovulatory follicle marked the ovulation). An increase in number of steps was defined as oestrus if it occurred for two consecutive periods of two hours. The actual ovulation occurred 29.3 +/- 3.9h after the onset of the increased walking activity (Roelofs *et al.* 2005).

It is assumed that P4 tests in milk or blood are the most accurate way to detect ovulations. But in the current study P4 tests are not an option because of the broad design and the large number of farms and animals included. Firk *et al.* (2002) compared several studies which investigated the sensitivity of pedometer systems, used for heat detection. The detection rate varied between 68% and 100%, depending on the threshold being used. Galon (2010) described a wide range of heat detection results with pedometer systems in Israel. He suggests that the settings of the pedometer system should be adjusted to the specific farm conditions. Herd size, walking distance and milking frequency for example, all effect the results of the pedometer system (Galon 2010).

Objectives

According to various reports, uterine diseases may cause the failure of ovulation of the first dominant follicle pp, and consequently delay the resumption of cyclicity. The aim of this study is to determine if there is a relationship between the moment of FH, detected with a pedometer, and the incidence of RDs (like metritis, clinical endometritis, ovarian cysts, and abnormal involution or tone of the uterus). Furthermore, to investigate if it is possible to use the calving to first heat interval (CFHI) to predict the manifestation of RDs. We hypothesize that there is a correlation between RDs and the moment of FH, detected with a pedometer. Cows without a RD ('healthy') at week 4 pp will have a shorter CFHI than cows with a RD.

Methods

Farms and animals

Data is collected from eight free-stall housing dairy farms spread throughout the Netherlands, including six Holstein-Friesian herds, one herd of Fleckvieh cows and one rotational crossbred herd. All farms have slatted floors and cubicles. More information about the various farms is shown in table 1. The participating farms were considered as average farms, regarding the production and fertility results. From November 2016 till September 2017 each cow in its transition period was used for this study. Two farms were added to the project in May and June 2017. A total of 605 cows was used for the data analysis as show in table 4 (results), containing 1st till 13th lactation cows. The animals were followed during the complete transition period, from the beginning of the dry period until 8 weeks pp. Once a week the farms were visited.

Farms	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number of lactating cows[1]	167	179	204	128	148	140	107	127
Breed	Holstein-Friesian	Holstein-Friesian	Fleckvieh	Holstein-Friesian	Holstein-Friesian	Holstein-Friesian	Rotational crossbred	Holstein-Friesian
Milk production, rolling herd average[1] (Kg)	8474	10024	8911	9307	10755	9523	8866	8995
Milking system Conventional = C Robot = R	C	R	C	C	R	C	C + R	C
Bedding	Rubber mats	Deep litter bedding	Deep litter bedding	Rubber mats	Deep litter bedding	Rubber mats	Rubber mats	Deep litter bedding
Pasture grazing	Yes	No	Yes	Yes	No	Yes	No	Yes
Expected calving interval[2] (days)	395	397	376	402	403	402	392	403
Interval calving to first insemination[2] (days)	73	80	60	82	82	83	77	72
Number of inseminations per inseminated cow[2]	2.39	2.16	2.44	2.00	2.30	2.24	1.75	2.48
Start data collection for this research project	November 2016	November 2016	November 2016	November 2016	November 2016	June 2017	May 2017	November 2016

Table 1: *General information about the participating farms. [1]Based on the rolling herd average, determined at September 2017. [2]Based on the management program calculations made in September 2017, covering a full year.*

Heat detection

All cows were equipped with a Nedap[1] Smarttag Leg pedometer on a front leg, with which the walking activity was measured. An antenna attached in the stable, received the information of the sensor and displayed it on a computer. The FH was detected, using the walking activity and the algorithm created by Nedap[1], as explained below:

There is no fixed threshold for a heat alert, because not every cow is equally active. First a determination should be done to evaluate the variation in the normal activity of an individual, wherefore the activity of a certain period can be compared with the expected activity.

This was done by dividing a day in periods of two hours. Of every two-hour-period, the number of steps was measured. The mean and the standard deviation (SD) of the same two-hour-period in the previous 10 days were defined. Then the activity of a certain period minus this mean, is divided by the previously mentioned SD, the outcome is the increase factor. There is an increased activity during this period if the increase factor is >1.5. A heat attention ('1'-alert) is given if the increase factor is >1.5 for three consecutive two-hour-periods and if the mean of the increase factor of these three periods is >3.0. A suspicious attention ('2'-alert) is given if the increase factor is >1.5 for two consecutive periods. The latter is a more sensitive but a less specific method. Both methods are used in this study.

The above resulted in a daily-total data file with a summary of the heat alerts generated for a certain animal on a certain day between November 2016 and September 2017. The following alerts were used:

0 = No attention
1 = Attention
2 = Suspicious
-1 = Missing value

A missing value could be the result of a cow not wearing a pedometer. It is possible that the number of the sensor was not connected to the number of the animal in the animal identification system. Or the sensor had been out of reach of the antenna for more than 24 hours. The settings of the system were identical on every farm.

Reproductive tract examination

At week 4 pp (day 22-28) and week 8 pp (day 50-56), the reproductive tract was rectally palpated to check for uterine diseases, once a week. Both ovaria were palpated to assess the (in)activity, to check for cysts and to evaluate at which day of the cycle the cows were. Furthermore, the degree of involution and the tone of the uterus were determined. This examination was done manually and thereafter with use of an ultrasound scanner (Tringa Linear, Esaote Pie Medical), with a 7.5 MHz linear transducer. Based on the paper of Sheldon *et al.* (2006), but with a few adjustments, a differentiation was made between the following uterine diseases:

- Metritis: Animals which may or may not be systemically ill, have a fetid watery red-brown or a purulent vaginal discharge, within 21 days pp.
- Clinical endometritis (V): Animals with purulent vaginal discharge and/or mucopurulent or purulent fluids in uterine lumen, detectable after 21 days pp.
- Subclinical endometritis is not included in this study, because no cytology had been done.

