Progesterone in milk - investigations on practicability as a functional trait in dairy cows

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Abstract

The interval from calving to commencement of luteal activity (CLA) was determined by progesterone measurements from milk samples obtained once weekly until 14[th] week postpartum in 678 lactations of 513 German Holstein cows in first to third parity. Milk samples were analyzed by an "on-farm" device (eProCheck®, Minitüb) and simultaneously by RIA. The threshold value for CLA was determined at a progesterone concentration >5 ng/ml milk. Milk progesterone concentrations of "on-farm" measurements correlated with measurements done by the RIA-method significantly ($r=0.72$; $P<0.001$). Within the analyzed herd interval from calving until first rise of progesterone averaged 5.6 ±2.4 weeks. 100-d milk yield of cows was used to analyze the impact of performance. Milk protein content was determined on basis of data from the first milk recording postpartum which were standardized on the day of lactation by linear regression. To estimate the influence of body condition on CLA change in back fat thickness (BFT) from 1[st] to 2[nd] milk recording was used. There was not found a significant influence of milk yield on CLA. Cows with lower 100-d milk yield (<3,000 kg) tended to have a 1 week earlier first luteal activity than cows with 3,000 to 4,000 kg milk ($P=0.1$). However, milk protein content showed a significant impact on CLA. Cows with a protein content of ≤3.5 % at 1[st] milk recording revealed first luteal activity 1.3±0.3 weeks later than cows that had a content of >3.75 % protein ($P<0.01$). Change in BFT also had a significant influence on CLA ($P<0.05$). Cows with decreasing back fat (more than 7 mm) showed first luteal activity 1.1 weeks later than cows with increasing BFT. In conclusion, milk progesterone is a practical indicator for fertility in dairy cows. Phenotypic impact of milk yield on fertility cannot be confirmed regarding to CLA. Negative energy balance after calving is more detrimental for the cyclical activity as was shown by the parameters milk protein content and change in BFT.

Keywords: dairy cow, milk progesterone, commencement of luteal activity, milk yield, protein content, body condition

Introduction

In dairy herds noninvasive methods to identify functional traits have a great importance. These methods cause no further stress for cows and it is an easy way to generate data for

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analysis. Such a noninvasive method is the measurement of progesterone concentration in milk for detailed information about fertility status of a cow.

It is a crucial aim of cattle breeding to characterize significant reproductive performances to find suitable traits to evaluate fertility. More frequently used reproductive traits are age at first calving, calving interval, days open, number of inseminations, and insemination interval etc.. However these traits reflect also the influence of management factors. Different references (Forster et al., 2007; Stevenson et al., 2007; Sheldon and Dobson, 2004) discuss ovarian, behavioral or uterine traits to evaluate more accurate the phenotypical reproductive status of cows. Observed traits should have high heritabilities which could be used for genetic selection. Such a parameter could be "commencement of luteal activity" which is determined by progesterone measurements. Estimates of heritability for CLA range from 14 to 28 percent (Darwash et al., 1997; Veerkamp et al., 1998; Royal et al., 2002). Consequently it is four to five times higher than fertility indices which are introduced in estimation of recent breeding value. Scientific studies discuss milk yield, protein content and body condition as crucial parameters on CLA (Patton et al., 2007; Wathes et al.; 2007; Windig et al.; 2008). Major aim of this study was to characterize the effect of milk yield, protein content and body condition on development of CLA. Furthermore, the repeatability of CLA in next lactation should be considered. Additionally influence of parturition and occurrence of endometritis are of substantial interest.

Material and method

Animals and sample collection

From November 2009 to December 2011 milk samples were taken from German Holstein cows for progesterone (P4) measurement. These cows were kept on a dairy farm with free-stall housing in Mecklenburg-Western Pomerania/Germany. Starting from the 3rd week until the 14th week post partum premilking samples from 513 cows of the first up to the third lactation were taken once a week. A total amount of 678 lactations was analyzed. In 2011 the herd reached a mean performance of 10,602 kg milk per cow containing 4.07 % fat and 3.39% protein.

