Animal (2018), **12:2**, pp 398–407 © The Animal Consortium 2017
doi:10.1017/S1751731117001975

Review: Behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle

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(Received 23 August 2016; Accepted 8 July 2017; First published online 15 August 2017)

Efficient detection of estrus is a permanent challenge for successful reproductive performance in dairy cattle. In this context, comprehensive knowledge of estrus-related behaviors is fundamental to achieve optimal estrus detection rates. This review was designed to identify the characteristics of behavioral estrus as a necessary basis for developing strategies and technologies to improve the reproductive management on dairy farms. The focus is on secondary symptoms of estrus (mounting, activity, aggressive and agonistic behaviors) which seem more indicative than standing behavior. The consequences of management, housing conditions and cow- and environmental-related factors impacting expression and detection of estrus as well as their relative importance are described in order to increase efficiency and accuracy of estrus detection. As traditional estrus detection via visual observation is time-consuming and ineffective, there has been a considerable advancement of detection aids during the last 10 years. By now, a number of fully automated technologies including pressure sensing systems, activity meters, video cameras, recordings of vocalization as well as measurements of body temperature and milk progesterone concentration are available. These systems differ in many aspects regarding sustainability and efficiency as keys to their adoption for farm use. As being most practical for estrus detection a high priority – according to the current research – is given to the detection based on sensor-supported activity monitoring, especially accelerometer systems. Due to differences in individual intensity and duration of estrus multivariate analysis can support herd managers in determining the onset of estrus. Actually, there is increasing interest in investigating the potential of combining data of activity monitoring and information of several other methods, which may lead to the best results concerning sensitivity and specificity of detection. Future improvements will likely require more multivariate detection by data and systems already existing on farms.

Keywords: behavioral estrus, herd management, estrus detection, activity monitoring, multivariate analysis

Implications

Comprehensive detailed knowledge – considering cow-, environmental- and management-related factors – of behavioral signs of estrus is crucial for the refinement of (fully) automated technologies for identifying estrual cows. The primary focus is on activity monitoring to detect the significant increase in activity levels that occurs during proestrus. For further improvement of estrus detection there is increasing interest in studying the potential and the benefits of multivariate detection. A combination of activity monitoring and several other methods may lead to acceptable estrus detection rates and thus to optimize reproductive management in dairy farms.

Introduction

Detection of estrus is one of the most important factors impacting the reproductive efficiency in dairy cattle, especially in farms using artificial insemination (AI). Reproduction management directly affects the calving-to-conception interval, thus affecting the calving interval and milk production, which impacts profit. However, in several studies, researchers have reported a serious decline in fertility, occurring simultaneously with increased milk yields which can be attributed to the genetic selection for higher milk yields as well as nutritional and management factors. The relationship between milk yield and characteristics of estrus has been the subject of numerous investigations (Lopez *et al.*, 2004; López-Gatius *et al.*, 2005). Reports have demonstrated that variation in cycle length, duration and intensity of estrus has significantly increased (Kerbrat and Disenhaus, 2004).

Traditionally, estrual cows were identified by visual observation. As herd size increases, visual observation of individual cows is not practical within the available time of the herd manager, resulting in unobserved estrus and remarkable economic losses. Detection efficiency is often below 50% in dairy herds (Van Vliet and Van Eerdenburg,

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1996). Although poor reproductive performance causes the highest culling rate, few cows are described to be infertile. About 90% of the factors for low detection rates can be attributed to management and 10% to the cow (Diskin and Sreenan, 2000). Due to the high variability in duration and intensity of the expressed estrous signs among individuals and the great influence of a number of various factors, detection of estrual cows is still a major problem.

(Fully) Automated sensor-based technologies that continuously monitor and record detailed information about the cow have been developed and greatly refined to attenuate further reproductive declines.

The purpose of this paper is to review relevant estrus characteristics to gain detailed knowledge of behavioral alterations at the onset of estrus as a necessary basis for developing technologies to improve the reproductive management on dairy farms. Factors affecting estrus expression and its detection are described in order to improve the balance between sensitivity and specificity of detection. The most successful methods are based on automated estrus detection. Thereby, a high priority is given to detection based on sensor-supported activity monitoring and to the potential of combination of activity measurement and several other strategies to identify cows in estrus.

Behavioral signs of estrus

Ovarian functions (follicle development, ovulation, luteinisation and luteolysis) are regulated by endocrine hormones secreted by the hypothalamus (gonadotropin-releasing hormone (GnRH)), anterior pituitary (FSH and LH), ovaries (progesterone, estradiol and inhibin) and the uterus (prostaglandin $F2_{\alpha}$ ($PGF2_{\alpha}$)) (Aungier *et al*., 2015). Elevated concentrations of estradiol secreted by the pre-ovulatory follicle in turn promote a GnRH surge and allow – when progesterone levels are low – the expression of behavioral estrus and the release of LH to cause ovulation (Figure 1). Estrous behavior can be classified on the basis of primary and secondary signs.