[1] *Nedap Livestock Management., Groenlo, The Netherlands*

The possible outcomes of the reproductive tract examination are displayed in table 2.
No differentiation was made between a pyometra and clinical endometritis. Both were all classified as U=V. The reproductive tract examination was done by the same veterinarian every time.

Uterus (U)	S = clean, no fluids (mucous/purulent) in the uterine lumen and no vaginal discharge		V = purulent vaginal discharge and/or mucous or purulent fluids in the uterine lumen
Tone (TON)	+	+/-	-
Involution (INV)	+	+/-	-
Left ovary (LO)	A = active	I = inactive	C = cystic
Right ovary (RO)	A = active	I = inactive	C = cystic

Table 2: *Possible outcomes of the reproductive tract examination at week 4 and 8 pp.*

Further registration

Every week the farmers were asked if any abnormalities happened at their farms the previous week. Diagnosed, treated, dried-off, calving, culled or dead animals were noted. Visits from a veterinarian or a hoof trimmer, and the start of the pasture grazing season were noted. With this information, a disease registration could be made for each cow. An overview was created of the incidence of all diseases during the transition period. Furthermore, some false-positive heat attentions could be excluded with this information (described below).

At week 1 pp (day 1-7) and week 2 pp (day 8-14), once a week the cows were visually checked for vaginal discharge. If there was any white/yellow purulent discharge or fetid red/brown discharge this was defined as metritis in the disease registration. All observations and diagnoses mentioned in 'Further registration' were done by different people.

Data analysis

For data analysis, the computer program Excel 2016 was used. Of each cow, the calving date and FH attention was registered. The latest FH alert was at 187 days. If a cow had no FH attention during this experiment and if that cow was followed for less than 70 days, the cow was excluded from the experiment. A differentiation was made between FH attentions with only '1'-alerts and FH attentions with both '1'- and '2'-alerts included. In case of alerts on consecutive days, only the first alert was used in the analysis. This was considered as the start of the heat. Heat attentions from day 1 till day 7 pp were not taken in account. After calving, the cows were moved from the dry/calving group to the lactating group. During the first days in the new group, a higher activity can be expected because of the changing hierarchy. Animals which had a missing value ('-1') between day 8 pp and the FH attention, were excluded from the experiment. Days at which an unlikely large number of cows was in heat at the same time, were deleted. The cut-off value was set to the mean number of cows in heat plus 3× SD. If the consecutive two days (after the deleted days) counted more than the mean number of cows in heat plus 1× SD, these days were also deleted. The latter was done because the average number of steps of the previous 10 days contained the peak activity of the deleted day. Most of the time, the deleted days were the days at which the hoof trimmer came by or the cows went on the pasture for the first time this season. It was assumed that because of that (and not because of oestrous behaviour), the activity was increased. In total 39 days were deleted.
For each RD, the mean and the median of the CFHI was determined and compared to the CFHI of cows without any of the RDs ('healthy').

Statistical analysis

For statistical analysis SPSS statistics 24.0 was used. First a transformation had been done to create binary values of the various RDs, as shown in table 3. Also, a log-transformation of the CFHI (days) was done. The normality of the CFHI was checked using a q-q plot, as shown in attachment 1. An ANOVA-model was performed, to investigate which of the RDs affects the moment of the FH. The factor 'lactation' (primiparous or multiparous) and the factor 'farm' were also put into the ANOVA-model, to check whether these factors affect the moment of FH. A difference was said to be significant if $P<0.05$.

	Binary value	
	1	0
U	V	S
LO	C	A, I
RO	C	A, I
TON	-, +/-	+
INV	-, +/-	+
Metritis	Yes	No
Lactation	Primiparous	Multiparous

Table 3: *Transformation of the various RDs and the factor 'lactation', to binary values.*

Results

After exclusion of cows with missing data or a '-1'-alert, 605 cows remained for data analysis. Table 4 shows the exact number of animals used per farm, including the number of primiparous versus multiparous cows. The number of cows that were used at farm 7 and 6 was small, because these farms were added to the research project in May and June respectively.

Farm	1	2	3	4	5	6	7	8	Total
Excluded because of a '-1' alert or missing data	24	22	7	7	27	6	26	7	126
Used for the data analysis	89	94	134	96	77	27	10	78	605
Primiparous	17	13	36	32	12	11	1	16	138
Multiparous	72	81	98	64	65	16	9	62	467

Table 4: *Number of animals used in this study, per farm.*

Type of alert

Figure 1 displays at which day pp the FH generally occurred with only taken the '1'-alerts in account. Figure 2 shows the combination of '1'- and '2'-alerts. The '2'-alert is the most sensitive one, it could be that with this method, more cows with less expression of the oestrous behaviour or cows with a short oestrus are detected. Figures with only '2'-alerts are not shown because the specificity of the '1'-alert is higher and therefore in every '1'-alert lies a '2'-alert. Figure 2 shows the most realistic curve of the moment at which the FH takes place. Therefore, in all the following results, the method with the combined alerts was used. In the description of every following figure, the mean, SD, median and number of animals (N) is shown. The number of cows in table 4 and the number of cows in the figures can differ, because some cows which were in the experiment for at least 70 days didn't have a FH alert.

