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## Original article

## A field study on estrus detection in lactating beef cattle

Christian Ducrot<sup>a\*</sup>, Yrjö T. Gröhn<sup>b</sup>, Françoise Bugnard<sup>c</sup>, Yves Senlis<sup>d</sup>, Philippe Sulpice<sup>e</sup>, Robert O. Gilbert<sup>f</sup>

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Abstract – Estrus detection efficacy and heat detection protocol were studied by means of a field study carried out on 878 lactating beef cows in 60 French herds. Average herd size was 48, and 75 % of the farmers partly or exclusively used artificial insemination. The cows were calved between October 1992 and March 1993. Estrus was recorded daily by the farmers. Cycling status was determined by progesterone radioimmuno-assay 2 months after calving. The relationship between the estrus detection protocol and the delay period from calving to first observed estrus was analysed using survival curves and the Cox proportional hazard model, adjusting for confounders. Seventy-one percent of the cows were seen in estrus by the farmers; the interval between calving and the first observed estrus ranged from 9 days to more than 5 months and the median was 56 days. Two months after calving, 44 % of the cycling cows had not been seen in heat by the farmers and 11 % of the non-cycling cows had been reported to have been in estrus. The heat detection protocol varied widely between farmers, depending on the considered estrus signs, schedule and time spent looking for signs. Two factors were significantly related to a shorter interval from calving to first observed heat: the use of artificial insemination (which relates to the farmer's interest in heat detection) and an overall daily time spent for heat detection greater than 1 h. Cows in tie stalls had a delayed interval to the first observed estrus. These results show that many farmers did not adapt their reproduction practice sufficiently to an earlier calving period. There is room for improvement since in many cases the heat detection protocol does not match the required standards for optimal heat detection. © Inra/Elsevier, Paris.

#### beef cattle / estrus / reproduction / epidemiology / risk factor

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Résumé – Étude de terrain sur la détection de l'œstrus en élevage bovin allaitant. Les méthodes de détection de l'æstrus et leur efficacité ont été étudiées en conditions d'élevage, à partir d'une étude de terrain conduite dans 60 troupeaux français, sur 878 vaches allaitantes ayant vêlé entre octobre 1992 et mars 1993. Les troupeaux comptaient 48 vaches en moyenne; 75 % des éleveurs utilisaient l'insémination artificielle partiellement ou en totalité. Les œstrus étaient notés quotidiennement par les éleveurs et l'existence de cycles sexuels a été analysée par dosage de progestérone deux mois après vêlage. L'effet du protocole d'observation de l'œstrus sur le délai vêlage - premier œstrus a été étudié à l'aide de courbes de survie et du modèle de Cox à risques proportionnels, avec ajustement sur les facteurs confondants. Soixante et onze pour cent des vaches ont été vues en œstrus par l'éleveur ; l'intervalle vêlage - premier œstrus a varié de 9 j à 5 mois, avec une médiane de 56 j. Deux mois après vêlage, 44 % des vaches cyclées n'avaient pas été vues en œstrus et 11 % des vaches non cyclées avaient été déclarées vues en æstrus. Le protocole d'observation des æstrus variait considérablement entre éleveurs (signes considérés, horaires et temps passé). Deux facteurs étaient statistiquement liés à une diminution de l'intervalle vêlage - premier œstrus détecté : l'usage de l'insémination artificielle (qui traduit l'intérêt de l'éleveur pour la détection de l'œstrus) et une durée quotidienne d'observation de l'œstrus au moins égale à une heure. Les vaches en stabulation entravées ont été vues en chaleurs en moyenne plus tard que les autres. Du point de vue du développement agricole, les résultats montrent que de nombreux éleveurs n'ont pas adapté suffisamment leurs pratiques de reproduction à une période de vêlage plus précoce. Il y a matière à amélioration, dans la mesure où le protocole d'observation des œstrus ne remplit pas les conditions optimales dans de nombreux cas. © Inra/Elsevier, Paris.

bovin allaitant / œstrus / reproduction / épidémiologie/ facteur de risque

#### 1. INTRODUCTION

Date of birth is one of the major components of the market value of beef calves in France [10]. Therefore, calvings are planned earlier and earlier in the winter and even in the fall, compared to the traditional calving period which is in the spring. The consequence is an increasing problem of postpartum anestrus during the housing period [21], which affects fertility [27] and results in an extended calving interval [3].

