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Estrus detection tools and their applicability in cattle: recent and perspectival situation

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Abstract

Good reproduction is key for successful dairy farming. Detection of estrus is the first step in getting a cow pregnant. Visual detection of estrus is a challenging job, to aid the farmer, estrus detection tools (EDT), such as pedometers, neck mounted collars to measure activity and pressure sensing devices to measure standing estrus, have been developed. EDT have proven useful in practical dairy farming, however, studies from the last five years reveal a great variation in sensitivity, specificity and positive predictive values. In research, the standard that is used to define a true estrus period can affect the performance of the EDT under investigation. Cow factors that can affect performance of EDT are number of ovulation after calving, milk production, lactation number, body condition score and lameness. The second step in getting a cow pregnant is insemination at the correct time. With EDT it is easier to determine optimal insemination time, which is 12 to 24 h before ovulation. The optimal time interval in which to inseminate seems to be about 5 to 17 h after an increase in activity as measured by pedometers or neck mounted collars. Novel measurements, such as rumination time, eating time, lying behavior, ultra-wide band technology to measure mounting and standing-to-be-mounted behavior and infrared thermography to measure temperature are being studied to further aid estrus detection.

Keywords: dairy cattle, estrus, insemination, technology.

Introduction

Good reproduction is key for successful dairy farming. Detection of estrus is the first step in getting a cow pregnant. Visual detection of estrus is a challenging job. The expression of standing estrus is only shown by about 50% of cows in estrus and lasts for a short period of time of about 5 to 7 h (Roelofs *et al*., 2005b; Sveberg *et al*., 2011). To aid farmers in detecting estrus and determining the optimal insemination time, many estrus detection tools (EDT) have been developed (reviewed by Roelofs *et al*., 2010; Saint-Dizier and Chastant-Maillard, 2012). For example, an increase in activity associated with estrus can be measured by pedometers or neck mounted collars and pressure sensing devices are on the market to detect cows expressing standing estrus.

A true estrus period can be detected by an EDT (true positive alert: TP) or not detected (false negative alert: FN). Outside a true estrus period, an EDT can give no alert (true negative alert: TN) or can give an alert (false positive alert: FP). To assess the performance of an EDT, sensitivity [TP/(TP+FN)], positive predictive value [TP/(TP+FP)] and specificity [TN/(TN+FP)] are often used (Roelofs *et al*., 2010). Rutten *et al*. (2014) concluded that an investment in activity meters for estrus detection is likely to be profitable for most dairy farms; however, this strongly depends on the increase in sensitivity that activity meters achieve, as compared with visual estrus detection. Although automated activity monitoring systems have proven useful as EDT in practical dairy farming (Michaelis *et al*., 2013; Neves and LeBlanc, 2015), studies from the last five years reveal a great variation in sensitivity and positive predictive values.

The second step in getting a cow pregnant is insemination at the correct time. The optimal time for insemination is 12 to 24 h before ovulation (Trimberger, 1948; Roelofs *et al*., 2006). Pedometers and neck mounted collars can be used to predict the time of ovulation (Roelofs *et al*., 2005a; Hockey *et al*., 2010) and therefore aid the farmer in deciding when to inseminate a cow.

In this review the performance and factors affecting the performance of different EDT will be discussed. Because this is elaborately reviewed by Saint-Dizier and Chastant-Maillard (2012), only studies performed over the last five years will be discussed in this review. The timing of insemination based on EDT and the effect on pregnancy rate will be discussed. New measurements that can aid in the detection of estrus will be reviewed.

Performance of EDT

Sensitivity and positive predictive value (PPV) varies between studies and EDT. In Table 1 the performance of different EDT is presented. Sensitivity ranged from 36 to 78% and is in all studies greater than the sensitivity of visual observations (range: 20 to 59%). PPV ranged from 74 to 97% and is not consistently better or worse thanvisual observations (Palmer *et al*., 2010; Holman *et al*., 2011; Michaelis *et al*., 2014). When pedometers were compared with neck mounted collars,