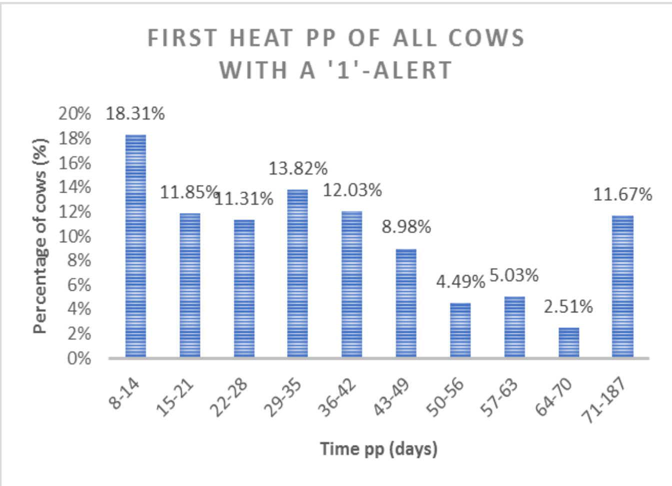

Figure 1: *FH of all cows with a '1'-alert. Mean=38.82; SD=27.65; median=33.00; N=557.*

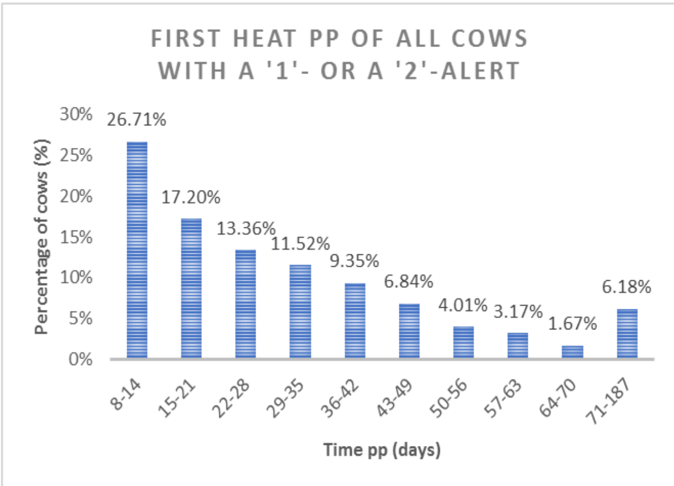

Figure 2: *FH of all cows with a '1'- or '2'-alert. Mean=31.11; SD=23.78; median=25.00; N=599.*

Descriptive analysis

RDs at week 4 pp

In table 5, the outcomes of the reproductive tract examination and the incidence of metritis are displayed. A single cow can be classified in several categories. That is why the total percentages in the last row are not shown. In total, 201 out of 605 cows (33.22%) were not diagnosed with any of the RDs ('healthy'). And 307 cows (50.74%) were diagnosed with 1 or more RDs ('unhealthy'). The remaining 97 cows (16.04%) were not scored for the tone and involution of the uterus and therefore could not be subdivided into the two groups mentioned above.

Primiparous cows had a much higher incidence of inactive ovaria (31.88%) compared to multiparous cows (14.56%). The incidence of cysts is slightly higher among primiparous cows. Multiparous cows have higher incidences of metritis, clinical endometritis, decreased tone (- and +/-) and decreased involution (- and +/-) compared to the primiparous cows.

RD \ Farms	1	2	3	4	5	6	7	8	Total	Total primiparous	Total multiparous
U = V	9	16	15	12	17	4	1	13	87 (14.38%)	12 (8.70%)	75 (16.06%)
LO = i	8	13	12	9	12	0	1	12	67 (11.07%)	20 (14.49%)	47 (10.06%)
RO = i	7	5	7	10	4	1	1	10	45 (7.44%)	24 (17.39%)	21 (4.50%)
LO = C	3	1	2	2	0	0	1	1	10 (1.65%)	2 (1.45%)	8 (1.71%)
RO = C	2	1	5	2	1	0	0	0	11 (1.82%)	3 (2.17%)	8 (1.71%)
TON -	6	4	6	4	6	2	0	7	35 (5.79%)	10 (7.25%)	25 (5.35%)
TON +/-	15	21	19	16	10	2	0	17	100 (16.53%)	14 (10.14%)	86 (18.42%)
INV -	4	5	6	5	1	2	0	5	28 (4.63%)	5 (3.62%)	23 (4.93%)
INV +/-	12	8	9	8	12	0	1	10	60 (9.92%)	8 (5.80%)	52 (11.13%)
Metritis	11	10	24	12	19	6	4	18	104 (17.19%)	22 (15.94%)	82 (17.56%)
Total	77	84	105	80	82	17	9	93	547	120	427

Table 5: *Outcomes of the reproductive tract examination per farm and in total, at week 4 pp. Metritis was diagnosed within 21 days pp. The percentages in the 'Total' columns are established by diving the incidences by the number of all cows (N=605), all primiparous cows (N=138) and all multiparous cows (N=467) respectively.*

The distribution of the CFHI of 'healthy' and 'unhealthy' cows are shown in figure 3 and 4. There is a small difference between the CFHI, comparing the 'healthy' and 'unhealthy' cows. Cows without a RD have a shorter CFHI (30.88 days) compared to cows with a RD (31.85 days).

Primiparous and multiparous cows with a RD have a mean CFHI of 30.85 and 32.13 days pp respectively (figure 6 and 8). Primiparous and multiparous cows without a RD have a mean CFHI of 25.23 days and 32.32 days respectively (figure 5 and 7). The difference between 'healthy' and 'unhealthy' primiparous cows is bigger (5.62 days) than the difference between the multiparous groups (0.19 day). 'Unhealthy' multiparous cows have a slightly shorter CFHI than 'healthy' multiparous cows.

The mean CFHI of the different groups can be influenced by cows with a very late FH. The median is more robust in presence of these outlier values. Although, when comparing the medians, the same conclusions can be drawn. The median of all 'unhealthy' cows (25.50 days) is 0.50 day higher than 'healthy' cows (25.00 days). Primiparous cows without a RD (19.50 days) have their FH 8 days earlier than primiparous cows with a RD (27.50 days). Multiparous cows with a RD (25.00 days) have their FH 3 days earlier than multiparous cows without a RD (28.00 days). No differentiation between multiparous and primiparous cows was made for the individual RDs, because of the relatively low number of primiparous cows (table 4).