To analyze the impact of performance 100-d milk yield was used. Milk yield for each cow was measured daily by a milkmeter and all records for every lactation were summarized to 100-d milk yield. Milk protein content was determined on the basis of data from first milk recording which were standardized on day of lactation by linear regression. Furthermore, diagnosis and treatment data which concern endometritis were extracted from herd management software (AGROCOM SUPERKUH, CLAAS Agrosystems, Germany) and also included into evaluation.

To estimate influence of body condition on CLA changes in Body-Condition-Score (BCS) and back fat thickness from first to second milk recording were used. These official milk records were performed once a month by the LKV Mecklenburg-Vorpommern (Güstrow, Germany) to evaluate the herd within the breeding program of the breeding organization. Average first and second official milk records were on d 23 and d 57 post partum respectively. This represents the most sensitive time at the beginning of the lactation. BCS was assessed by using a 1 to 5 scale where a score of 1 indicated a skinny condition and a score of 5 an obese condition (Edmonson et al., 1989). BFT was measured by ultrasound examination at the sacral region as described by Staufenbiel (1992). A portable ultrasound generator with a linear transducer (Proxima Pavo, Proxima Medical Systems; Germany) and a frequency of 6.0 MHz was applied. Both body condition parameters were determined once a month by the same person at each milk recording. Evaluation of the parturition was included in the study. It was distinguished between spontaneously delivered calves and those delivered by mild to severe extractions or caesarean section, respectively. Additionally these observations were accompanied by control of the puerperium of cows. Involution of uterus and presence of pus in vagina were used for diagnosis of endometritis (Sheldon and Dobson, 2004).

Progesterone analysis

In total, 7,662 milk samples were analyzed by an "on-farm" device (eProCheck®, Minitüb, Germany) which is based on an ELISA method. Simultaneously 7,650 milk samples were analyzed by using RIA as described in Blödow et al. (1988). Threshold value for CLA was determined at the first week post partum where the P4 concentration exceeded 5 ng/ml milk. Intra-assay and inter-assay coefficients of variation ranged from 4.5-9.5 and 9.7-15.6 respectively for the "on-farm" ELISA. The intra-assay and inter-assay coefficients of variation were 8.0 and 9.6 respectively for the RIA. Limit of sensitivity for RIA, using a 50 µl milk sample, was 8 pg/ml.

Statistical analysis

All calculations were carried out using SAS version 9.2 (SAS Institute, 2008). Evaluation of factors associated with CLA was performed by MIXED procedure of SAS. The model included fixed effects on milk yield, protein content, parity, calving ease and endometritis as well as a combined year-season effect. A second model included change in BFT and BCS from first to second milk recording and also veterinarian treatments because of endometritis and year-season as fixed effects. Models were adjusted for multiple lactations within the same cow by use of a compound symmetry covariance structure. Multiple comparison adjustment for the pairwise difference in least square means was performed by using the Tukey-Kramer option in SAS. Statistical significance was considered at P-value <0.05.

Results

Results for P4 concentrations of both methods are presented in Table 1. The difference in means between RIA- and "on-farm"-progesterone concentrations in premilking amounted to 1.5 ng/ml. Milk progesterone concentrations of "on-farm" measurements correlated with measurements done by the RIA-method significantly ($r=0.72$; $P<0.001$). Within the analyzed herd the interval from calving until the first rise of progesterone averaged 5.6 ±2.4 weeks. Repeatability of CLA showed a coefficient of $\omega^2=0.34$.