Primary sign of estrus

In various studies, standing to be mounted was the primary and most characteristic external sign for determining when a cow is in estrus and considered sexually receptive for AI. But, an advancing decrease in the number of cows showing standing estrus is well documented (At-Taras and Spahr, 2001). In a number of previous studies, <50% of the cows stood to be mounted on the day of estrus (Peralta *et al*., 2005). The duration of estrus based on standing mounts averaged 7.6 mounts/cow with a mean duration of 4 s (Sveberg *et al*., 2013) and 8 to 9 h (Walker *et al*., 1996) but it could be <6 h in some dairy herds (At-Taras and Spahr, 2001). Duration is significantly affected by daily milk production. To characterize the relationship between milk yield and duration of estrus, Wiltbank *et al*. (2006) noted a correlation coefficient of $r = -0.51$. This may be the result of a lower serum estradiol concentration on the day of high-yielding cows' estrus (Lopez *et al*., 2004) due to increased metabolic clearance rate of steroid hormones (Wiltbank *et al*., 2006). Length of time during which high-yielding cows (⩾39.5 kg/day) expressed estrous signs was 6.2 h compared with the duration of 10.9 h in cows with lower milk yields (<39.5 kg/day) (Lopez *et al*., 2004). Peralta *et al*. (2005) found a significantly lower number of standing events for cows in the third lactation (5.6 ± 2.8) compared with those in the second (6.2 ± 3.5) and first lactations (9.2 ± 6.6). Over the past several years, there has been a clear trend toward the use of technological methods for accurate detection of estrus in dairy cattle. Mount detector are used to record standing events, whereas pedometers and accelerometers are based on detection of increased activity during proestrus. Table 1 demonstrates the differences in the length of time cows displaying estrus behaviors depending on the detection method. Duration

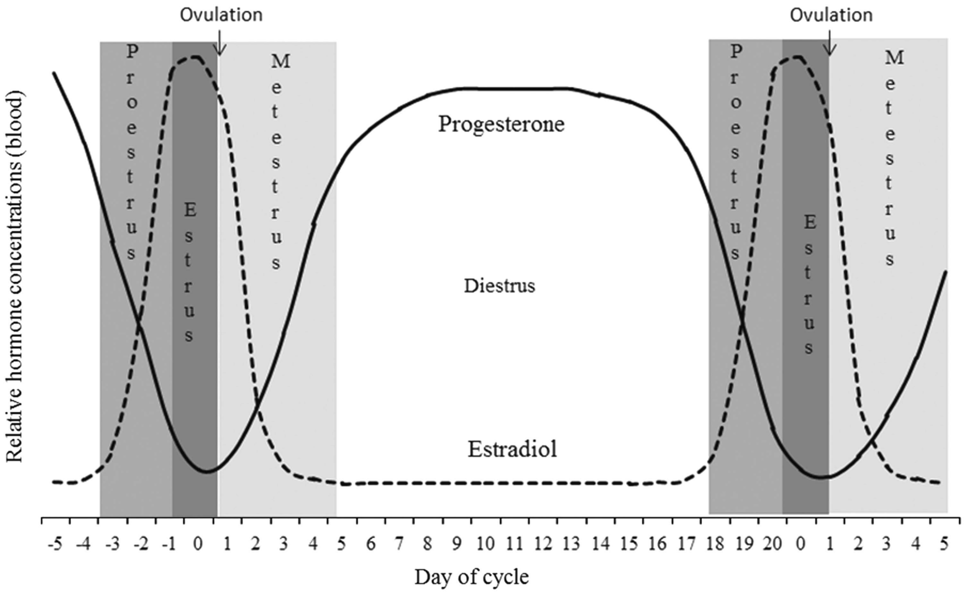

Figure 1 Hormone patterns of cow's estrous cycle, modified from Senger (2003).

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Table 1 *Mean duration of cow's estrus in dependence on the detection method and the housing type (References since 2000)*

References	Mean (h)	Detection method	Housing type
At-Taras and Spahr (2001)	5.83 ± 0.78 (SE)	HeatWatch	Free-stall barn/concrete flooring
Lopez *et al.* (2004)	8.7 ± 0.6 (SE)	HeatWatch	Free-stall barn/concrete flooring
Roelofs *et al.* (2005)	11.8	Pedometer	Free-stall barn/slatted floor
	10.0	Visual observation (30 min every 3 h)	
Valenza *et al.* (2012)	16.1 ± 4.7 (SD)	Accelerometer system	Free-stall barn
Aungier *et al.* (2015)	11 ± 1 (SE)	Accelerometer system	Free-stall barn/slatted flooring
Silper *et al.* (2015)	14.3 ± 4.1 (SD)	Accelerometer system	Free-stall barn/rubber flooring

and these behavioral symptoms of cows' estrus reveal a dependency on housing system and floor surfaces.

The number of standing mounts was significantly reduced under housed (52% of cows) than under pasture conditions (91% of cows) – irrespective of the detection method (Palmer *et al.*, 2010). Because not all estrual cows expressed standing estrus (Britt *et al.*, 1986), Kerbrat and Disenhaus (2004) focused on secondary signs to enhance detection of estrus.

Secondary signs of estrus

Mounting behavior. Researchers published many secondary symptoms of estrus which seem more indicative than standing behavior. According to studies of Yoshida and Nakao (2005) differences in secondary signs occurred on average 9.6 h before the onset of standing estrus and persisted until 18.4 h after the end of this primary sign. Mounting or attempting to mount other cows have a high frequency during estrus compared with other days (Kerbrat and Disenhaus, 2004). A significant increase in the frequency of mounting was detectable in the period between 6 to 1 h before and 3 h after standing estrus (Sveberg *et al.*, 2013). Mounting behavior was observed in 80% of the cows with an average number of mounts of 2.9 (Van Vliet and Van Eerdenburg, 1996). Front mounts were observed rather infrequently as Britt *et al.* (1986) found only 3.4% of the cows attempting to mount another cow from the front. In a recent report, the average duration of mounting estrus was 12.9 h (Sveberg *et al.*, 2013). The mean number of mounts – with a total standing time between 21.7 and 28.2 s – was lower for cows with milk production above than for cows with milk yields below the herd average (6.3 ± 0.5 *v.* 8.6 ± 0.5) (Lopez *et al.*, 2004). Because mounting activity was lowest in heifers (5.5 mounts/h) and increased to 7.9 mounts/h for cows in the fourth lactation, Gwazdauskas *et al.* (1983) suggested an association with sexual experience.

