### ESTRUS DETECTION IN DAIRY CATTLE: HOW TO BEAT THE BULL

Oestrusdetectie bij melkvee: Hoe we de stier kunnen verslaan

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#### **ABSTRACT**

Prior to ovulation, cows display certain behaviors that we characterize as estrous behavior. The standing reflex, a behavior that by definition accompanies heat, is important for determining the moment of insemination. These animals, which are ovulating, can then be inseminated with success. Only 50% of the cows display this standing reflex, however, so it is necessary to formulate a better and more conclusive definition of estrus.

An effective and valid visual detection scoring system has been developed. With this system, the intensity of estrous behavior can be expressed numerically both for individual cows and at the herd level. Other ways of detecting cows in estrus are discussed, such as using pedometers, body temperature, electrical conductivity and heat mount detectors. Most of the aids that have been developed are not reliable or sensitive enough to relieve the farmer from frequent visual observation of the herd. Pedometers and heat mount detection devices seem to be the most promising detection aids.

### **SAMENVATTING**

Voorafgaand aan een ovulatie vertonen koeien bepaalde gedragingen die we als oestrusgedrag omschrijven. Belangrijk voor de bepaling van het inseminatiemoment is de stareflex, het gedrag dat per definitie met bronst gepaard gaat. Deze dieren ovuleren en kunnen vervolgens met succes geïnsemineerd worden. Slechts 50% van de koeien vertoont echter deze stareflex waardoor het formuleren van een betere en meer sluitende definitie van oestrus noodzakelijk is.

Een gevalideerd visueel scoringssysteem werd daarvoor ontwikkeld. Met dit systeem kan de intensiteit van het oestrusgedrag worden weergegeven in punten, zowel op individueel niveau als op koppelniveau. Andere manieren van oestrusdetectie worden besproken, zoals stappentellers, lichaamstemperatuur, elektrische geleidbaarheid en apparaatjes die registreren of een koe besprongen werd. De meeste van de ontwikkelde methoden zijn niet betrouwbaar of gevoelig genoeg om de veehouder te ontlasten van een regelmatige observatie van zijn kudde. Stappentellers en apparaatjes die registeren of een koe besprongen werd, zijn de meest belovende alternatieven.

### INTRODUCTION

Estrus is the period in which a cow can be successfully bred by a bull or AI (Sturman *et al.*, 2000; Waldmann *et al.*, 2001) and is defined as the period in which a cow stands when she is mounted by a bull or another cow. The detection of estrus is one of the key components in the management of dairy farms with respect to fertility. The availability of time to spend on the detection of estrus, but also adequate knowledge of its symptoms, are often lacking. This results in a low detection rate and poor fertility indices (O'Farrell, 1978; Reimers *et al.*, 1985; Opsomer *et al.*, 1996; Webster *et al.*, 1997). Recent studies carried

out by our group have revealed that estrus detection rates in dairy cattle are low (< 50%) not only because of the two factors mentioned above, but also because of the fact that cows do not show the proper behavioral symptoms (Heres *et al.*, 2000; Van Vliet and Van Eerdenburg, 1996). Most of the older studies report a mean duration of estrus of around 18h. However, for dairy cattle, recent reports mention shorter periods of around 13h and, for individual animals, even as short as 4h (Van Vliet and Van Eerdenburg, 1996) (Figure 1).

The length of the cycle for dairy cows is 18-24 days, although for more than 20% it is longer than 25 days (c.f. Trimberger, 1956). For a dairy farm, poor detection rates

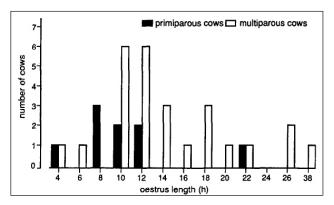


Figure 1. Number of primiparous and multiparous cows grouped per category of estrus length (after Van Vliet and Van Eerdenburg, 1996).

result in substantial financial losses due to: 1) unexploited potential of milk and calf production caused by prolonged calving intervals, 2) expenditure on excessive replacement heifers and on unsuccessful inseminations, and 3) the reduced rate of genetic progress (Dijkhuizen *et al.*, 1991; Lehrer *et al.*, 1992; Senger, 1994).