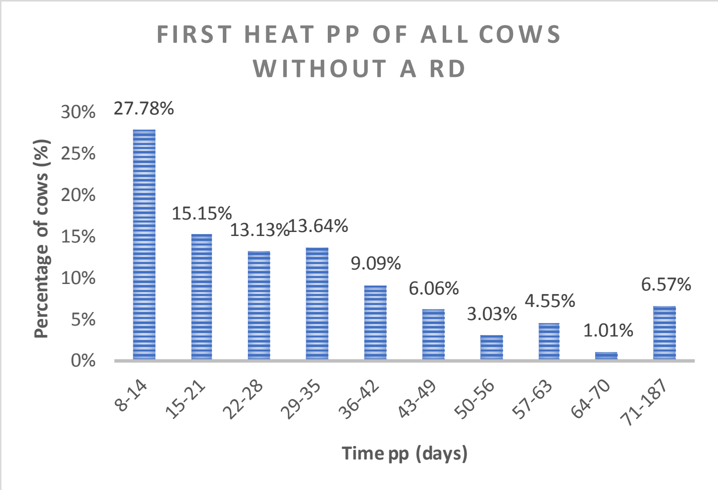

Figure 3: *FH of all cows without a RD at week 4 pp. Mean=30.88; SD=22.17; median=25.00; N=198.*

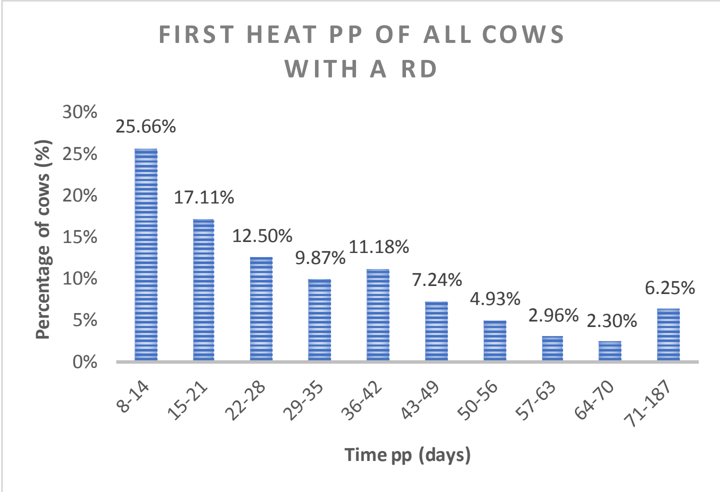

Figure 4: *FH of all cows with one or more RDs at week 4 pp. Mean=31.85; SD=23.83; median=25.50; N=304.*

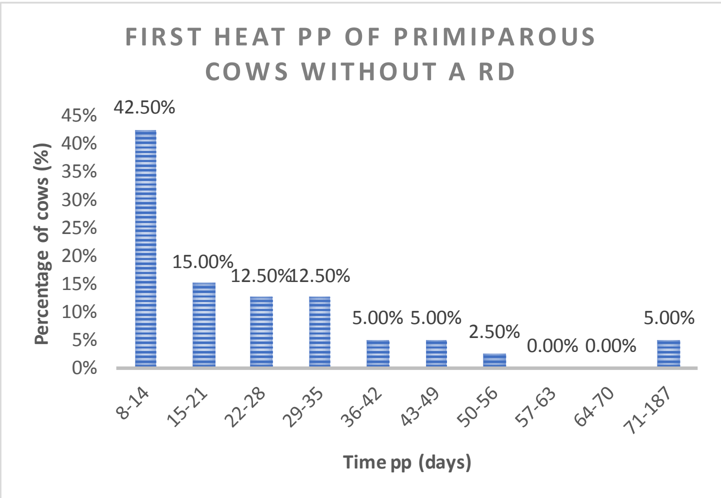

Figure 5*: FH of all primiparous cows without a RD at week 4 pp. Mean=25.23; SD=23.02; median=19.50; N=40.*

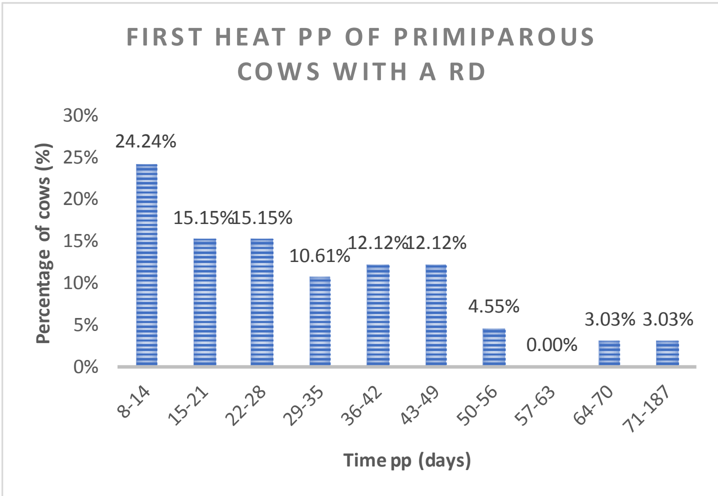

Figure 6: *FH of all primiparous cows with one or more RDs at week 4 pp. Mean=30.85; SD=22.92; median=27.50; N=66.*

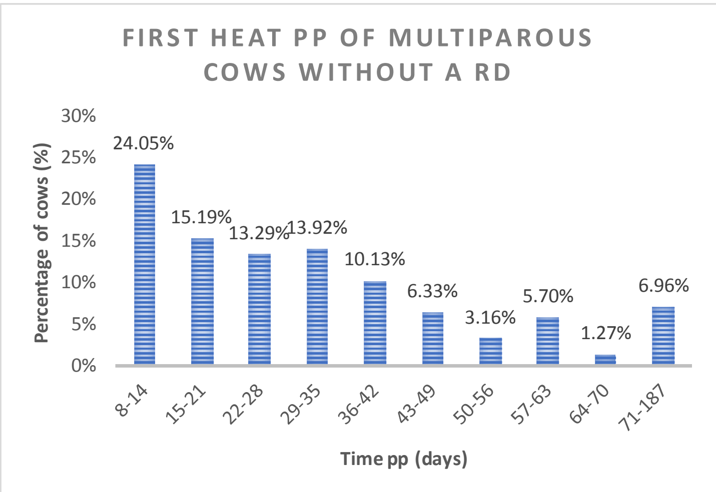

Figure 7: *FH of all multiparous cows without a RD at week 4 pp. Mean=32.32; SD=21.79; median=28.00; N=158.*