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sensitivity was greater but PPV was less for pedometers (Holman *et al*., 2011; Chanvallon *et al*., 2014). A better sensitivity means less false negative alerts, resulting in more detected true estrus periods. A better PPV means less false positive alerts, so less alerts are given when a cow is not in estrus. The design of the study can influence the number of false positive and false negative alerts. The number of FP and FN alerts as generated by the EDT depends on the definition of a true estrus period. Measurements of milk progesterone concentration are often used as golden standard for a true estrus period. Milk samples are collected 2 to 3 times weekly and a period of low progesterone, followed by a period of high progesterone, is considered to be a true estrus period. Based on individual progesterone profiles TP, FN and FP alerts from the EDT are assigned (Palmer *et al*., 2010; Holman *et al*., 2011; Kamphuis *et al*., 2012; Chanvallon *et al*., 2014). Using this definition for true estrus, cows that do not show any estrous behavior before ovulation (silent ovulation) will have more FN alerts resulting in lower sensitivities. This is not a malfunction of the EDT, but rather a physiological issue. Factors that can play a role in these FN alerts are discussed further on in this review. Another golden standard that is used for a true estrus period is the day of an insemination which led to a pregnancy (Jónsson *et al*., 2011). Using this definition silent ovulations do not generate a FN alert, because a cow is not inseminated when she is not detected in estrus at all. Sensitivity is likely to be greater when this golden standard is used. Michealis *et al*. (2014) used 21-day cow-periods according to the cycle length. After the voluntary waiting period, every cow started into a first 21-day cow-period and cows were observed for numerous cow-periods. When a cow was reported to be in estrus by visual observation or activity as measured by a neck mounted collar, estrus was confirmed by rectal palpation, ultrasonography and a blood sample for progesterone analysis. FN alerts were assigned when no alert was generated in a 21-day cow-period. Besides silent ovulations which generates a FN alert, the luteal phase can be prolonged or a cystic ovarian follicle can develop, which means that no ovulation occurs in a 21-day period (Lamming and Darwash, 1998). Therefore, an overestimation of FN alerts will probably occur by using 21-day cow-periods as golden standard. This might explain the low sensitivity found in the study of Michaelis *et al.* (2014) by neck mounted collars (36%).

Table 1. Sensitivity and positive predictive value (PPV) of different estrus detection tools (EDT).

References	EDT	Sensitivity %	PPV %	Housing	GS
Palmer *et al*., 2010	pressure sensing device	69	97	pasture	P4
	tail paint	65	94		
	VO[1] (3 times/day, 20 min.)	59	97		
Palmer *et al*., 2010	pressure sensing device	37	77	indoors	P4
	tail paint	26	92		
	VO[1] (3 times/day, 20 min.)	20	100		
Holman *et al*., 2011	neck mounted collar	59	94	indoors	P4
	pedometer	63	74		
	VO[2] (6 times/day, 10 min.)	57	93		
Kamphuis *et al*., 2012	neck mounted collar	78	78	pasture	
	tail paint	91	95		
					P4
Chanvallon *et al*., 2014	neck mounted collar	62	83	indoors	
	pedometer	71	71		
Michaelis *et al*., 2014	neck mounted collar	36	84	indoors	21dp
	VO[2] (2 times/day, 30 min.)	34	75		
Hockey *et al*., 2010	neck mounted collar	90	76	pasture	P4
Jónsson *et al*., 2011	pedometer	89	84	indoor	preg
Aungier *et al*., 2012	neck mounted collar	72	67	pasture	P4
Talukder *et al*., 2015	neck mounted collar	80	67	pasture	P4

VO = visual observation;[1]Visual observation of standing to be mounted;[2]Visual observation of vulva sniffing/being sniffed, chin-resting/being chin-rested on, mounting other cows and standing to be mounted, mucoid or bloody vaginal discharge; GS = golden standard for true estrus period; P4 = 2 or 3 times weekly milk sampling for progesterone concentrations; 21dp = 21-day cow-periods according to the cycle length; preg = confirmed pregnancy.

Factors influencing the performance of EDT

Different factors play a role in the number of false positive and false negative alerts generated by an EDT. It is clear that the threshold at which an estrus alert is generated by an EDT has a great impact on the sensitivity and PPV (Roelofs *et al*., 2005a; Hockey *et al*., 2010; Kamphuis *et al*., 2012). Physiological factors might also play a role in the performance of an EDT. Factors that decrease the expression of estrus will also decrease the sensitivity of an EDT when progesterone analyses are used as golden standard for a true estrus period. The first ovulation after calving is often not accompanied by an increase in activity or standing heat. Sensitivity of neck mounted collars for first ovulations after calving were found to be 23 and 30% in two studies (Aungier *et al*., 2012; Chanvallon *et al*., 2014). Sensitivity increased to 80% for second and later ovulations after calving. The same was found for estrus detection with pedometers (Chanvallon *et al*., 2014), where sensitivity for first ovulations after calving was 40% compared with 86% for subsequent ovulations. Ranasinghe *et al*. (2010) studied sensitivity of first, second, third and fourth ovulations after calving, which resulted in sensitivities of 45, 76, 79, and 89%, respectively. In normal, healthy cows, first ovulation occurs on average 28 days after calving (Johnson *et al*., 2012; Chanvallon *et al*., 2014). In practice, the voluntary waiting period is usually around 50 days. So, the low sensitivity of estrus detection found for first ovulations is not really an issue in practice. When however, many cows in a herd have an extended post partum anestrus, the performance of an EDT will be less. In interpreting and comparing research findings, it is important to take into account whether or not first ovulations were included in the calculations of sensitivity.