### BEHAVIORAL CHARACTERISTICS

Cows can be detected in estrus because they display specific behavior. Most of the detection systems and aids use the following behavioral characteristics:

- Mucous vaginal discharge, although often used by farmers, is unreliable as an indicator for estrus. When a long string (> 50 cm) of clear, viscous mucus hangs from the vagina, the cow can be considered to be in estrus. As a key symptom for insemination it is of low value because it can be seen for several days in certain animals. Furthermore, these marked vaginal discharges are rarely seen in cows in loose housing systems (Loeffler et al., 1999; Van Eerdenburg et al., 1996). For an extensive review of this symptom, see Holtz and Meinhardt (1993).
- Cajoling (= flehmen), although often observed during diestrus as well, has a high frequency during estrus. It is therefore a relevant symptom. Since cajoling is difficult to distinguish from other types of behavior that are unrelated to estrus (e.g. sniffing for fresh air in the air inlets), it can be considered to be of minor importance for determining whether a cow is in estrus or not (Van Eerdenburg et al., 1996)
- Mooing, sometimes continuously, can be a symptom of estrus. However, in dairy cattle, only few animals show this symptom and if they do so, they also show other symptoms in high intensity as well. The selective potential for dairy cattle is therefore low. It is more of-

ten seen as a nuisance by farmers and people who live in the vincinity of a farm. Cows with COF can also display this type of behavior.

- Restlessness is a very subjective symptom. However, a skilled observer who knows his cows will be able to score signs of unrest, such as ear play and mooing. Cows will move a little away from the herd when in estrus, if possible. Also they will walk more. Because cows can show unrest for various other reasons, and in most cases will show it several times during a particular observation period, this symptom is of relatively low selective importance (Holtz and Meinhardt, 1993; Van Eerdenburg et al., 1996).
- Sniffing the vulva of another cow occurs during estrus as well as in between estrous periods. It is often followed by cajoling.
- Resting with the chin on another cow occurs during estrus as well as during diestrus. However, the frequency during estrus is substantially higher and is therefore a good indicator of estrus (Holtz and Meinhardt, 1993; Van Eerdenburg et al., 1996). Often it is followed by an attempt to mount the other cow.
- Mounting, or attempting to mount, other cows is one of the most accurate external signs of estrus in dairy cows (Holtz and Meinhardt, 1993). However, mounting also occurs in diestrus. Esslemont and Bryant (1976) considered a cow as being in estrus when she mounted another cow at least six times a day. With the system described below, a cow can be considered in estrus when she mounts another cow twice in 24 hours. According to Roelofs et al. (2005a), the onset of mounting behavior is the best predictor for the time of ovulation.
- Being mounted, but not standing, is a symptom that occurs with cows during and between estrous periods.
   Cows close to estrus are more attractive to estrous cows, resulting in a higher frequency of being mounted. It can therefore serve as an indication of (pro)estrus (Holtz and Meinhardt, 1993; Van Eerdenburg *et al.*, 1996).
- When a cow is mounted, the hairs at the pelvic region will be disturbed. Even though one did not see the cow being mounted, the rough hairs will give an indication that the animal was mounted. Sometimes even the skin can be damaged and a little blood might be present.
- Mounting the head side of another cow is, according to our results, highly discriminative of the cow being in estrus (Van Eerdenburg et al., 1996).

• Standing estrus has always been the most discriminative sign of estrus (Holtz and Meinhardt, 1993). However, in recent reports low numbers of standing estrus have been noted—even as low as in 37% of the cows in estrus (with 12 observations of 30 min. during 24 h. per day) (Van Eerdenburg et al., 1996; Heres et al., 2000; Lyimo et al., 2000). The type of floor influences the occurrence of mounting behavior. Slippery, wet, concrete (slatted) floors are not encouraging for cows to mount each other. Dirt floors (especially in outside paddocks) or thick straw bedding are preferred (Britt et al., 1986; Dozier-Vales and Britt, 1990).