True anestrus, expression of estrus by cows and heat detection efficacy by farmers all influence post-partum observation of estrus [1]. Among them, a poor estrus detection is said to be one of the main causes of long calving intervals [11], and its effect on heat detection is even higher when considering post-partum depression of estrous behavior. Various environmental and social factors [1] can partially inhibit estrous behavior and these negative effects have to be counterbalanced by a very accurate and successful heat detection protocol. Estrus detection in beef cows was not a major con-

cern when calvings occurred during the spring because breeding was mostly with the bull during the pasture period. However, the quality of heat detection becomes a key factor in a winter calving option; in that case, breeding must take place during the housing period and the farmer plays an important role. Cattle are known to have variable post-partum anestrous periods [3], and suckled beef cows are said to have a longer anestrous period than milking cows [8]: however, in beef cattle, few studies [7] have described the distribution of the interval from post-partum to heat detection. Given the reproductive physiology and the estrus behavior of the cow, different authors have proposed guidelines for heat detection protocol [1, 11]. Some studies have been carried out to assess the sensitivity and specificity of estrous detection by farmers [18], mainly in dairy cattle. In beef cattle, neither the current practices of farmers nor their effect on heat detection have been reported.

The purpose of this large-scale field study was to analyze beef-cow post-partum

anestrus under field conditions. The study focused on the winter period when the cows are housed, because post-partum anestrus is an acute problem during that period, before cows are turned out to pasture [23]. The first part of the study which concerned the risk factors for anestrus (determined by the progesterone assay) was published previously [6]. Estrus detection by the farmer, based on his current and usual practices, was the subject of interest for the second part of the study. The goals were two-fold: first, to describe the initial time of estrus detection after calving, and the relationship between heat detection and true cycling status 2 months after calving and second, to examine specific estrus detection strategies practised by farmers, and their relationship to the initial estrus detection after calving.

#### 2. MATERIALS AND METHODS

#### 2.1. Study design

A longitudinal and prospective study [17] was carried out under field conditions to analyze both anestrus and heat detection. The study involved a pluriprofessional workgroup composed of veterinarians, inseminators, technicians and scientists. They defined the hypotheses, assembled the protocol and the questionnaires and tested them for comprehension and accuracy, and discussed the results. The data collection on the farms was performed by 44 participants (same professions as the workgroup) that were given a 1-day training session about the study goals, the protocol and questionnaires, and the body condition scoring.

The study was carried out from the fall of 1992 to the end of the spring of 1993. The subjects of interest were the cows that calved in fall and winter. Cows that were housed at calving and were turned out to pasture within 2 months of calving were excluded from the study. Thus, two kinds of cows were represented; those that were housed for a 2-month period after calving and those that had access to pasture throughout the study.

#### 2.2. Study population

A sample of 60 farms was selected [25] from the farms proposed by 44 surveyors (veterinarians, inseminators, advisors and scientists) who participated in the project. All farmers were volunteers and, in order to be included, each farm had to have at least ten calvings between October and February and facilities to confine the cows for sampling. Farms using artificial insemination (AI) (75 %) as well as farms using bulls for breeding were included in the study. Even farmers using a bull had to observe the heat when the cows were housed, especially in tie-housed farms. This was the case for the winter, the period of interest for this study. These farms were located in the Rhône-Alps region in France, in a traditional area for beef-cattle production.

On each farm, all cows calving between October and March (up to a maximum of 30 per farm) were followed up if they were still housed 2 months after calving.

#### 2.3. Data collection

For each cow, the farmer noted breed, parity, date of calving, calving circumstances (difficulty, complications), suckling type, housing characteristics and feeding method. In addition, he reported the daily observed heats on a calendar posted in the barn. The farmer also gave descriptive information about his farm, his beefcattle enterprise and the feeding and breeding methods. Among others, he completed a questionnaire about his practices in heat detection. This was performed at the beginning of the calving period in the presence of the surveyor. The usual habits were explored. They concerned the persons involved, the daily observation schedule, and those important signs considered for heat diagnosis (excitability, vulva characteristics, immobilization response [1], mounts others, etc.). Each farm was followed up by a surveyor who visited the farm once a month to check the quality of the farmer's records and, during the requisite period, also took blood samples for progesterone measurement.