It is well known that the number of mounts per cow and the length of mounting revealed a significant dependency on housing conditions. According to Britt *et al.* (1986) floor type was the most important factor affecting estrous behavior. Cows showed a clear preference for mounting – 3- to 15-fold greater – and further secondary signs (butting, sniffing, licking, chin-resting) on soft than on concrete surface. Equally, the time during which cows displayed standing and mounting behavior was longer on soft than on concrete surfaces (13.8 *v.* 9.4 h) (Britt *et al.*, 1986). Mounting activity was markedly inhibited by slippery floors, especially in cows that previously sustained a fall when attempting to mount another cow during estrus (Palmer *et al.*, 2010). The climate in the livestock house, where temperature and relative air humidity are important factors, becomes an additional stressor. Expression of mounting activity was not inhibited as long as the maximum environmental temperature on the estrous day remained within the cows' thermoneutral zone. Beyond 30°C, as observed by Gwazdauskas *et al.* (1983), temperature negatively impacted the number of mounts. There was a reduction in LH secretion leading to suppressed synthesis of follicular steroids thus, reduced plasma estradiol concentrations contributing to impaired detection of estrus (De Rensis and Scaramuzzi, 2003). Use of artificial cooling methods including installation of shaded areas, fans, sprinkler systems allowed overcoming the detrimental effects of hyperthermia on fertility in dairy cattle, but the improvement of fertility did not correspond with normal winter fertility (De Rensis and Scaramuzzi, 2003).

Activity. Activity increases markedly in cows approaching estrus (e.g. Valenza *et al.*, 2012; Reith *et al.*, 2014a; Madureira *et al.*, 2015), indicating a reliable prediction of sexual restlessness. Cows were between 2.3 and 6 times (Redden *et al.*, 1993; Silper *et al.*, 2015; Gaillard *et al.*, 2016) as active at the time of estrus – mostly defined as day 0 – as when not in estrus. Compared with the day before estrus, the time spent walking increased by 342% with a range from 21% to 913% on the estrous day for each cow, lasting from 8 h before to 5 h after the onset of estrus (Kerbrat and Disenhaus, 2004). Activity – measured by an accelerometer system fitted on the neck collar of each cow – was 17 ± 1 movements/h higher at the time of estrus compared with the 5 days before the estrous day (Gaillard *et al.*, 2016). They further found more cows showing activity at the 8th than at the 2nd observed estrus (63.3% and 45.9%, respectively). Duration of activity episodes varied between 11 and 19.1 h (Table 1). Thereby, multiparous cows expressed lower intensity (Reith *et al.*, 2014a) and peak activity (Madureira *et al.*, 2015) as previously shown by López-Gatius *et al.* (2005) who calculated that each additional lactation number caused a 21.4% decrease in locomotion. Negative effects of high milk production on activity were reported by Yániz *et al.* (2006) and López-Gatius *et al.* (2005) who observed a

https://doi.org/10.1017/S1751731117001975 Published online by Cambridge University Press

decrease of 1.6% in walking activity when a cow's milk production increased by 1 kg. Referring to investigations of various researchers, activity correlates positively with most of the other behavioral symptoms including standing estrus, mounting behavior, chin-resting, sniffing, butting (Van Vliet and Van Eerdenburg, 1996; Roelofs *et al.*, 2005), as well as with the occurrence of another cow displaying estrous behavior. According to Yániz *et al.* (2006) expression of walking activity increased by 6.1% for each additional estrual cow. Most estrual cows were more restless between 0200 and 0800 h (Reith *et al.*, 2014a). But, in farms, cows expressing estrus at nighttime and in the morning hours could remain undetected by herd managers when only visual observation of estrus is used.

Hot climatic conditions are major factors depressing reproductive efficiency due to reduced duration and intensity of estrus and a larger range in cycle length contributing to low detection and pregnancy rates. Using pedometers for estrus detection Schüller *et al.* (2016) found a lower conception rate – cows were between 63% and 80% less likely to get pregnant – during long- and short-term heat stress independent of the type of semen employed compared with animals without stress. López-Gatius *et al.* (2005) detected a significantly lower increase in walking activity during the summer season (May to September) than that measured during the period from October to April (369 ± 152% *v.* 384 ± 156%). Similarly, an increase in mean relative humidity higher than 95% was associated with a decrease in walking activity at estrus (Yániz *et al.*, 2006). In correlation with activity, heat stress indirectly affected reproductive performance by reduced appetite and dry matter intake (DMI) which prolonged the period of negative energy balance (NEB) in early lactation (De Rensis and Scaramuzzi, 2003).