As for the performance of mounting behavior, it is important to know that cows preferably mount other estrous cows. A minimum of two cows in or near estrus is thus required. Herd size and calving management are thus of major influence. When problems exist in a small herd, synchronization protocols can be a solution.

#### OTHER CHARACTERISTICS

Several farmers use the reduction in food intake and milk production during estrus as a determinant in their detection protocol (Britt *et al.*, 1986; Holtz and Meinhardt, 1993; Van Asseldonk *et al.*, 1998). However, the drop in milk production often occurs after the reduction in food intake (concentrates) and is not overt in all animals (Holtz and Meinhardt, 1993). These can be important factors, however, if combined with others (De Mol and Woldt, 2001).

#### **SEASON**

Although the domesticated cow is not considered to be a seasonal breeder, since she ovulates year round, certain influences of the season can be observed. Reproductive efficiency is generally lower in winter at northern latitudes, whereas at latitudes closer to the equator reproductive efficiency is lower in summer. Seasonal variations in ambient temperature, photoperiod, humidity and feed supply contribute to seasonal variances in reproductive efficiency. Heat stress lengthens the estrous cycle and decreases the duration and intensity of estrus (Tucker, 1982; Moore *et al.*, 1992; Orr *et al.*, 1993).

#### ESTRUS DETECTION

#### **Detection by observation**

Visual observation is the most common way of detecting cows in estrus (Rae *et al.*, 1999). However, as described above, recent reports indicate that the intensity of the symp-

toms shown by dairy cows is not as high as reported in older studies. Especially the lack of standing events is a serious problem, since it is the key symptom for defining a cow in estrus. For this reason, we have developed a validated system that includes other behavioral characteristics of estrus as well (Van Eerdenburg *et al.*, 1996; Heres *et al.*, 2000). With this system, a cow scores points for each behavioral symptom that she shows (Table 1), based on the frequency of these behaviors during estrus and diestrus. The points are summed up over a rolling 24 hour period. If a farmer observes his cows two or three times a day for 30 minutes per observation period, the threshold for determining a cow in estrus is 50 points.

Because the estrous signs are less overt, the farmer has to pay more attention to his detection protocol and include other signs of estrus as well (Stevenson *et al.*, 1983; Cowen *et al.*, 1989). Frequency, duration and time of observation are important (see below). Furthermore, the method of observation also influences the detection rate. The observation period should not be combined with another task, such as feeding or cleaning. It must be fully dedicated to watching the cows. The observer should also not walk through the herd but stay quiet outside the cow area. In this way the animals show the most estrous signs (unpublished results). Many false positive indications are obtained when the cows are gathered before milking (Williamson *et al.*, 1972). Last but not least, the observer should be trained well and know the animals and their behavior.

## Frequent and long observations

Cows do not show their estrous behavior at a specific time of the day, and estrous periods are sometimes very

Table 1. Scoring scale for estrous behavior.

Behavior	Points
Mucous vaginal discharge	3
Cajoling (= flehmen)	3
Restlessness	5
Being mounted but not standing	10
Sniffing vulva of another cow	10
Resting with chin on another cow	15
Mounting (or attempting to mount) other cows	35
Mounting head side of another cow	45
Standing heat	100

Table 2. Effect of number, time and duration of observations on estrus detection rate.

No. of	Time of observation					Detection rate (%)			
Observations						30 min	20 min	10 min	
2	06:00	20:00				62.9	37.1	14.3	
2	06:00	22:00				48.6	31.4	8.6	
2	10:00	20:00				74.3	48.6	25.7	
2	10:00	22:00				62.9	42.9	22.9	
3	06:00	12:00	22:00			62.9	48.6	17.1	
3	10:00	12:00	20:00			77.1	54.3	22.9	
4	06:00	12:00	16:00	22:00		74.3	57.1	31.4	
4	06:00	10:00	16:00	20:00		82.9	57.1	34.3	
5	06:00	10:00	14:00	18:00	22:00	85.7	62.9	37.1	

short. It is therefore important to watch the cows frequently and for a substantial period of time. In Table 2 the results of frequent observations are presented, (milking was at 07:00 and 16:30 and lasted for about 1 hour). It is evident that the time of the day and duration of the observations are the most important factors for a high detection rate. More observations per day, even when resulting in a larger total amount of time spent are of lesser importance. (e.g. compare 2 observations at 10:00 and 20:00 for 30 min with 4 observations at 06:00, 10:00, 16:00 and 20:00 for 20 min). The cows can be observed best after milking and feeding in the morning, in the early afternoon and in the evening (around 20:00 h). Observation periods should last more than 20 minutes to be effective.