#### 2.4. Samples and assays

The anestrus determination was based on progesterone measurements [22, 23], either from serum (n = 744) or milk (n = 134) samples [24].

The method used for these assays from milk [28] or serum [14] samples has been detailed in a previous paper [6]. The first progesterone measurements were performed  $60 \pm 5$  days after calving. If the result was not positive (progesterone concentration < 2 ng/mL), a second measurement was performed  $9 \pm 2$  days later. The cow was considered to have cycled if at least one of the two measurements was positive, and was otherwise considered to be anestrous.

#### 2.5. Analysis

Estrus detection after calving was described at the animal level by the time interval between calving and first heat observed by the farmer. Cows that were not seen in heat by the farmer (due either to anestrus, silent heat or poor observation) were classified as 'no heat observed'. Data about the first observed heat was then compared to referenced progesterone measurements performed 2 months after calving. The percentage of cows that were not seen previously in heat was determined, based on progesterone assays, among cycling cows. The percentage of those

cows that the farmer reported to have seen in heat was determined, based on progesterone assays, among anestrous cows.

The protocol for estrus detection used by the farmer was described at the farm level. An analvsis was then performed at the cow level, to study the relationships between factors relative to the heat detection protocol and the time interval from calving to the first observed heat. Main factors were the number of heat observation periods per day, time of the first daily observation, time of the last daily observation, first-to-last daily observation delay, length of each observation period, overall daily time spent for heat detection, number of people involved in heat detection, breed, parity, body condition at calving and change in body condition within 2 months of calving. Those cows that were never seen in heat after calving were declared censored 6 months after calving, when heat detection was no longer an important issue.

A two-step analysis was performed, using the hazard ratio [2] as the relationship estimator. The hazard ratio is interpreted similarly to a risk ratio [16]. A hazard ratio of 1.3 for the daily time spent for heat detection (*table I*) indicates that cows

**Table I.** Distribution of factors and conditional hazard ratios for the interval from calving to first observed estrus.

Factor	Category	No. of cows	Hazard ratio <sup>a</sup>			nce P
Daily time spent						
J 1	0-30 min	370	1.3	1.0	1.6	0.02
	31–60 min	335	1.4	1.1	1.7	0.003
	more than 60 min	173	1 (ref)			
Anestrus (pro	gesterone)					
	no	593	1 (ref)			
	yes	285	2.5	2.1	3.0	0.0001
Two main estr	rus signs					
	immobilization	501	1 (ref)			
	excitab, or vulva characteristics	312	1.0	0.9	1.2	0.8
	mounts or no specific sign	65	2.4	1.6	3.6	0.0001
Housing type						
2 71	tie housing	229	1.6	1.3	2.0	0.0001
	loose housing	355	1.0	0.8	1.2	0.9
	access to pasture	294	l (ref)			

ref: reference category of the factor.

Sample: 878 cows (621 events, 257 (29.3 %) censored).

Likelihood ratio test = 182, 7 df, P = 0.0001.

<sup>&</sup>lt;sup>a</sup> The hazard ratio is analogous in interpretation to a risk ratio [16] (see explanation in section 2.5).

being observed for heat from 0 to 30 min per day have a 1.3 times higher probability not to have been seen in estrus compared to cows that have been observed for more than 60 min every day (reference group). If the confidence interval of the hazard ratio of a category includes 1, the hazard for this category is not significantly different from those of the reference category. A univariate analysis was first carried out in order to screen the existing relationships. The variable of interest was a time interval (days) and was checked using survival curves [25]; for each category studied, these curves described the percentage of cows not yet seen in estrus, against the time since calving. Log-rank statistics [25] were used to test the difference in the curves and were performed with SAS software (LIFEREG procedure [26]).