Rumination time. Cows spend about one-third of the day ruminating. Using a microphone-based system for automatic recording of cow's individual rumination time Reith *et al.* (2014a) observed an average daily rumination time of 442 min. The circadian rhythm of rumination time was found to be bimodal. Maximum levels were measured between 0200 and 0400 h and around noon between 1200 and 1400 h. Compared to behavior on non-estrous days cows initiating estrus showed a reduction of rumination time. Duration decreased gradually starting 2 days before the onset of estrus. The minimum level was identified on the estrous day, after which rumination time returned to base level again. On average, data of daily rumination time were reduced by 19.6% (83 min/day) with lowest values between 0400 and 1000 h on the day of estrus. These cows with less time spent ruminating at estrus were characterized by tendential higher activity. Rumination time during estrus was associated with average daily milk yield. Cows with a daily milk yield >40 kg exhibited the greatest decline compared with those with a production ⩽40 kg/day (Reith *et al.*, 2014a).

Besides rumination time, DMI and cow's water consumption which were automatically measured by troughs placed on an electronic floor scale are reduced at estrus. With a decline of on average 14.6%, most cows (85%) consumed significantly less dry matter of the forage ration – concentrate intake was not affected by estrus – during estrus in comparison with non-estrous days (20.4 *v.* 23.0 kg). Similarly, estrual cows drank less water. Water intake was reduced by 15.3% in 67% of all cows, with the lowest value determined on the day before estrus relative to the reference period (Reith *et al.*, 2014b).

Agonistic interactions. In the period of estrus, the cows are more motivated to involve in agonistic interactions than during di-estrus. Aggressive interactions were exhibited more intensively on the day of estrus than on all other days. With an incidence of 73.4% the most frequent agonistic behavior was head-to-head butting. Thereby, the number of butts was correlated positively with approach-walking (Kerbrat and Disenhaus, 2004) and pedometer readings, respectively (Van Vliet and Van Eerdenburg, 1996). Butting occurred at high incidence at the same time as mounting before standing estrus in the pre-ovulatory period (Sveberg *et al.*, 2011). Push-away behavior, during which the initiating cow pushes the receiving cow with its head, was the only agonistic behavior displayed relatively infrequently in estrual cattle (Sveberg *et al.*, 2011).

Social interactions. Chin-resting/chin-rubbing, sniffing/licking the anogenital region (vulva) of another cow and orientation are classified as social behaviors. Chin-resting and sniffing/licking represented 48.0% and 21.7%, respectively, of all sexual interactions on the day of estrus (Kerbrat and Disenhaus, 2004). In order to determine important symptoms for detection of estrus, they analyzed correlations between estradiol concentration and some typical symptoms. Differences in correlation factors indicated that mounting, unrest and chin-resting are more indicative of estrus than sniffing vulva (Kerbrat and Disenhaus, 2004). Increased frequencies of these signs were found especially during standing estrus (Sveberg *et al.*, 2011).

Generally, social as well as agonistic interactions are mainly affected by stocking density. It was found that increasing stocking density enhanced the number of cows meeting and interacting sexually as well as that overcrowding reduced the display of estrous signs because of no adequate space in housing systems (Diskin and Sreenan, 2000). Previous studies have demonstrated that the number of cows simultaneously in estrus affected both intensity of sexual activities (Britt *et al.*, 1986; Diskin and Sreenan, 2000) and duration of behavioral signs (Van Vliet and Van Eerdenburg, 1996; Roelofs *et al.*, 2005). The length varied between 11.6 ± 4.9 and 16.1 ± 8.2 h with one or more cows becoming estrous (Van Vliet and Van Eerdenburg, 1996). Cows receive some sexual stimulation by the estrual group, contributing to the manifestation of estrous behaviors. Thus, cows often participate in sexually active groups during estrus, in which some cows are more attractive and sexually active than other animals in the herd (Sveberg *et al.*, 2013).

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Management-related factors affecting estrous behavior

Nutrition

Fertility of modern dairy cows is affected by the process of *postpartum* metabolic adaptation regulating the resumption of estrous activity. As milk yield of dairy cows is closely related to DMI, nutritional requirements increase rapidly in the early lactation (Wiltbank *et al*., 2006). The most important factor to explain impaired reproductive performance is the cow's energy balance, the difference between the available energy from feed intake and the amount of energy needed for maintenance and milk production. To meet the huge demands of lactation cows usually enter a period of NEB causing – dependent on the extent and duration of NEB – inhibited expression of estrous behaviors and development of further reproductive dysfunctions. Estrus is negatively affected by alterations in blood metabolites and hormone profiles including glucose, insulin, IGF-I, non-esterified fatty acids, β-hydroxybutyrate (Wathes *et al*., 2007). A status of NEB decreases hypothalamic production of GnRH and, in turn, suppresses pulsatile LH secretion and circulating estrogen and progesterone concentrations (Wiltbank *et al*., 2006; Wathes *et al*., 2007), explaining the decrease in duration and intensity of estrus (Lopez *et al*., 2004). Body reserves are mobilized to compensate for NEB and contribute to higher loss of BW and body condition score (BCS) (Liefers *et al*., 2003) which in turn affects fertility by fewer cows showing initiated estrus. In a recent study conducted by Madureira *et al*. (2015) the BCS of an estrual cow affected significantly peak activity, as animals with BCS $\leqslant 2.5$ expressed less intense estrus patterns. In addition, NEB has been related to delayed resumption of ovarian activity, prolonged *postpartum* anestrus (Liefers *et al*., 2003), a greater incidence of irregular cycles (Wathes *et al*., 2007), decreased conception rates and increased pregnancy loss (Wiltbank *et al*., 2006). In contrast, cows in a positive energy balance were found to have 11.3 days less until first *postpartum* luteal activity reducing calving-to-conception interval (Liefers *et al*., 2003).

