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Overview of progesterone profiles in dairy cows

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ABSTRACT

The aim of this study was to gain a better understanding of the variability in shape and features of all progesterone profiles during estrus cycles in cows and to create templates for cycle shapes and features as a base for further research. Milk progesterone data from 1418 estrus cycles, coming from 1009 lactations, was obtained from the Danish Cattle Research Centre in Foulum, Denmark. Milk samples were analyzed daily using a Ridgeway ELISA-kit. Estrus cycles with less than 10 data points or shorter than 4 days were discarded, after which 1006 cycles remained in the analysis. A median kernel of three data points was used to smooth the progesterone time series. The time between start of progesterone rise and end of progesterone decline was identified by fitting a simple model consisting of base length and a quadratic curve to progesterone data, and this luteal-like phase (LLP) was used for further analysis. The data set of 1006 LLP's was divided into five quantiles based on length. Within quantiles, a cluster analysis was performed on the basis of shape distance. Height, upward and downward slope, and progesterone level on Day 5 were compared between quantiles. Also, the ratio of typical versus atypical shapes was described, using a reference curve on the basis of data in Q1–Q4. The main results of this article were that (1) most of the progesterone profiles showed a typical profile, including the ones that exceeded the optimum cycle length of 24 days; (2) cycles in Q2 and Q3 had steeper slopes and higher peak progesterone levels than cycles in Q1 and Q4 but, when normalized, had a similar shape. Results were used to define differences between quantiles that can be used as templates. Compared to Q1, LLP's in Q2 had a shape that is 1.068 times steeper and 1.048 times higher. Luteal-like phases in Q3 were 1.053 times steeper and 1.018 times higher. Luteal-like phases in Q4 were 0.977 times steeper and 0.973 times higher than LLP's in Q1. This article adds to our knowledge about the variability of progesterone profiles and their shape differences. The profile clustering procedure described in this article can be used as a means to classify progesterone profiles without recourse to an a priori set of rules, which arbitrarily segment the natural variability in these profiles. Using data-derived profile shapes may allow a more accurate assessment of the effects of, e.g., nutritional management or breeding system on progesterone profiles.

1. Introduction

Increases in genetic potential for milk production, together with changes in nutritional management and herd size, have been associated with a decline in fertility of dairy worldwide [1,2]. Impaired fertility on a dairy farm is an increasingly important economic burden [3], and in conventional dairy herds, it is the main reason for culling [4]. To help improve fertility, progesterone measurements have been used to assess the reproductive status of the cow and to diagnose pregnancy [5]. These have now become

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available for on-farm use. The interpretation of progesterone measurements generally uses a set of a priori rules to convert the progesterone levels into the different stages of the reproductive cycle (e.g., luteal phase, follicular phase), and an evaluation of the "normality" of the cycle. In the late 1970s, these rules were on the basis of expert opinion, but the development of ELISA methods and later automated in-line systems made it possible to process data creating more explicit reference profiles [6,7]. These can be used to determine whether a cow is in estrus or not, and what the quality of that estrus is, on the basis of what is assumed to be a normal or reference progesterone profile.

The earlier studies that have examined progesterone profiles generally discarded abnormal cycles according to the a priori rules without describing them [8,9]. This may lead to the discarding of a substantial part of the data set: [10] reported an incidence of abnormal progesterone profiles of 44%, an increase of 12% compared to 20 years earlier. Given, the increase in abnormal progesterone profiles with increasing milk yield and dry matter intake [11], these profiles matter in practice (maybe even more so in the future). Therefore, there is a need to consider such cycles and to re-evaluate the global variability in cycle length and shape. Accordingly, this study uses a naïve approach to study progesterone profiles, i.e., with as few a priori rules as possible. The aim of this study is to gain a better understanding of the variability in the shape and features of all progesterone profiles during estrus cycles in cows and to create templates for estrus cycle shapes and features. In the future, these templates may be used as a benchmark for other farms or studies using progesterone profiles.