The significant factors (P < 0.20) linked to the calving-to-first-observed-estrus interval were then incorporated in a multivariable analysis performed with the Cox proportional hazards model [2] (SAS software, PHREG procedure [26]). Several potential confounders were introduced in the model because they can interfere with the effect of heat detection procedure: cycling status (based on progesterone assays) determined 2 months after calving, in order to adjust for a real anestrus problem; the housing type and the most important signs considered by the farmer to diagnose estrus. The assumption of equality of the hazard ratio over time was checked graphically [12] with a Log[–Log (survival function)] versus Log (time) plot. The curves for the different cow categories were roughly parallel and the hazard ratio was assumed to be equal. Only confounders and factors linked to the calving-to-firstobserved-estrus interval that were significant (P < 0.05) were considered in the final result.

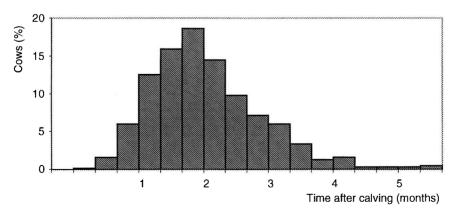
#### 3. RESULTS

#### 3.1. Description of the farms and cows

The average herd size was 48, and 12 % of the herds had more than 70 cows. Only 33 % of the farms were specialized in beef production; the others had complementary operations such as dairy or poultry enterprises. Among the 878 cows followed, 304 (34.6 %) were primiparous; the others ranged from parity 2 to 12. Ninety-one percent were of the Charolais breed, 5 % of the Limousine breed, and the others of mixed breeds. Eighty-nine percent of the cows calved before February. Different housing types were represented: 26 % of the cows were kept in tie housing, 40 % in loose housing and 34 % had free access to pasture.

#### 3.2. Estrus detection after calving

The distribution of the calving interval to the first observed estrus is shown in *figure 1*. Seventy-one percent of the 878 cows followed up were seen in heat by the farmer within 6 months of calving. This figure



**Figure 1.** Distribution of the interval from calving to first observed estrus (878 cows).

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relates to the observation made by the farmer; it does not represent the real anestrus rate. The interval from calving to the first observed heat ranged from 9 days to more than 5 months. Among the cows that were seen in estrus by the farmer, half had their first observed heat less than 56 days after calving and the average date was 61 days post-calving.

Among the 593 cows that cycled within 2 months of calving, based on progesterone assays, 44 % were not seen in heat by the farmer within 2 months post-calving. Furthermore, among the 285 anestrous cows, 11 % were judged by the farmer to be in estrus within 2 months of calving.

#### 3.3. Heat detection protocol

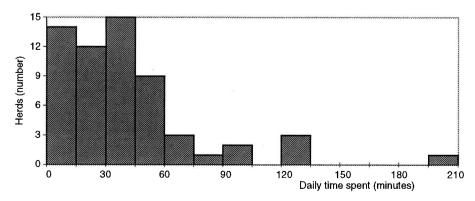
Among the 60 surveyed farms, 25 % used mating with a bull exclusively (mainly pasture-bred), 23 % used artificial insemination exclusively and 52 % used both. The estrus detection protocol varied considerably from farm to farm. The two main signs of estrus considered by the farmer were related to the immobilization response (51 % of the farmers), to physical characteristics of the vulva only (congestion, clear or bloodtinged mucous discharge) (22 % of the farmers), to excitability only (irritability, bellowing) (3 % of the farmers), or to physical

characteristics of the vulva and excitability (12 % of the farmers). The remaining 12 % of the farmers either considered those cows that mounted others to be in heat, or they did not pay much attention to heat detection. The number of persons from the farm involved in heat detection ranged from zero to five and the average was 1.4.

The heat detection schedule also differed greatly from farm to farm. The number of daily periods devoted to heat detection ranged from zero to eight; 22 % of the farms passed less than two times a day and the median was between 2 and 3. The hour of the first daily period ranged from 6 a.m. to 2 p.m.; the hour of the last period ranged from 8 a.m. to 10 p.m. (which also includes those farmers who had only one period of observation). The interval between the first and last daily observation periods ranged from zero to 14 h, and the median was 8 h. The overall time spent each day in heat detection is shown in figure 2. It ranged from zero to more than 3 h and was on average 40 min. Nearly half of the farmers (43 %) spent less than 30 min per day checking the cows.