Nevertheless, López-Gatius *et al*. (2005) expected no effect of NEB on the intensity of estrus expression and there have been, indeed, some high-yielding cows being able to maintain high fertility in spite of the described influence of milk production on reproductive function.

Hormonal therapy

Synchronization of estrus by reproductive hormones has been used to stimulate herd fertility and to increase the efficiency of estrus detection rates in dairy herds (De Rensis and Scaramuzzi, 2003). But, duration and intensity of estrus were highly variable and were not different between estrous cycles induced by $PGF2_{\alpha}$ and those occurring spontaneously (Walker *et al*., 1996). Using a progesterone-releasing intravaginal device (PRID) for estrus synchronization López-Gatius *et al*. (2005) compared the effect of natural and PRID induced estrus on activity behavior and found cows treated with the PRID expressing similar walking activity to cows in natural estrus.

The use of synchronization of ovulation that allows for fixed timed AI eliminates the need for detection of estrus (Dolecheck *et al*., 2016). The goal is as early as possible to simultaneously and blindly inseminate all injected cows. The administration of GnRH results in ovulation and formation of a new or accessory corpus luteum (CL) and coincides with initiation of a new follicular wave. The CL regresses after the injection of $PGF2_{\alpha}$ which follows 7 days later. Cows receive a second injection of GnRH 48 h after the luteolytic treatment to induce a fertile ovulation followed by timed AI 24 h later. Dolecheck *et al*. (2016) found no difference in probability of pregnancy or pregnancy loss between cows with estrus detected by increased pedometer activity and cows which were subjected to an ovulation synchronization program. Fricke *et al*. (2014) who also compared the effectiveness of timed AI with or without detection by an automated activity measurement showed that supplementing timed AI with activity monitoring resulted in reduced time to first service by 7.5 to 12.4 days.

In the United States pre-synchronization is widely used in dairy farms, whereupon in a study conducted by Fricke *et al*. (2014) the accelerometer attached to the neck collar detected 70% of the cows. Pregnancy rate of cows with enhanced activity after completing the second $PGF2_{\alpha}$ injection of a Presynch-Ovsynch protocol and following AI was 27% whereas pregnancy rate was increased to 40% in cows with enhanced activity and additional timed AI after pre-synchronization. According to Valenza *et al*. (2012) using activity monitoring systems and heatmount detectors for identifying cows in estrus, increased activity and standing behavior was detected in only 71% and 66% of synchronized cows. Multiple reproductive management programs can be economically feasible in dairy farms. However, for success timed AI demands strict observance and labor (Dolecheck *et al*., 2016).

Fully automated systems for detection of cow's estrus

Pressure sensing system

Electronic pressure-sensitive devices such as HeatWatch® (DDx Inc., Boulder, CO, USA) (Walker *et al*., 1996; At-Taras and Spahr, 2001) or DEC® (IMV Technologies, L'Aigle, France) (Saumande, 2002) are based on detection of onset and length of standing mounts accepted by estrual cows. The system consists of a pressure-sensitive transmitter which is embedded in a burlap pouch and glued to the sacral region anterior to the tail head (Walker *et al*., 1996; Saint-Dizier and Chastant-Maillard, 2012). This on-cow sensor is activated by the weight of a mounting animal for a minimum of 2 s to limit the number of false-positive results, although it has been found that up to 40% of mounts lasted <2 s (Walker *et al*., 1996). Via radio signal data (date, time, cow ID, number and duration of mounts, signal strength) are sent within a 1200-m radius to a receiver and recorded by the management software on a farm computer (At-Taras and Spahr, 2001; Saint-Dizier and Chastant-Maillard, 2012). A defined algorithm analyzes each cow's mounting profile with the software classifying a 'standing' as three or more

https://doi.org/10.1017/S1751731117001975 Published online by Cambridge University Press

standing events in any 4-h period (Diskin and Sreenan, 2000; Peralta *et al*., 2005). Initiation of estrus is confirmed by the first activation of the sensor (Lopez *et al*., 2004). The software provides various reports including lists and graphs of cows defined as standing or suspected of standing – depending on whether cows receiving or not receiving three or more mounts within the 4-h period (At-Taras and Spahr, 2001). Use of that system resulted in detection of 82.1% of the ovulations (Lopez *et al*., 2004) and improved detection of estrus compared with visual observation. In two different trials, At-Taras and Spahr (2001) found efficiencies of 86.8% and 71.1% for detection based on HeatWatch® in comparison with 54.4% and 54.7% provided by visual observation of cows. However, similar efficiencies – 48.0% identified by the system *v*. 49.3% by visual observation – were indicated in a study conducted by Peralta *et al*. (2005). The efficiency of the DEC® system was reported to be considerably lower, that is to say approximately only 50% of the efficiency obtained from visual observation (35.4% *v*. 68.8%) (Saumande, 2002). The potential of pressure-sensitive systems was affected significantly by housing conditions (Palmer *et al*., 2010), type of flooring (Britt *et al*., 1986), weather (Peralta *et al*., 2005) and difficulties in maintaining the sensors in the proper position (Diskin and Sreenan, 2000). Displacements or losses of sensors up to 40% were described by Saumande (2002).