2. Materials and methods

2.1. Data description

Data were collected from milking cows in a research herd at the Danish Cattle Research Centre in Foulum, Denmark, between 2002 and 2009. The cows on this farm were Holstein, Danish Red, or Jersey cows. Cows were milked by an automatic milking system, with a mean number of 2.4 visits per day. During the first 120 days from calving, one progesterone measure was to be made daily in 2002 to 2005 and once every other day in 2006 to 2009. In 2002 to 2005, for the remainder of the lactation, cows were sampled every other day.

Progesterone was analyzed using the Ridgeway ELISA-kit (Ridgeway Science Ltd, Gloucestershire, UK). Milk samples were pipetted, diluted, and distributed using a Biomek 2000 (Laboratory Automation Workstation, Beckman Coulter, Fullerton, CA, USA). Milk samples (25L, diluted 1 + 2with water) were handled according to the manufacturer's instructions; however, incubation receptor 4 was increased to 1 hour 30 minutes. Plates were read using a spectrophotometer/fluorometer at 575 nm (Fluostar, BMG Labtechnologies, Offenburg, Germany). Analyses were performed in 96-well plates; two sets of seven standards (0–30 ng/mL), locally made using milk from an ovariectomized cow and ethanolic progesterone solutions, and for every analysis and plate two sets of two control samples were used. For the low and high controls, the intra-assay precision (coefficient of variation %) was 14.9 and 1.4, respectively; the interassay precision (coefficient of variation %) was 32.7 and 20.1, respectively; and the average inaccuracy (bias) was +0.82 and 0.60 ng/mL, respectively. Because the measurement method is optimized for low values of progesterone, values higher than 30 ng/mL were replaced by 30 ng/mL.

When a gap of over 14 days without progesterone measurements was present in a given lactation, data after this gap were excluded. Lactations with less than five measurements after progesterone increase (i.e., after detecting the end of the postpartum period) were excluded from further analyses. This step discarded 5.8% of the lactations.

Farm staff did not have access to the progesterone data for the purpose of reproductive management, thus decisions to inseminate were independent of progesterone records. Estrus was detected using activity meters (DeLaval, Tumba, Sweden) and two times per day 20 minutes visual detection. Estruses that occurred within 35 days from calving were not inseminated. When cows were detected to be in heat in the morning, they were inseminated in the afternoon. Otherwise, they were inseminated the next morning.

2.2. Estrus cycle detection

All statistical analyses were performed with R (R Core Team [2013]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/). For the sole purpose of detecting estrus cycles, all data points in one lactation were smoothed using b-splines (see R/smooth R for detail). B-splines take given data points and use them to create a smoothed curve. As shown in Supplementary Figure 1, this smoothing method is accurate for detecting inflection points (i.e., points where the velocity or the acceleration is null). These inflection points can be used to cut the lactation into cycles. Smoothing failed on a single lactation because this lactation did not contain enough data to compute b-splines, and this lactation was discarded.

As described by Gorzecka et al. (2011), estruses were identified as points of zero velocity with the velocity crossing from negative to positive values at a smoothed progesterone value of less than 8 ng/mL [8]. To filter out trivial undulations, points of zero velocity were only considered if velocity had exceeded a threshold of 1 ng/mL/day because of the preceding zero velocity point. The end of the postpartum anestrus period was defined as the first point on the smoothed profile, where progesterone was greater than 3 ng/mL immediately before the first two consecutive progesterone measures greater than 3 ng/mL. Throughout the following text, "estrus" refers to points in the progesterone profiles identified with the previous procedure.

Per lactation, all estrus cycles from the end of the postpartum anestrus until the last estrus were detected. Cycles with less than 10 data points and cycles shorter than 4 days were discarded because they contained too few data points for analysis. Also, cycles at the end of lactation were discarded if measurement stopped before the cycle ended. The remaining cycles were kept.