### 3.4. Estrus detection efficacy

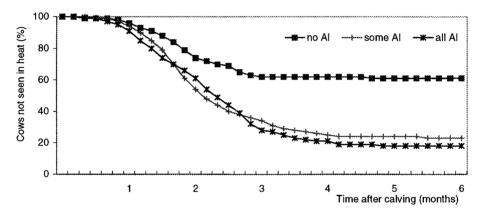
The use of artificial insemination obviously appeared to be closely linked to estrus



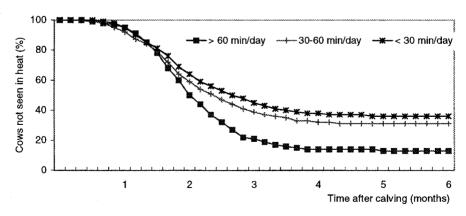
**Figure 2.** Distribution of the overall daily time spent by farmers in estrus detection (60 farms).

detection (*figure 3*). Only half as many cows were seen in heat, and at later periods, by the farmers who did not use artificial insemination compared to farmers using artificial insemination systematically or from time to time. This relationship remained significant when adjusted for confounding factors.

Among factors related to estrus detection strategy such as the number of observation periods or the number of persons involved, the overall daily time spent for heat detection was the only factor significantly linked to the calving-to-first-observed-heat interval (*figure 4*). The relationship remained significant when adjusted for real anestrus status (determined by progesterone assays 2 months after calving), housing type and main signs of estrus considered by the farmer (*table I*). The longer the time spent in heat detection, the smaller the interval from calving to first observed heat, and 1 h or more gave the best heat detection.



**Figure 3.** Non-adjusted survival curves for calving to first observed estrus by herd breeding policy (878 cows).



**Figure 4.** Non-adjusted survival curves for calving to first observed estrus by the overall daily time spent for heat detection (878 cows).

#### 4. DISCUSSION

#### 4.1. Farm characteristics

Farms followed up were not chosen at random, the agreement and the participation of the farmer being necessary to collect the data. Farms varied with respect to the level of specialization in beef cattle, herd size and technological standards. However, they were, on average, larger than most beef farms in France; 53 % of the surveyed farms had more than 40 beef cows, compared to 10 % of all French beef farms in 1990 [9]. Farmers were also younger; only 15 % were more than 50 years old in the surveyed farms compared to 56 % in all French beef farms in 1990 [9] and, hence, they could be assumed to be technically more trained than the average. Therefore, caution is needed in extrapolating these data to all farms.

# **4.2. First estrus observation after calving**

Estrus detection was performed by the farmer under regular farming conditions. The 60 farmers used their habitual heat detection method, in terms of signs observed and detection schedule. We observed a wide range of more or less complicated methods used by the farmers, ranging from those who fulfilled a heavy and demanding schedule, to those who did not pay much attention to heat detection but noted the heat that they happened to observe when they fed or cleaned the cows.

Given these facts, the interval from calving to first observed heat ranged from 9 days to 6 months (recordings were up to 6 months), and averaged 2 months. This range is in agreement with Garverick and Smith's report in dairy cattle [8], even though estrus is usually said to be delayed in beef cattle, compared to dairy cattle [8]. Twenty-nine percent of the cows were not seen in heat after calving. However, the

cullings which occurred after 2 months postcalving were not recorded. There is no doubt that some cows were culled during this period, whereas they could have been in heat if not removed from the herd: therefore, some cows should have been considered in heat during the study. Thus, the percentage of cows not seen in heat may be overestimated. By first considering an annual culling rate of 18 %, previously reported in the same kind of herds [5], and second a uniform distribution of culling over the year, which is exaggerated because culling usually occurs just after calving or after weaning, we would obtain an estimated culling rate of 6 % (one third of 18 %) between 2 and 6 months post-calving (one third of the year). In that case, only 24 % instead of 29 % of the non-culled cows would not have been seen in heat in the study. This, however, does not strongly modify the result.