Activity monitoring

Pedometer. Pedometers attached to the leg of the cow record the number of steps taken per unit time as an indicator of walking activity being markedly increased during proestrus and estrus of dairy cows (López-Gatius *et al*., 2005; Roelofs *et al*., 2005; Yániz *et al*., 2006). Various researches evaluated these commercially available systems as a reliable method of identifying estrual animals as well as useful for prediction of ovulation time (Roelofs *et al*., 2005). Further, López-Gatius *et al*. (2005) found a positive relationship between walking activity and pregnancy rate of dairy cows.

More precisely, cows coming into estrus are identified by an increase in locomotion above the mean activity value recorded – during the same time period – for preceding days (Roelofs *et al*., 2005; Yániz *et al*., 2006). The system described by Dolecheck *et al*. (2016) calculated a 10-day backward moving mean steps per hour after each data download. Pedometer recordings showed a diurnal rhythm in the number of steps which is important for the development of algorithms considering within-cow comparisons. Alerts are generated using different algorithms and are set off if weighted activity has exceeded a user-defined threshold value (Roelofs *et al*., 2005, Dolecheck *et al*., 2016). The detection rates and error rates for the different thresholds used to study the increase in the number of steps around estrus have been reported (Redden *et al*., 1993; Roelofs *et al*., 2005). Data stored in a memory are transferred to receivers usually placed near the milking system and sent to the management software (Roelofs *et al*., 2005) enabling herd managers to review the reproductive status of individual cows.

Accelerometer system. Activity meters using acceleration technology are attached to the neck collar of each cow (Madureira *et al*., 2015; Gaillard *et al*., 2016) and measure continuously horizontal accelerations related to upward movements of cow's head and neck during walking and mounting behavior (Reith *et al*., 2014a). Data present average activity shown as a general activity index (Silper *et al*., 2015) which can be stored in 1-h (Gaillard *et al*., 2016) or 2-h intervals each day (Reith *et al*., 2014a; Madureira *et al*., 2015). Specially developed algorithms based on deviations of the current measured data from the stored activity pattern are used to separate cow's day-to-day activity from activities associated with estrous behavior. Herdsmen receive an alert after cows have exceeded a user-defined threshold. Data are read by an antenna and automatically transferred via IR signal to the herd management software providing lists and graphs to control reproductive (and health) status of individual cows (Reith *et al*., 2014a) (Figure 2).

Aungier *et al*. (2015) showed that the start of estrus-related behavior was 6 h before the start of an activity cluster recorded by Heatime® (SCR Engineers Ltd, Netanya, Israel) and finished 3 h after the start of the activity cluster. In their investigation the system alerted estrus in 90% of cows and incorrectly identified 17% of the total number of clusters. Further, Valenza *et al*. (2012) verified that the percentage of cows detected in estrus did not differ between the accelerometer system and the heatmount detectors (71% *v*. 66%, respectively). Thus, accelerometer systems are described as a useful tool to detect estrus and to improve fertility in dairy cattle (Valenza *et al*., 2012). The technology is commercially available for measurement of activity only or in combination with rumination characteristics (Reith *et al*., 2014a).

Video camera

The use of video systems for estrus detection relies upon identification of the standing mount position. So, the length of time during which cows exhibit standing estrus is comparable with the average duration measured by pressure sensing systems. Cameras fixed preferably in the upper corners at a height of 3 m are connected to the video management software providing visualization of stored video sequences. Detection is affected by camera resolution, as low resolution may result in difficulties in reading of the ear-tag number and, thus, identifying the cow (Saint-Dizier and Chastant-Maillard, 2012), disposition and the used threshold value. Although these systems are equipped with IR technology, artificial lighting is necessary at nighttime (Bruyère *et al*., 2012). Compared with a duration of 40 min/day (four periods of 10 min) needed for visual observation, the time exposure to analyze the video sequences varied between 8 and 32 min, depending on the number of cows that were simultaneously in estrus (Bruyère *et al*., 2012). Due to investigations conducted by Bruyère *et al*. (2012) the efficiency for detection based on video recording was higher in comparison with the detection rate obtained from classical visual observation (80% *v*. 68.6%). They concluded that using video cameras for detection of estrus can replace visual

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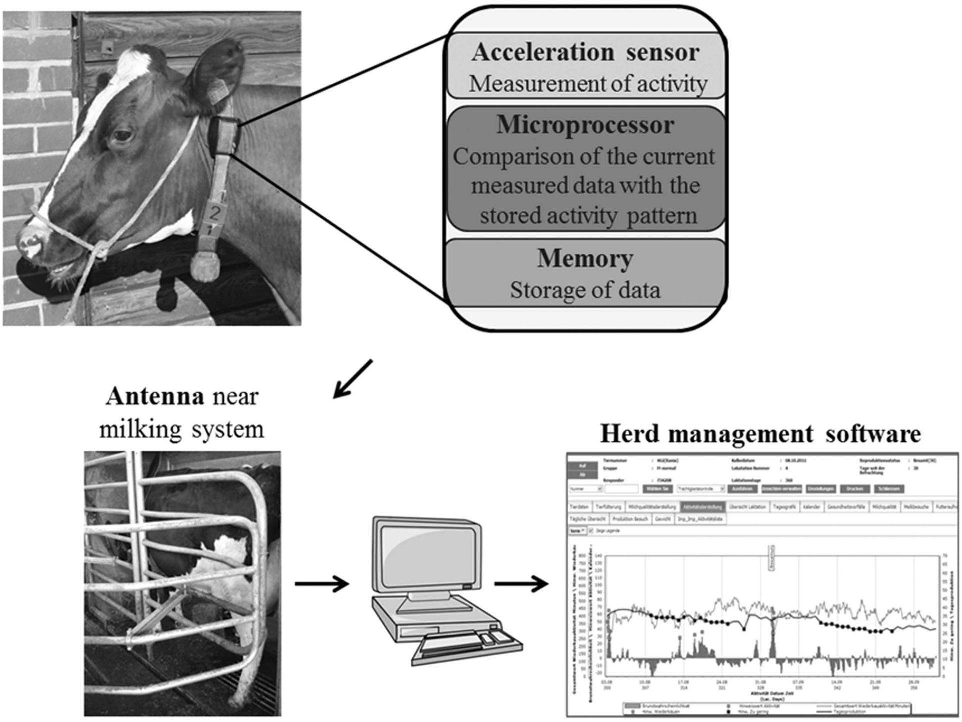