# **Detection aids**

Slenning and Farver (1990) already indicated that currently recommended procedures for estrus detection, namely visual observation of mounting and standing, may be inappropriate as a routine screening test for estrus. A large variety of aids have been developed with occasional (local) success, but more often with poor detection results due to the large number of false positive indications. Apparently, the ideal detection aid has not yet been invented. The ideal aid should provide continuous surveillance (24h/day) of the cows, and accurate and automatic identification of the cows in estrus. It should also operate for the productive lifetime of

the cow, and it should have minimized labor requirements and a high accuracy in identifying the appropriate physiologic or behavioral events that correlate highly with ovulation (Senger 1994). Most of the aids in use possess several of the above mentioned aspects but none possess all of them.

### Teaser animals

Of course a bull, or rather a vasectomized bull, is the best detector. They can be equipped with a chin ball device for marking the estrous cows or buffaloes (Lang *et al.*, 1968; Hill *et al.* 1992; Zicarelli *et al.*, 1997). Caution is needed to interpret the markings on a cow because bulls often 'try' a cow by placing their chin on the pelvic region. So only cows with markings at their shoulder region have indeed been mounted by the bull. When bulls are able to enter the vagina of the cow with their penis the risk for sexually transmitted diseases exists. In order to prevent this, various surgical procedures have been designed (Donaldson, 1968; Foote, 1975). However, all of these should not be, or are not allowed to be, performed for ethical and legal reasons. The ratio between bulls and potentially estrous cows should not be larger than 1:30-40 (Foote, 1975; Varner, 1986)

Keeping a bull has its dangers and costs. Bulls with good libido are often aggressive. To reduce the costs, a dairy farmer might choose a beef breed. This, however, could be a problem for the heifers because of their size. A smaller breed is therefore preferred (Holtz and Meinhardt, 1993).

Estrous or pregnant cows can also serve as teasers (Esslemont and Bryant,1976; Dijkhuizen and Van Eerdenburg, 1998; Thomas and Dobson, 1989). The size and composition of the herd can be relevant in this respect. Cows with COF or hormonally treated cows are also a possibility (Signoret, 1975; Kiser *et al.*, 1977; Mortimer *et al.*, 1990). However, these cows can be very active, causing a lot of unrest in the herd.

The presence of a bull can also stimulate the cows to show their estrous state more overtly. Mating stimuli as given by sterile bulls can improve pregnancy rates (Zicarelli *et al.*, 1997; Rodriguez and Rivera, 1999). However, the period to ovarian reactivation postpartum may be extended, though without long-term effects on reproductive performance, when a bull is present in a dairy herd (Shipka and Ellis, 1999).

## Tail painting/heat mount detectors

One cheap and effective aid in an estrus detection protocol is tail painting (Macmillan and Curnow, 1977; Slenning and Farver, 1990; Xu et al., 1998). The pelvic region of the back of the cows that may be in estrus is painted with a stroke of an easily removable type of paint, such as chalk. When the cow is mounted by another cow or teaser bull, the paint is disturbed or wiped off. With twice daily observations (e.g. when walking to the milking parlor), quick detection is possible. The method has some drawbacks. In freestall barns with brushes to let the cows keep themselves clean, the animals may wipe off the paint themselves. In wet countries, the paint needs to be waterproof. In large herds or in synchronized herds, when there is a fair probability of having more than one cow in estrus on the same day, this method can be successful. In smaller herds, however, it can easily be that only a single animal is in estrus at a given time, and therefore it will not be mounted. Furthermore, as stated in the section on Behavioral Characteristics above, many cows no longer show the typical symptom of standing heat and therefore will be missed. The risk of false positive indications exists because cows are also mounted by estrous animals when they themselves are not in estrus. They will not stand, but the tail paint does not discriminate.