Lactation rank, milk protein content, body condition score, milk production, lameness and somatic cell count are studied for their effect on the performance of EDT. A high peak milk production as well as an above average daily milk yield and high production at the time of a preovulatory follicular phase were found to negatively affect sensitivity of neck mounted collars or pedometers. Sensitivity of neck mounted collars was 36% for cows with a peak milk production of more than 40 kg, whereas sensitivity was 68% for cows with a peak milk production of less than 35 kg (Chanvallon *et al*., 2014). Another study that investigated neck mounted collars and pedometers found a sensitivity of around 37% for cows with above average daily milk yield compared with around 60% sensitivity for all cows for both EDT (Holman *et al*., 2011). Aungier *et al*. (2012) concluded that if a cow was producing 10 kg less than another cow that was also in a preovulatory follicular phase, the odds of her preovulatory phase being detected by a neck mounted collar were greater by 67%.

Body condition scores of less than two resulted in a very low sensitivity for neck mounted collars (0%) and pedometers (20%; Holman *et al*., 2011). Only a few cows were in this category, so firm conclusion could not be drawn. Aungier *et al*. (2012) found that detection of a true estrus period by a neck mounted collar increased by a factor of 1.383 for each additional 0.25 BCS unit. No effect of somatic cell count (Holman *et al*., 2011; Aungier *et al*., 2012) on sensitivity of EDT was found. Milk protein content did (Talukder *et al*., 2015) or did not affect (Aungier *et al*., 2012; Chanvallon *et al*., 2014) sensitivity of EDT. Aungier *et al*. (2012) did not find a lower sensitivity in lame cows compared with non lame cows, whereas others did find a lower sensitivity in lame cows (Holman *et al*., 2011; Talukder *et al*., 2015). Lactation number did not affect sensitivity of neck mounted collars (Aungier *et al*., 2012) but did affect sensitivity of pedometers (Chanvallon *et al*., 2014). For cows in their first lactation, a higher sensitivity (77%) was found compared with cows with higher lactation number (52%). This is in agreement with the study of Roelofs *et al*. (2005a) in which cows in their first lactation had a longer duration of increase in activity and higher maximum steps during the increase in activity compared with cows with higher lactation number. Depending on the threshold calculation of an estrous related activity increase, a longer period of increased activity and more steps are more likely to give an alert.

Timing of insemination

To be able to give accurate insemination advice based on oestrus detection technologies, the parameters that are measured by the EDT to indicate onset of estrus should have a strong correlation with the time of ovulation and should be consistent between animals. A few studies have looked at the time of ovulation relative to the onset of estrus. The time of ovulation relative to the onset of estrus as measured by EDT is quite consistent between different studies. Intervals of 29.3 ± 3.9 h (n = 63 ovulations) and 30.2 ± 0.6 h (n = 20 ovulations) between the onset of oestrus based on pedometer measurements to ovulation were found in Holstein-Friesian and Japanese black cows, respectively (Roelofs *et al*., 2005a, Yoshioka *et al*., 2010). This agrees with the interval of 28.7 h (n = 60 ovulations) between onset of estrus based on neck mounted collars and ovulation in synchronized dairy cows (Valenza *et al*., 2012). An interval of 33.4 ± 12.4 h (n = 94 ovulations) was found between the onset of estrus as detected by neck collars and ovulation (Hockey *et al*., 2010). The interval between the first standing estrus as detected by a pressure sensing system and the time of ovulation was found to be 27.6 ± 5.4 h (n = 67 ovulations, Walker *et al*., 1996) and 29.0 ± 0.6 h in Japanese black cows (n = 20 ovulations; Yoshioka *et al*., 2010). The consistency in these intervals indicates that activity meters or pressure sensing systems can be used to predict time of ovulation and advise on optimal time of insemination.