Reproductive efficiency requires that cows exhibit estrus cycles shortly after calving. For reproduction management efficiency, it is recommended that cows should be seen in heat within 60 days after calving [8]. Only 40 % of the cows in our study fulfilled this condition and the percentage was still poor (56 %) according to Cupps' recommendation within 80 days [3]. These poor results, however, should be relativized since the study was focused on the fall and winter periods, when calvings are usually followed by a longer anestrous period than calvings occurring in the spring.

# **4.3.** Estrus observation versus ovarian cyclicity

The comparison between estrus detection and ovarian cyclicity, determined by progesterone assays, showed that 44 % of the cycling cows were not seen in heat by the farmer by 2 months post-partum. Silent ovulation and quality of heat detection must be considered. Silent ovulation, also called silent heats [7], occurs when post-partum

ovulation is not associated with estrous behavior [1], and can reach 50 % for the first post-partum ovulation in dairy [15] and suckling cow [19]. This phenomenon may explain a large part of the 'missed estrus' because the time when the progesterone assay was performed corresponds to the peak (median and mode) of estrus detection (figure 1); however, given the study design, there is no precise way to evaluate this effect. Many inhibitory influences are reported to determine silent ovulation [1]. most of which are environmental and social factors. The second factor explaining that nearly half of the cycling cows were not seen in heat is the sensitivity of heat detection by the farmer. This aspect is discussed in section 4.4.

It also appeared that 11 % of the noncycling cows, based on progesterone assays performed 2 months after calving, were reported to be in estrus by the farmer. This indicates lack of specificity of the heat diagnosis which was reported to be equal to 87 % in dairy cattle [13]. Specificity problems are higher if farmers consider behavioral signs of estrus other than immobilization responses [1], such as mounting activity or irritability. This is particularly the case in tie housing [21]. Another possibility is that some cows might have cycled for a while, expressed estrus, and then moved to a second anestrous period; this situation has been reported for dairy cows by Opsomer et al. [20] but may be rare.

Another explanation for the difference between the progesterone assay and the observation of heat may be the quality of the progesterone assay, especially the sensitivity of the milk assay compared with the blood assay. The cows with the milk assay were at five times greater risk of real anestrus than were cows with the blood assay [6]. However, these assays were comparable in a preliminary study [24]. The main reason for the difference may be that some farmers with heavy anestrus problems preferred the milk sampling which was performed for all their cows. This may have

built an artificial relationship between the type of sampling and the anestrus risk.

#### 4.4. Heat detection protocol

The heat detection protocol used by the farmer differed greatly from farm to farm. For example, a quarter of the farmers had four or more estrus detection periods a day and spent more than 45 min per day; on the other hand, some farmers did not schedule any specific time for heat detection; they just looked at the cows during cleaning or feeding periods. Allrich [1] presents the usual recommendations for estrus detection: "to perform estrus observations three times per day for 20 to 30 min at each observation period". Considering these recommendations, it appeared that only 50 % of the surveyed farmers visited their cows a sufficient number of times each day, and only 17 % of them spent enough time observing. This can explain partly why nearly half of the cycling cows were not seen in heat by the farmers.

The breeding policy appeared to be of importance in explaining the heat detection efficacy. Farmers who did not use artificial insemination did not pay a lot of attention to heat detection, and hence did not detect heat successfully. An easier way for them to get rid of the task of heat detection was to use a bull, but this method cannot be applied easily when the breeding period is going on during the housing period, especially in tie housing. This observation, though obvious, is nevertheless important to take into consideration for continuing education and extension purposes. It means that planning the calvings earlier in winter does force traditional farmers to revolutionize their opinions and improve their practices in heat detection. Although the breeding policy was linked to the heat detection efficacy, it was not accounted for in the multivariate model. First, the breeding policy is highly related to the heat detection method: taking into account both variables should have induced

multicollinearity problems. Second, the breeding policy induces a more or less strong need for good heat detection, but choosing between AI or bull-mating does not represent a technical answer to better heat detection. However, if one includes the breeding system in the model, the factors relative to the heat detection method do not remain significant. Apart from the statistical problems exposed above, this may reflect that practices differ between farms practising AI versus farms not practising AI, the factors of interest in our study being only part of them.