Some of those problems can be overcome by using heat mount detectors (e.g. the Kamar heat mount detector). These consist of a paint filled (plastic) tube that can be glued on the pelvic region of a cow. When another animal mounts the cow, either the color of the tube changes or the tube of paint ruptures. In this way cows can be detected at long distances. The problems with these detectors are loss of the tube, false positive indications due to scratching, and the failure of other cows to mount (Foote, 1975; Holtz and Meinhardt, 1993).

#### Electronic devices

Pedometers are electronic devices attached to a leg of the cow that count the number of steps an animal takes. During estrus, females tend to walk more, which is recorded by the pedometer. The pedometer can be read by a computer, which calculates the increase in activity as compared with the previous day(s). A large number of algorithms have been developed (Hurnik et al., 1975; Kiddy, 1977; Peter and Bosu, 1986; Lehrer et al., 1992; Liu and Spahr, 1993), but none of them provides the ideal combination of a large number of indications and a low number of false positives. The pedometer combined with monitoring measuring of progesterone levels in the milk can eliminate most of the false positive indications (Moore and Spahr, 1991). Recently, pedometer readings have been combined with other estrous parameters as well, resulting in improved detection rates (Maatje et al., 1997; De Mol and Woldt, 2001). One important factor for achieving high pregnancy rates is the timing of insemination (Roelofs et al., 2005a). Reading the pedometer at milking, which is common in practice, can result in a considerable time lag between the maximum level of behavior, which is highly correlated with the estradiol level, and the indications provided by the pedometer (Lyimo et al., 2000). More frequent readouts of the pedometer will provide more precise indications of when estrus has started, thus enabling a more precise determination of the moment of insemination. This results in high pregnancy rates (Maatje et al., 1997; Roelofs et al., 2005b).

The Heatwatch system is an electronic pressure sensitive device that is fixed on the pelvic region of the cows. A subcutaneous implantable device (SQUID) with similar function is also available. Both Heatwatch and SQUID are equipped with a clock and memory chip. Through radiotelemetry, the moments of first and last mount on a cow—and thus the start of estrus—can be recorded. Thus an accurate timing of insemination can be achieved (Senger 1994; Walker *et al.*, 1996; Xu *et al.*, 1998; Rae *et al.*, 1999)

The electrical resistance of the vaginal mucus and mucosa changes during estrus (Canfield and Butler, 1989; Phillips *et al.*, 1991; Kitwood *et al.*, 1993). The lowest resistance coincides with the maximum of the preovulatory LH surge and can thus be an excellent predictor for ovulation. However, efficiency and accuracy vary between studies and are not always 100%. Electrodes have been implanted that can be read by radiotelemetry, but were not practical (Lehrer *et al.*, 1991; Senger 1994). The Ovatec , a commercially available probe, measures the conductivity of the vaginal mucus. It is claimed that this probe can also be used to determine the sex of the offspring (Wehner *et al.*, 1997). However, Rorie *et al.* (1999) could not repeat this effect of insemination timing.

The detection of systematic changes in the Fourier transform of the infrared (FT-IR) spectra in milk could be a way for detecting cows in estrus, but so far the results are not yet ready for practice (Norup *et al.*, 2000).

#### Video camera

Video cameras can also be used to detect cows in estrus. However, for large herds more cameras are needed because of the resolution of the equipment. The recognition of the cows can sometimes cause problems (own observations), certainly in herds of single color breeds. The system can be equipped with a time lapse VCR, thus reducing the time spent on detection. Another option is to connect the VCR to a laser beam that is directed over the walking area of the cows. When the beam is interrupted by the mounting behavior of a cow the VCR starts running. In this way the amount of time needed to watch the video tapes is minimized (Boyd, 1984; Hurnik and King, 1987)

#### Electric nose

Cows excrete pheromones during estrus, which are meant to be detected by the bull (Chenoweth, 1983; Kiddy, 1984; Paleologou, 1977; Dehnard and Claus, 1996). Dogs can be trained to detect these pheromones (Jeziersky, 1991). Recently, an artificial method (the 'electronic nose') has been developed to detect the estrous pheromones (Lane and Wathes, 1998). Although the results are promising, practical applications of the system have not yet been reported.