The optimal time of insemination relative to ovulation was found to be 24 to 12 h before ovulation (Trimberger, 1948; Pursley *et al*., 1998; Roelofs *et al*., 2006).

In 1948 the a.m. - p.m. guideline for time of insemination was established. This guideline recommends that cows observed in estrus in the morning should be inseminated in the afternoon, and cows observed in estrus during the afternoon should be inseminated the following morning (Trimberger, 1948). Since then several studies have examined the optimal time for insemination relative to the onset of estrus as detected by an EDT (Table 2). Combining the optimal time of insemination relative to ovulation, with the time of ovulation after detection of the onset of estrus, will give an optimal estrus to insemination interval. Roelofs *et al*. (2005a) calculated this interval to be 5 to 17 h after the onset of increased activity as measured by pedometers. This interval is comparable to the interval found in other studies on pedometers, electric pressure sensing systems or neck mounted collars (Table 2). However, the interval that Hockey *et al*. (2010) found is noticeably different. Even though they found about the same interval between onset of estrus and ovulation as in other studies, the optimal time for insemination relative to ovulation was much later (16-0 h before ovulation). This could explain why the optimal interval between onset of estrus and insemination is later. The reason for this discrepancy in optimal timing of insemination relative to ovulation is not clear.

Table 2. Optimal insemination intervals after onset of estrus as detected by different estrus detection tools (EDT).

References	EDT	Optimal insemination interval after onset of estrus (h)
Maatje *et al*., 1997	Pedometers	6 - 17
Roelofs *et al*., 2005	Pedometers	5 – 17
Yoshioka *et al*., 2010	Pedometers	10 -18
Stevenson *et al*., 2014	Neck mounted collars	13 -16[1] 9 -12[2]
Hockey et *al*., 2010	Neck mounted collars	24 - 40
Dransfield *et al*., 1998	Pressure sensing device	4 -12
Xu *et al*., 1998	Pressure sensing device	12 – 18
Dalton *et al*., 2001	Pressure sensing device	12

[1]Primiparous cows; [2]Multiparous cows.

Stevenson *et al*. (2014) found a difference in optimal interval for insemination relative to the onset of estrus based on neck mounted collars, between primiparous and multiparous cows. In primiparous cows, inseminations between 13 and 16 h after onset of estrus resulted in the highest conception rates, where as in multiparous cows insemination less than 12 h after onset of estrus resulted in the highest conception rates. Primiparous cows had a longer lasting increase in activity as measured by pedometers compared with multiparous cows, but the interval between onset of increased activity and ovulation was not different (Roelofs *et al*., 2005b). This would mean that in primiparous cows, the interval in which an insemination results in comparable conception rates is larger than in multiparous cows. Consequently, insemination shortly before ovulation does not compromise conception rates in primiparous cows as it does in multiparous cows. A possible explanation could be a difference in quality and thereby the fertile lifespan of an oocyte. Primiparous cows have lower NEFA concentration after calving compared with multiparous cows (Wathes *et al*., 2007). Elevated NEFA exposure can compromise follicle growth and result in inferior quality oocytes (Van Hoeck *et al*., 2014). When the fertile lifespan of the oocyte is compromised, it is more important to have the sperm at the site of fertilisation ready when ovulation occurs. The difference in optimal insemination interval between primi- and multiparous cows is worth further investigation.

A study with sex-sorted semen in dairy heifers resulted in the highest conception rates for inseminations performed between 20 and 24 h after the onset of estrus as detected by a pressure sensing device (Sá Filho *et al*., 2010). Further research on optimal insemination intervals for heifers as well as for the use of sex-sorted semen is needed to optimise reproductive efficiency on dairy farms.