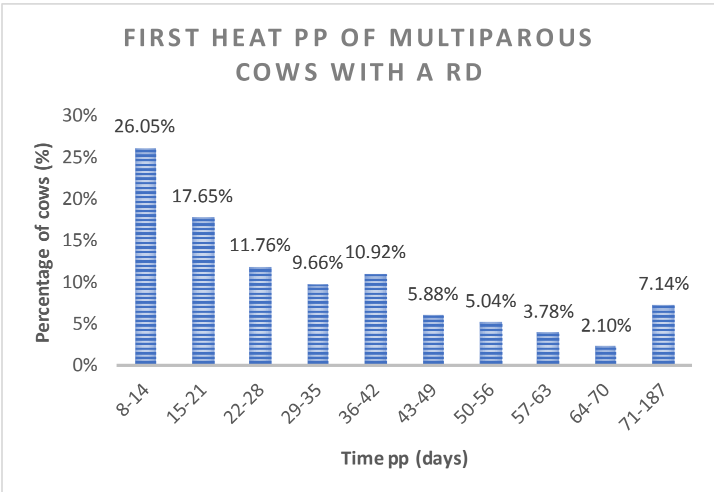

Figure 8: *FH of all multiparous cows with one or more RDs at week 4 pp. Mean=32.13; SD=24.11; median=25.00; N=238.*

Clinical endometritis

According to the descriptive statistics, the mean day at which cows with clinical endometritis (U=V) have their FH (figure 9) is higher than the FH of 'healthy' cows: 36.69 and 30.88 respectively, with a difference of 5.81 days. When looking at the median (28.00 days), the difference is 3 days, compared to 'healthy' cows (25.00 days). Still, 45 cows (52,33%) with clinical endometritis have their FH within 28 days.

Of the 87 cows with U=V, 44 cows (50.57%) also had a decreased involution of the uterus (– or +/-). And 29 cows (33.33%) with U=V, also had a decreased tone of the uterus (– or +/-).

Cystic ovaries

Of all cows with a cyst (LO or RO) at week 4 pp, 90.47% had their FH within 28 days pp (figure 10). The mean day was 17.62, this is 13.26 days lower than the 'healthy' cows (30.88 days). The difference between the median, of the CHFI of cows with a cyst (15.00 days) and 'healthy' cows (25.00 days), is 10 days.

No data analysis was performed on cows with inactive ovaria. Only 4 cows had two inactive ovaria at week 4 pp. The other cows with an inactive ovarium were still cyclic, because of the other active ovarium. Thus, compared to the cows without an inactive ovarium no difference was expected.

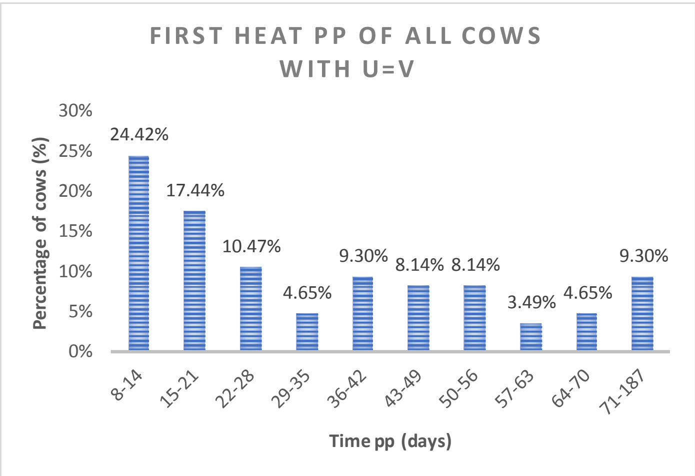

Figure 9: *FH of all cows with clinical endometritis at week 4 pp. Mean=36.69; SD=30.19; median=28.00; N=86.*

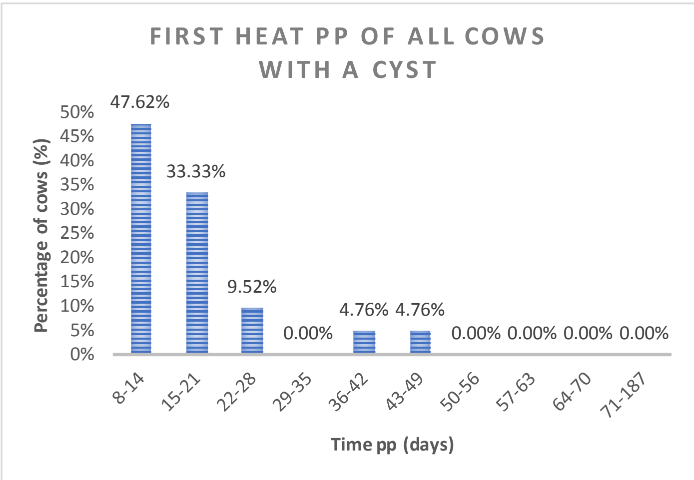

Figure 10: *FH of all cows with a cyst (LO or RO) at week 4 pp. Mean=17.62; SD=9.92; Median=15.00; N=21.*

Decreased tone and involution of the uterus

Because of the low number of cows with TON -, TON - and TON +/- were combined. For the same reason, INV - and INV +/- were combined. The mean day at which cows with a decreased tone of the uterus (- or +/-) had their FH is 31.66 (figure 11). This is 0.78 day higher than the 'healthy' cows (30.88 days). The median of the CFHI for cows with a decreased tone is 26.50, compared with 'healthy' cows (25.00 days) this is 1.50 day higher.