The interval from calving to the first observed heat is influenced by three main factors: cycling status of the cow (related to true anestrus), estrus behavior (related to silent ovulation) and heat detection quality [1]. In order to study the last factor, we adjusted for the cycling status of the cow in the analysis, based on progesterone assays 2 months after calving. The second one (silent heats) could not be taken into account, and it is therefore possible that this decreased the ability of the study to identify the factors that influenced the quality of heat detection. Factors such as parity, calving assistance, suckling and nutrition were not taken into account because their role in heat detection is thought to be due to their effect on true anestrus. This was already considered in the analysis (a model including these variables instead of the estrus status gave exactly the same results). Furthermore, the analysis was also adjusted for housing type, which can affect the ease of heat detection [11], and for the signs of estrus considered by the farmer, in order to keep their effect on heat detection constant. Herd was not accounted for in the model. Taking it as a random effect in the Cox proportional model is not possible with usual statistical software, and considering it as a fixed effect requires 59 dummy variables that should have decreased the power of the analysis strongly. No interaction was analyzed because we did not identify any plausible hypotheses in the studied data. Among

factors related to heat detection protocol, only the overall daily time spent in heat detection by the farmer was significantly linked to the date of first observed heat after calving. The best heat detection occurred when farmers spent at least 1 h each day observing the cows. This fits in well with Allrich's recommendations (three times, 20–30 min each time) [1].

We did not find a relationship with the number of observations performed each day. even if the short duration of cattle estrus suggests that three observations or more are needed each day to avoid missing estrus [1]. McDougall and Hampson [18] reported the same lack of relationship with the number of observations. No effect of the number of persons involved in the heat detection, nor of the hours of first and last daily observations was found. Many environmental and social factors influence estrus expression, which ranges from long and well-expressed estrus to silent ovulation [1]: these factors differ from farm to farm. There is hence an interaction between estrus expression and heat detection protocol which greatly modifies their effects on heat detection. No definite recommendation can be made for heat detection schedule and method. As Allrich reports [1]: "so many factors change from one livestock enterprise to the next that cases have to be looked at individually. This is an important concept to remember because techniques that work well for one person will fail for his or her neighbor down the road". Hence it is not surprising to find contradictory results [4, 18] that depend on the study design and on the number of farms and observers involved.

#### 4.5. Method

From a methodological point of view, two aspects must be discussed. The first is that some cows might be lost to follow-up between 2 and 6 months post-calving because of culling; however, this information was not reported. As discussed previously, less than 6 % could have been in this situation. It was possible to study the potential bias that these lost-to-follow-up cows could induce in the analysis by considering what the survival curves should look like if all the culled cows belonged to one particular category of the studied variables. In fact, it appeared that it would not have significantly changed the results. A similar case is that of the undetected bull-bred pregnancies concerning those cows that were not seen in heat by the farmer. These data do not reflect the actual numbers of bull-bred cows at risk of first observed heat. This could explain why the curve remains horizontal after 3 months post-calving in 'no AI' farms (figure 3).

The second aspect of the methodology is the choice of the unit of interest in the analysis. One may suggest that the heat detection protocol is related to farm management and hence to farm level; it would, perhaps, have been preferable to study that question at the farm level instead of the cow level. However, there is no good farm-level outcome that properly reflects the interval from calving to first observed heat of the different cows in the herd. One way to perform such an analysis is to consider the percentage of cows that were seen in heat in the herd at a given date after calving. But, this leads to an important loss of information about the actual individual intervals. Nevertheless, a complementary farm-level study was performed and showed the same relationship between heat detection schedule and heat detection. However, this relationship was not significant, because the sample size of 60 farms did not allow sufficient statistical power for the analysis.

This field study clearly illustrates that part of the farmers did not adapt their reproduction management practices adequately to an earlier period of calving that implies a winter period of breeding, during the housing period. The consequence is that 44 % of cycling cows were not seen in heat by 2 months post-partum. In many cases, the heat detection protocol does not match the

required standards for optimal heat detection. There is room for improvement, based on heat detection efficacy and according to estrous behavior in the farm. Some of the farmers have better results, mostly those who were forced to improve heat detection efficacy in order to use AI. Among factors related to heat detection protocol, the daily time spent for heat detection appears to be important. These results need to be considered when extension is planned.

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