Figure 2 Acceleration technology attached to cow's neck collar.

observation. Nevertheless, as with visual observation, only cows with obvious behavioral estrous signs are detected.

Recording of vocalization
The vocal behavior of cattle gives information on the reproductive status of the vocalizing animal and may bear upon estrus advertisement. Near the time of estrus vocalization rate was found to be increased (Schön *et al.*, 2007), with the extent of vocalizations depending on the status of the estrous cycle: di-estrus < pro- and postestrus < estrus (Dreschel, 2014). Vocalizations are recorded continuously by a clip-on microphone attached to a neck harness of the animal. Via a transmitter the recordings are transferred to a stationary receiver being connected to the sound card of the computer. By use of the available algorithm, serial signal windows are generated from the sound recording and only those with means exceeding a defined threshold are considered for detection of estrus. However, large individual variability of absolute vocalization rate might reduce the suitability of this trait for practical application (Schön *et al.*, 2007).

Measurement of body temperature
Automated systems of monitoring body temperature around estrus are based on radiotelemetric transmission of information. The temperature rhythms have been recorded by rectal (Piccione *et al.*, 2003) and vaginal thermometry (Fisher *et al.*, 2008). According to Fisher *et al.* (2008), the vaginal temperature decreased slightly 2 days before the day of estrus followed by an increase at the time of the LH peak. In their study, the average temperature increase was 0.48°C ranging from 0.40°C to 3.22°C in estrous Holstein Friesian cows. In a study conducted by Redden *et al.* (1993), transmitters enclosed by a support anchor with finger-like projections were inserted into the vagina to a depth of 20 cm. Transmitter signals were picked up by specific receivers which were connected to a computer. Others used microprocessor-controlled temperature loggers (size = 92 × 20 mm; weight =40.5 g) placed in the vaginal cavity (Suthar *et al.*, 2011) or on-chip temperature sensors implanted in the cow's vulvar muscle – connected with receivers located in the collar (Morais *et al.*, 2006). Piccione *et al.* (2003) used a rectal probe inserted 15 cm into cow's rectum. With small seasonal variations, increases in body temperature occurred every 21 days on the day of estrus. Nevertheless, the records of the body temperature of four representative cows resulted in a detection rate of only 78% and a false positive rate of 12%. As the interval between the onset of increasing temperature and the time of ovulation was found to be consistent, the use of this predictor may be a reliable indicator of ovulation and the time of the LH surge (Fisher *et al.*, 2008). However, limitations may be due to variation in environmental temperature, disease-related hyperthermia, or some systemic or local inflammation, increasing the incidence of false positive results (Fisher *et al.*, 2008).

Measurement of milk progesterone concentration
As the blood concentration of progesterone is closely associated with its concentration in milk, progesterone analysis of representative milk samples can be used to determine the reproductive status of the dairy cow. The development of in-line and real-time automatic monitoring systems such as Herd Navigator® (DeLaval, Glinde, Germany) replace the manual collecting of progesterone information. The samples taken during the milking session are collected in a sample intake unit and transferred automatically to the analyzing unit connected

https://doi.org/10.1017/S1751731117001975 Published online by Cambridge University Press

to a computer. The frequency of progesterone assays can be varied according to the stage of the estrous cycle (Saint-Dizier and Chastant-Maillard, 2012). Before being processed in a biological model developed by Friggens and Chagunda (2005) the milk progesterone values prepared over the last few days are smoothed using an extended Kalman filter, with the algorithm distinguishing between different categories of cows: *postpartum* anestrus, estrus cycling and potentially pregnant. Alerts are generated by the software in case of milk progesterone concentrations <4 ng/ml (Friggens and Chagunda, 2005). Roelofs *et al.* (2006) found large inter-individual variation in timing of decreased levels and noted values <5 ng/ml 80 h (range: 54 to 98 h) before ovulation. Except comparatively major investment costs, in-line measurements of milk progesterone may have the potential to be a reliable tool in reproduction monitoring (Friggens and Chagunda, 2005; Saint-Dizier and Chastant-Maillard, 2012). The fully automated system of progesterone assay in milk (Herd Navigator®), presented by Saint-Dizier and Chastant-Maillard (2012), has been marketed in Denmark and is commercially available since 2010 with an average detection rate of 95%.