Cows with a decreased involution of the uterus (- or +/-) had a mean CFHI of 33.77 days, as shown in figure 12. This group had their FH 2.89 days later than the 'healthy' cows (30.88 days). The median of this group is 28.00 days, and the difference compared to the 'healthy' cows (25.00 days) is 3 days.

Cows with TON – and INV – (not taken the +/- score in account) showed doubtful results (table 6). The mean CFHI for cows with TON – (29.91 days) is lower than the mean CFHI of 'healthy' cows (30.88 days), while the median CFHI (26.00 days) is higher than the median CFHI of 'healthy' cows (25.00 days). The opposite applies to cows with INV -, with a mean CFHI of 30.89 days and a median CFHI of 22.00 days.

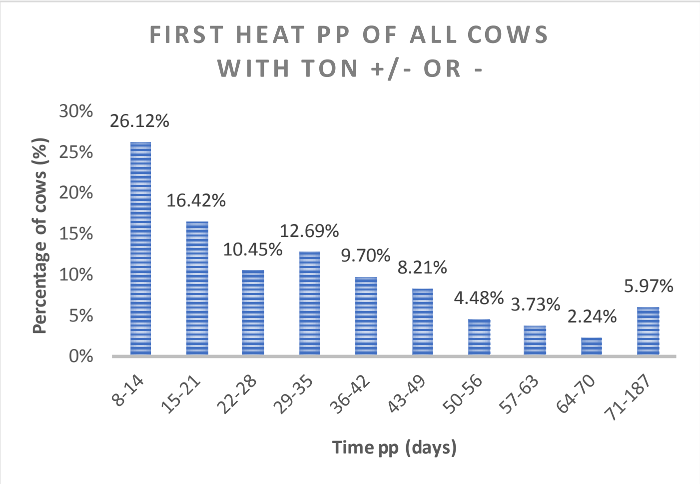

Figure 11: *FH of all cows with a decreased tone of the uterus at week 4 pp. Mean=31.66; SD=22.98; Median=26.50; N=134.*

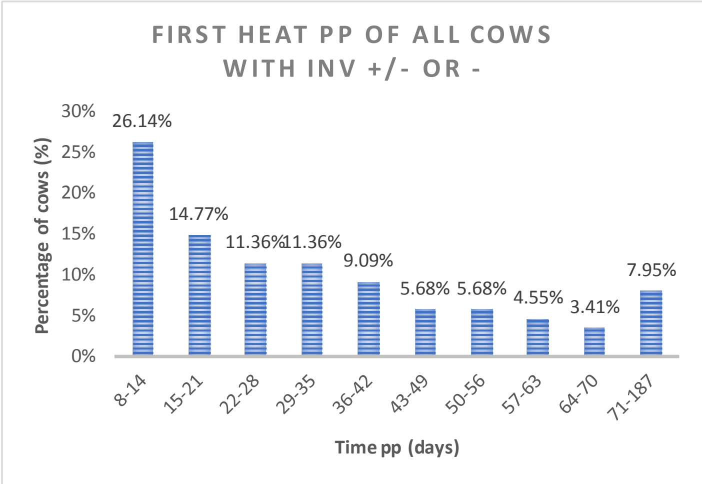

Figure 12: *FH of all cows with a decreased involution of the uterus at week 4 pp. Mean=33.77; SD=25.56; Median=28.00; N=88.*

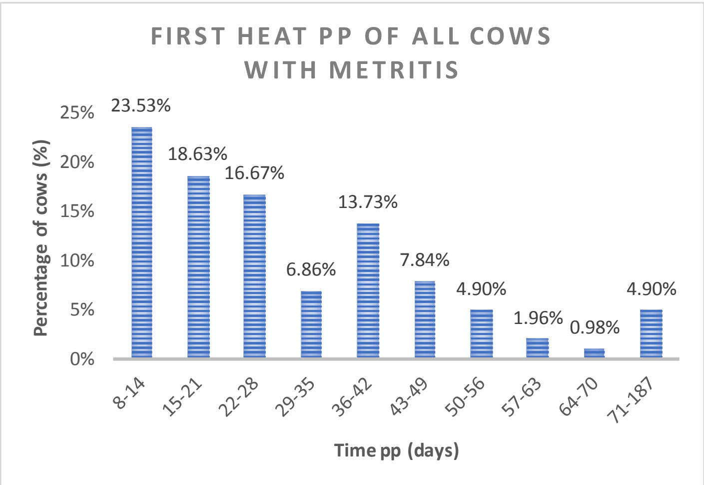

Figure 13: *FH of all cows with metritis. Mean=29.49; SD=18.97; Median=24.50; N=102.*

Metritis

Cows with metritis turned out to have a mean CFHI of 29.49 days (figure 13). This is 1.39 day lower than 'healthy' cows (30.88 days). The median CFHI of cows diagnosed with metritis was 24.50 days and differed 0.50 day with the 'healthy' cows (25.00 days).

Of the 103 cows with metritis (within 21 days pp), 20 cows (19.42%) developed clinical endometritis at week 4 pp.

A summary of the descriptive statistics, of the relationship between the CFHI and the various RDs on week 4 pp, is displayed in table 6.

RDs at week 8 pp

Only the RDs of week 4 pp were analysed. Not enough cows had an RD at 8 weeks pp which they did not already have at 4 weeks pp (table 7). The duration of the various RDs, was not the subject of this study.

FH per farm

A difference can be seen between the CFHI on the participating farms, as shown in table 8. The possible reasons for this difference will be discussed in the discussion.