### Measuring body/milk temperature

Increases in body temperature ( 0.3 - 0.9 °C) during estrus can be used to detect estrus (Ball *et al.*, 1978). Vaginal/rectal/ear skin temperature can be measured, even with implanted radio-telemetric devices (Redden *et al.*, 1993; Kyle *et al.*, 1998). Milk temperature has a high correlation with rectal temperature and can be measured online during milking (Maatje and Rossing, 1976; Fordham *et al.*, 1987). The detection rates may be at an acceptable level, but the method produces large numbers of false positive indications (Fordham *et al.*, 1988).

# MONITORING THE OVARIAN ACTIVITY

There are three ways of monitoring ovarian activity:

#### **Rectal palpation**

Rectal palpation is an action that is often performed by the veterinarian. It is a good way to obtain information about the status of the reproductive organs of female cattle. Regular palpations can confirm the cyclicity or pregnancy of a cow, predict a future estrus, etc. Palpation of the uterus at the moment of insemination by the inseminator can predict the success of the insemination (Loefler *et al.*, 1999; Sturman *et al.*, 2000).

### **Ultrasound imaging**

Images of the ovaries and uterus can be obtained using transrectal ultrasonography. Regular scanning will reveal detailed information about the reproductive status of the animals. Accurate diagnosis of pregnancy and follicular cysts is possible in this way. (Reeves *et al.*, 1984; Pieterse *et al.*, 1990; Kamimura *et al.*, 1993; Henao *et al.*, 2000). Frequent rectal ultrasound examinations do not interfere either with the estrous cycle or with the timing of ovulation (Roelofs *et al.*, 2004).

# Regular milk or blood samples

Milk or blood (or even saliva) samples that are taken regularly (e.g. 3 times a week) can be used to determine hormone levels in order to monitor the cycle of a cow. Progesterone is often used in scientific studies, but also in daily practice it can be of value. Several test kits for progesterone in milk have been developed and are commercially available (Brandes *et al.*, 1988; Gao, 1988; Elmore, 1989; Nebel *et al.*, 1989; Ruiz *et al.*, 1989; Schallenberger, 1990; Eldon, 1991; Williams and Mc Leod, 1992; Rajamahendran *et al.*, 1993). Claycomb and Delwiche (1998) developed a system for measuring progesterone levels online during milking. Not all the cow-side test kits are very reliable, and most of them are too expensive to use on a large scale.

### ESTROUS BEHAVIOR DURING PREGNANCY

Pregnant cows sometimes show estrous behavior, including even standing estrus (Donald 1943; Chauhan *et al.* 1976; Kaikini and Fasihuddin, 1984; Erb and Morrison 1985; Dijkhuizen and Van Eerdenburg, 1998; Thomas and Dobson, 1989). This can occur at a regular interval of around 21 days. In many cases the cows are reinseminated because the farmer supposes the cow is not pregnant. Around 4% of the calves in the Netherlands are the result of an insemination previous to the last one, 0.16% of the calves are the result of two inseminations before the last one (Dijkhuizen and Van Eerdenburg, 1998).

Since some pregnant cows show estrous behavior, they can also interact with estrous animals and help the farmer with estrus detection (Dijkhuizen and Van Eerdenburg, 1998; Thomas and Dobson, 1989).

### **CONCLUSION**

Estrus detection on the farm is mostly based on the behavior that cows express before they ovulate. Although the level of expression is substantially lower than it used to be several decades ago, a farmer can still detect when his cows are in estrus. Several aids have been developed, but most of them are not reliable or sensitive enough to relieve the farmer from frequent visual observation of the herd. Pedometers and mounting heat detection devices seem to be the most promising detection aids.

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