Potential of multivariate analysis including activity monitoring

Detection of cow's estrus is a balance of sensitivity and specificity. Correctly identified estrus events are classified as true positive. Non-alerted estrus events lead to false negative results. Alerts outside estrus events are considered true negative, and alerted non-estrus events are denoted as false positive. So, the sensitivity of a specific technology expresses the percentage of correctly detected estrus events, whereas the specificity is the probability of a missing alert when an event does not occur. The percentage of false estrus alerts in relation to the number of detected estrus events is indicated by the error rate (Firk *et al.*, 2002) (Table 2). Often, there is a contradiction between the sensitivity and the specificity, as an increase in the sensitivity provokes a decline in the second parameter.

Table 2 *Criteria for evaluation of methods for detection of estrus in dairy cows*

Criteria	
Detection rate/efficiency/sensitivity	TP/(TP + FN) × 100
Error rate/rate of false-positive results	FP/(TP + FP) × 100
Specificity	TN/(TN + FP) × 100

TP = true positive; FN = false negative; FP = false positive; TN = true negative.

To date, most technologies for identifying cows in estrus are based on automated activity measurement (Madureira *et al.*, 2015; Dolecheck *et al.*, 2016). This system is repeatedly considered as being suitable for estrus detection, and is likely to be gainful for most dairy farms (Rutten *et al.*, 2014). Analyzing the economic benefits of activity meters for detecting cows in estrus they showed that the increase in sensitivity of activity meters (80%) in comparison with the detection rate caused by visual observation (50%) was the most important determinant of the profitability of the investment in such a system. Furthermore, they calculated that investing in activity measurement is less expensive than increasing the detection rate of visual estrus detection by increasing labor input.

A combination of several estrus detection aids might lead to the best results concerning sensitivity of detection. Firk *et al.* (2002) postulated that the aim should be to achieve an efficiency higher than 90% and an error rate lower than 20% by combination of traits. Table 3 demonstrates the advantages of univariate and multivariate analysis of continuous and close activity monitoring, especially by neck-mounted accelerometer systems for estrus detection. According to Peralta *et al.* (2005), the analysis of activity measurement, visual observation and mounting detection alone resulted in low detection rates (37.2%, 49.3% and 48.0%, respectively).

Table 3 *Evaluation of activity measurement as well as combinations of methods including activity measurement for detection of estrus in dairy cows*

	Traits	Sensitivity	Error rate
Redden *et al.* (1993)	ACT + vaginal temperature	90.0	
Krieter (2005)	ACT + last estrus	77.5	9.1
Peralta *et al.* (2005)	ACT	37.2	
	ACT + VO + mounting	80.2	
Wangler *et al.* (2005)	ACT	78.1 to 94.7	44.7 to 54.1
Holman *et al.* (2011)	ACT	58.9	
	ACT + VO	74.4 to 75.0	
	ACT + mounting	75.6 to 75.9	
Jónsson *et al.* (2011)	ACT + lying	88.9	5.9
Dela Rue *et al.* (2014)	ACT	89.2	
Fricke *et al.* (2014)	ACT	56 to 70.0	
Reith *et al.* (2014a)	ACT	76.5	
	ACT + rumination	93.7	
Aungier *et al.* (2015)	ACT	90.0	17.0

ACT = activity; VO = visual observation.

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The subsequent combination of all three traits revealed an estrus-detection sensitivity of 80.0%. Not all combinations have practical significance due to the above-mentioned limitations. For use in the field it is important to utilize cost-effective methods with minimal labor requirements and a high degree of accuracy at identifying physiological or behavioral estrus signs. Variation in detection performance depends on the methods of calculation and definitions of algorithms as well as on differences in farm structure (housing system, health status, herd management). Setting a threshold for alerts requires a balance between false positives and false negatives. Estrual cows with modest increase in activity behavior, especially lame cows, remain undetected when the threshold is set too high to create alerts (Dolecheck *et al.*, 2016).

For practical application the automated combination of activity with data that are anyway available in most dairy farms (e.g. time since last estrus, information about previous estrus events) may be ideal (Krieter, 2005). In addition, data about cow's health status – rumination time is mainly used in dairy farms to predict impending metabolic disorders – can be useful for multivariate estrus detection. In their study carried out on five practical farms, Reith *et al.* (2014a) analyzed data of activity and rumination time which can be recorded exactly and automatically on a daily basis for individual cows by a microphone-based sensor system. They showed that simultaneous analysis improved the detection rate of cows starting estrus. Unexpectedly, the number of cows with enhanced activity at estrus was lower than that identified by rumination time (76.5% *v.* 86.2%), suggesting that measurement of rumination duration may detect more cows approaching estrus compared with measurement of activity. So, combined analysis greatly underscores the relevance of considering more than only one trait for identification of cows that would otherwise not be inseminated.

Conclusion

It is undisputed that detection of bovine estrus significantly affects reproductive efficiency and profitability of dairy herds. The development of improved methods of identifying estrual animals depends on the knowledge of behavioral alterations at the onset of estrus. Behavioral signs differ among individual cows in duration and intensity of estrus. Cow-related factors as well as environmental- and management-related factors influence the expression of estrus and are responsible for high inter-individual variations. Over the past several years, there has been a clear trend toward the analysis of routinely collected sensor-based data and constant surveillance of behavior. The focus is on secondary symptoms of estrus which are more indicative than standing behavior. A number of diverse automated detection systems have been refined and marketed to enhance detection of estrus. Relatively new measurements such as rumination time or feed intake are studied to further improve reproductive management in dairy farms. Prospective, biosensors for in-line measurement of bovine progesterone and combinations of several technologies including activity monitoring may promise the greatest success.

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