	H	UH	H prp	UH prp	H mp	UH mp	U=V	Metritis	Cysts	TON -	TON -, +/-	INV -	INV -, +/-
Mean	30.88	31.85	25.23	30.85	32.32	32.13	36.69	29.49	17.62	29.91	31.66	30.89	33.77
SD	22.17	23.83	23.02	22.92	21.79	24.11	30.19	18.97	9.92	18.17	22.98	20.63	25.56
Median	25.00	25.50	19.50	27.50	28.00	25.00	28.00	24.50	15.00	26.00	26.50	22.00	28.00
N	198	304	40	66	158	238	86	102	21	35	134	28	88

Table 6: *Summary of the descriptive statistics of the CFHI, including the mean, SD, median and the number of animals used (N). H = 'Healthy'/without RD; UH = 'Unhealthy'/with RD; prp = primiparous cows; mp = multiparous cows.*

RD	Incidence
U = V	10
LO = i	14
RO = i	12
LO = C	4
RO = C	1
TON -	8
TON +/-	59
INV -	8
INV +/-	12
Total	128

Table 7: *Incidence of new RDs at week 8 pp compared to week 4 pp.*

	1	2	3	4	5	6	7	8	All farms
Mean	24.67	37.87	23.65	35.91	36.83	26.59	39.00	32.56	31.11
SD	17.16	23.69	16.77	27.42	31.49	15.16	37.88	23.11	23.78
Median	20.00	33.00	16.00	29.00	33.00	22.00	19.50	23.00	25.00
N	89	93	134	94	77	27	8	77	599

Table 8: *CFHI per farm, including the mean, SD, median and the number of animals used (N).*

Statistical analysis

The results of the ANOVA-model are shown in table 9. Step by step the least significant factor was removed from the test. Eventually, only the factor 'farm' and 'cyst' turned out to be significant, with P-values of 0.000 and 0.005 respectively, as shown in table 10. From the estimated marginal geometric mean (table 11), it can be concluded that cows with a cystic ovarium have a 1.49 times (e^(3.245-2.846)) shorter CFHI than cows without a cyst. In addition to the significant results, no tendencies to significance were found, regarding the other factors.

Tests of Between-Subjects Effects

Dependent Variable: ln-firstheat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22,671[a]	13	1,744	4,168	,000
Intercept	310,310	1	310,310	741,612	,000
Farm	16,751	7	2,393	5,719	,000
U=V	,374	1	,374	,894	,345
TON +/- or -	,138	1	,138	,329	,567
INV +/- or -	,069	1	,069	,164	,686
Metritis	,059	1	,059	,140	,709
Cyste	3,176	1	3,176	7,591	,006
Lactation	,723	1	,723	1,727	,189
Error	195,405	467	,418		
Total	5263,782	481			
Corrected Total	218,077	480			

a. R Squared = ,104 (Adjusted R Squared = ,079)

***Table 9**: Statistical analysis of the influence of the various RDs at week 4 on the CFHI.*

Tests of Between-Subjects Effects

Dependent Variable: ln-firstheat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	24,804[a]	8	3,100	7,610	,000
Intercept	656,161	1	656,161	1610,499	,000
Farm	20,534	7	2,933	7,200	,000
Cyst	3,176	1	3,176	7,795	,005
Error	232,233	570	,407		
Total	6196,472	579			
Corrected Total	257,037	578			

a. R Squared = ,096 (Adjusted R Squared = ,084)

***Table 10:** Final ANOVA-model of the significant factors 'farm' and 'cyst'.*

Estimated marginal means

Dependent Variable: ln-firstheat

Cyst	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
,00	3,245	,039	3,169	3,321
1,00	2,846	,142	2,567	3,125

***Table 11:** Estimated marginal means of the factor 'cyst'.*

Discussion

For this study, the following assumption had to be made: RDs diagnosed at week 4 pp affect the resumption of cyclicity from day 1 pp. Because the diagnosis of the various RDs was made at week 4 pp, nothing can be said about when the RD started to develop exactly.

Descriptive analysis

Based on the descriptive statistics, clinical endometritis, a decreased tone (-, +/-) and a decreased involution (-, +/-) seem to extend the CFHI. Clinical endometritis seems to have the biggest effect, with a mean difference of 5.81 days, compared to 'healthy' cows. Metritis seems to shorten this interval, although the difference is minimal (1.39 days). Stangaferro *et al.* (2016) and Liboreiro *et al.* (2015) found that postpartum activity of cows with metritis is reduced. This may have affected the ability of the sensor to detect the FH of cows with metritis. Cows with TON – or INV – (not taken the +/- score in account) showed doubtful results, probably because of the low number of cows (N=35 and N=28 respectively).

Primiparous cows had a shorter CFHI compared to multiparous cows. Even the 'unhealthy' primiparous cows had a shorter interval compared to the 'healthy' multiparous cows. Sakaguchi *et al.* (2004) found similar results: Primiparous cows (N=26) and multiparous cows (N=24) had a calving to first ovulation interval of 26.7 days and 35.5 days respectively. And more primiparous cows ovulated after one follicular wave (50%) than multiparous cows (33%) did. This difference in the number of follicular waves can explain why, in the current study, the mean CFHI of primiparous cows is shorter than the CFHI of multiparous cows. This is in contradiction with the experiment of Tanaka *et al.* (2008), where a CFHI of 31.8 days for primiparous cows and a CFHI of 17.3 days for multiparous cows was reported. Also, multiparous cows seemed to have less follicular waves. However, in this experiment only 16 cows were used and the biparous cows were classified into a separate group. Beyond the contradictory results it needs to be noticed that, if the pedometer will be used for prediction of diseases in the future, distinction must be made between primiparous and multiparous cows.

The fact that 50.57% of the cows with clinical endometritis also had a decreased involution of the uterus, is in accordance with El-Din Zain *et al.* (1995). They concluded that cows with puerperal complications had a shorter calving to uterine involution interval than cows without complications. Furthermore, they found that primiparous cows had a shorter calving to uterine involution interval. Which is also in accordance with our findings. The overall incidence of RDs is higher among multiparous cows than primiparous cows, except for the slightly higher incidence of inactive and cystic ovaria in primiparous cows. The total incidence of metritis is 17.19%, this is much lower than the 32% what Stangaferro *et al.* (2016) reported. And the incidence of clinical endometritis (14.38%) is much lower than the incidence of 27% what Potter *et al.* (2010) found. This could be the result of the few moments we checked the cows for metritis and clinical endometritis (and the other RDs). Through this, we could have made an underestimation of the real incidence.