New measurements for estrus detection

Research on increased activity associated with estrus has already been performed more than 60 years ago (Farris, 1954). In the last five years, other measurements to aid in estrus detection have been studied. Among these new measurements are lying, eating and ruminating behaviour, feed intake, water intake, temperature measurements, body weight, sound and motion measurements. Jónsson *et al*. (2011) automatically recorded lying behavior as well as number of steps. True estrus periods (n = 18) were defined as periods around inseminations that led to confirmed pregnancy. Sensitivity was 50% when only lying behavior was used to detect estrus. A combination of number of steps and lying behavior, did not result in a higher sensitivity than using number of steps alone

(89%). Specificity was high for the number of steps (99.4%), lying behaviour (99.6%) and the combination (99.8%). PPV increased by 10% when lying behaviour was combined with the number of steps, so less false positive alerts were generated compared to using number of steps alone. Silper *et al*. (2015) studied lying and standing behaviour in heifers. An increase in activity (as measured by the number of steps) combined with ovarian ultrasonography was used to define a true estrus period. Both lying and standing behaviour differed on the day of estrus compared with non-estrus days. A large variation was found between heifers in both standing and lying measurements. Especially the length of the longest standing bout and its relationship with the time of onset estrus (as measured by increased number of steps) seems a promising aid in estrus detection. Measurements of lying behavior, standing behavior and number of steps can be combined in a sensor. The combination of these measurements is likely to result in less false positive alerts than measurement of increase in number of steps alone. This can lead to less inseminations performed on cows not in estrus.

Changes in rumination time around estrus have been studied in the last few years (Reith and Hoy, 2012; Reith *et al*., 2014a; Talukder *et al*., 2014; Pahl *et al*., 2015). One study found that measurement of rumination time alone or the combination of rumination time and activity did not result in a more accurate estrus detection performance than activity alone (Talukder *et al*., 2014). This finding does not agree with other studies that found that measurements of rumination time could aid in estrus detection (Reith and Hoy, 2012; Reith *et al*., 2014a; Pahl *et al*., 2015). In those studies rumination time was reduced by an average 20%, on the day before insemination (Pahl *et al*., 2015) or on the day of estrus as defined by activity measurements or visual observation (Reith and Hoy, 2012; Reith *et al*., 2014a). A >10% decrease in rumination time on the estrus day was found in more than 70% of the cows, whereas about 6% of the cows showed an increased rumination time on the estrus day. A high variation in the decrease of rumination time was found (Reith and Hoy, 2012). Feeding time and roughage intake decreased around estrus with approximately 20 and 10%, respectively (Reith *et al*., 2014b; Halli *et al*., 2015; Pahl *et al*., 2015). Concentrate intake was not affected by estrus (Reith *et al*., 2014b). Rumination time and eating time can be measured automatically by neck mounted collars (e.g. SCR heatime, Nedapsmarttag neck), but individual roughage intake is difficult to measure in practice. Therefore, measurements of rumination and eating time to aid in estrus detection are promising. More research on the factors affecting rumination and eating time and sensitivity, PPV and specificity, however, is needed.

Vaginal temperature increases before ovulation (Rajamahendran *et al*., 1989). Recently, studies were done to see whether infrared thermography could be used to detect estrus and predict time of ovulation. In one study, sensitivity of 75% was found with infrared thermography of the vulva and muzzle every four hours. This sensitivity was higher than the sensitivity with six times daily visual observations (67%). Specificity and PPV, however, were lower with infrared thermography (57 and 69%, respectively) compared with visual observations (86 and 89% respectively, Talukder *et al*., 2014). A study done by the same group in which eye, vulva and muzzle temperature were measured using infrared technology showed poor performance for detecting estrus (Talukder *et al*., 2015).

A novel approach to detect estrus is the use of ultra-wide band technology (UWB). This technology can measure 3-dimensional positioning and could be used to monitoring mounting and standing-to-be-mounted behavior. In a study, 9 out of 9 possible cows were detected in estrus automatically by UWB technology and 6 out of 6 cows were correctly identified as not in estrus (Homer *et al*., 2013). Roelofs *et al*. (2005b) found that 90% of cows in estrus showed mounting behavior, whereas only 58% of cows in estrus showed standing-to-be-mounted behavior. The first mount was displayed on average 29 h before ovulation. Automatic detection of mounting behavior could be a helpful tool in detection of estrus and determining optimal insemination time.

In conclusion

Performance of estrus detection tools varies between studies, but is overall better than visual observation of estrus. Taking into account factors that affect the performance of EDT such as first ovulations after calving, high milk production, lactation rank etc. and possibly adjusting the calculations for the threshold used to generate an alert might increase the performance of pedometers and neck mounted collars. Because the beginning of estrus is detected by the EDT, inseminations can be better timed, thus increasing conception rates. An interesting area of research is optimal insemination time in heifers, and when using sex-sorted semen. Other behavioural measurements, and measurements of physiological traits associated with estrus, are studied to aid in the detection of estrus and determining optimal insemination time. The combination of activity measurements and rumination, eating and lying time measurements seems promising.

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