Another thing to take in mind, is that cows can have multiple RDs at the same time. In the results of clinical endometritis for example, also three cows with a cyst were included. This may have affected the results. It can be an explanation for the fact that 'unhealthy' multiparous cows seemed to have a shorter CFHI than 'healthy' multiparous cows, because the cows with cystic ovaria may have lowered the mean CFHI.

Statistical analysis

The hypothesis of this study was that there is a correlation between RDs and the moment of FH. And it was expected that cows without a RD ('healthy') at week 4 pp will have a shorter CFHI than cows with a RD. The first part of this hypothesis is partly true. The only significant correlations found in this study were the relationship between the CFHI and cystic ovaria, and between the CFHI and the different farms. Based on the statistical analysis, the second part of this hypothesis can be rejected completely. Regarding the RD 'cyst', the results show the complete opposite. And, regarding the other RDs, no significant differences in the CFHI were found.
Cysts can suppress oestrous behaviour or can lead to excessive oestrous behaviour (Bierschwal *et al.* 1975, Savio *et al.* 1990). The latter can cause false positive heat alerts. It is possible that the cows with cysts show oestrous behaviour and get a heat alert, while they did not have an ovulation. This can explain the conflicting result in the current study compared to Savio *et al.* (1990), who reported a calving to first ovulation interval of 58.2 +/- 23.5 days in cows with cysts.

All farms were combined to test the hypothesis. In this way, it seems to be an adequate representation of the actual population of dairy cattle in the Netherlands. But it is also a disadvantage. The CFHI turned out to differ significantly between the farms. This could be the result of the different housing types, breeds, disease incidences, management systems, etcetera (Beam and Butler 1998, Roelofs et al. 2010). Thus, due to combining the farms, the number of variables will increase, this can lead to distorted outcomes in the descriptive analysis. The only farm with Fleckvieh cows (farm 3) turned out to have the shortest interval (Mean=23.65; Median=16.00; N=134) of all participating farms. This may be the result of a different oestrous expression between the breeds (Roelofs *et al.* 2010, Orihuela 2000). In the statistical analysis a correction was done to exclude the influence of the different farms. Therefore, the results from the statistical analysis can be generalized to other farms.

In this project, Nedap Smarttag Leg pedometers were used. The settings and the algorithm used in the Nedap sensors, are not necessarily the same as sensors of other companies used in livestock farming. Caution is required when extrapolating the results to farms with different types of sensors.

Recommendations for further research

In this study, the moment of first oestrus was detected instead of the actual ovulation. Cows with clear oestrous behaviour and therefore a clearly increased walking activity got a heat alert. Nevertheless, false positive and false negative heat alerts may have occurred.
For a more accurate detection of the first ovulation, P4 tests in milk or blood can be done. Then less false positive heat alerts, due to a higher walking activity at the beginning of the pasture season or due to visits of a hoof trimmer for example, can be expected. Or maybe in the future, it is possible to automatically delete these false positive alerts from the pedometer system.
Next to false positive, also false negative heat alerts can be expected with the current method. As mentioned above, heat expression can be reduced due to housing conditions, heat stress, breed differences, energy balance, lameness and other diseases (Beam and Butler 1998, Roelofs *et al.* 2010). For example, Liboreiro *et al.* (2015) found that cows with ketosis have reduced postpartum activity. Therefore, oestrous cows with ketosis may not be detected by the pedometer. While cows with diseases (RDs) are the cows we want to detect in this research. Furthermore, not every cow has the same duration of oestrous behaviour. A cow with a short expression of oestrous behaviour is easily missed with a method that is not sensitive enough. Another way to minimize the number of false negatives, next to P4 measurements, is to lower the average of 10 days used in the algorithm. In this study, the increased number of steps in a two-hour-period is compared with the expected activity of the previous 10 days. If this period of 10 days is shortened to 5 days for example, it will

make the algorithm more accurate. And, as mentioned in the introduction, the pedometer settings can be adjusted to each individual farm to get the most accurate results (Galon 2010).

Registration of the various RDs was done by the same person every time. Except for the diagnosis of metritis and all the parameters mentioned in ‘Methods - further registration’. This information was collected with help of the farmer, the local veterinarian and the students assisting in this research project. All these people have different ways of interpreting things. In the future, this should be done by a single person.
Furthermore, metritis is ideally diagnosed using cytology (Sheldon *et al.* 2006) instead of visual observation. If cytology can be done in the future, then the incidence of subclinical endometritis can also be determined. The incidence of subclinical endometritis was not included in this project.
To score the involution of the uterus it is more accurate to measure the diameter of the uterine horn, instead of the categorical score we gave de uterus using manual palpation. And besides that, to get a more realistic image of the real RD incidence and the onset of the various RDs, it is preferred to check the cows more often than once a week, starting from day 1 pp.

In conclusion, this research showed less relation between the FH and the various RDs than was expected. Many cows with a RD still had a relatively short CFHI. The difference in the CHFI between cows with a cyst and without a cyst was significant, but cows with this RD showed an even shorter CFHI compared to ‘healthy’ cows. Following the results of this research, it is difficult to say if the FH (detected with a Nedap Smarttag Leg pedometer) is a good predictor of RDs. But the fact that one of the RDs shows a correlation with the CFHI, is a promising result. Hopefully further research, with more accurate pedometer readings (preferably combined with P4 measurements) and a more precise RD registration, will contribute to a more realistic image of the opportunities of the pedometer in this area. Eventually, these data may be used in herd health management programs to improve the management during the transition period on dairy farms.

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Attachment

Attachment 1

Q-Q plot of the FH including ‘1’- and ‘2’-alerts, which shows a normal distribution.