Table 1: Means of progesterone concentration for "on-farm" ELISA and RIA

	Number of samples (n)	Mean (ng/ml)	Standard-deviation	Minimum (ng/ml)	Maximum (ng/ml)
on-farm ELISA	7,662	6.9	5.9	0	36.4
RIA	7,650	5.4	4.6	0	45.7
difference |on-farm ELISA-RIA|	7,640	1.5	4.1	0	38.6

Measured milk yield tended to have an influence on CLA (Table 2). Cows with low phenotypical 100-d milk yield (<3,000 kg) demonstrated first luteal activity earlier than cows that had an output of 3,000 to 4,000 kg milk (4.6±0.4 weeks vs. 5.6±0.3weeks; P=0.1). Within performance categories of 3,000-4,000 kg milk, 4,000-5,000 kg milk and above 5,000 kg milk within 100 days no significant differences could be observed. Milk protein concentration had a significant (P<0.01) impact on CLA. Cows with a protein content of ≤3.5 % revealed first luteal activity 1.3±0.3 weeks later than cows that had a content of >3.75 % protein (6.2±0.3 weeks vs. 4.8±0.2 weeks). Among categories 3.5-3.75 % protein and >3.75 % protein no significant difference could be observed.

Parity tended to have an effect on the CLA. Primiparous cows showed first luteal activity approximately one week later (0.8±0.4 weeks; P=0.06) than multiparous cows. Cows that were medicated due to endometritis within the first 100 days p.p. presented a significant longer interval until the CLA than cows without this diagnosis (5.1±0.2 weeks; P<0.05). Furthermore, cows with assisted calving or dystocia presented significantly later CLA than cows which required no help during calving process (5.8±0.2 weeks vs. 5.2±0.2 weeks; P<0.05). Change in BFT had a significant influence on CLA. Cows with decreasing back fat (≤7 mm) within the period from first to second milk recording, showed first luteal activity 1.1 weeks later than cows with increasing back fat (≥0 mm) (6.1±0.2 weeks vs. 5.0±0.3 weeks; P<0.05). Cows with moderate BFT decrease from -6 mm to -1 mm were not significantly different from mentioned categories. In contrast change in BCS from first to second milk recording had no significant effect on CLA.

Table 2: Influence of 100-d milk yield, milk protein content and change in BFT on commencement of luteal activity

100-d milk yield (kg)	n	CLA[1] (weeks postpartum)
< 3,000	57	4.8 ± 0.4
≥ 3,000 to < 4,000	187	5.6 ± 0.2
≥ 4,000 to < 5,000	160	5.7 ± 0.2
≥ 5,000	59	5,8 ± 0.3
Milk protein content (%)		
≤ 3.5	119	6.3 ± 0.3[a]
> 3.5 to ≤ 3.75	202	5.3 ± 0.2[b]
> 3.75	170	4.9 ± 0.2[b]
Change in BFT (mm)		
≤ -7	95	6.2 ± 0.2[a]
> -7 bis < 0	272	5.5 ± 0.2[ab]
≥ 0	62	4.9 ± 0.3[b]

[1]LSMeans ± SE

[a,b] Columns with different superscripts differ significantly (P<0.05)

Discussion

In the present study influences of production traits and body condition on commencement of luteal activity were observed. CLA determinations were realized by progesterone measurements in milk samples. Overall 7,662 milk samples were analyzed by “on-farm” ELISA and 7,650 milk samples by RIA. Correlation between these two methods was high (r=0.7). Chang and Estergreen (1983), Hoedemaker et al. (1984) and Mitchell et al. (2004) found similar correlations among progesterone concentration of the RIA- and ELISA-method ranging from r=0.7 to r=0.9 with regard to an ELISA Test Kit which was used under laboratory conditions. Nebel et al. (1987) tested an “on-farm” ELISA in comparison to RIA and detected a correlation of r=0.79.

Average interval until CLA of 5.6 weeks coincided with results of Martin et al. (2011). Patton et al. (2007) and Garmo et al. (2009) stated that cows had a shorter interval from calving to CLA (4.4 and 3.7 weeks, respectively) because of an earlier start of milk sampling for progesterone measurement to determine CLA in the first or second week after calving.

Another question was whether CLA of a cow is repeatable in following lactation. However, repeatability of CLA over cow’s lactations could not be confirmed. The separate consideration from 1st to 2nd and from 2nd to 3rd lactation showed moderate and low repeatability coefficients, respectively which might be due to small number of cows with two recorded lactations. Overall 49.2 % of cows showed deviations of CLA in following lactation of ± 1 week. Therefore, environmental influence on a cow’s CLA, such as puerperal diseases, is more likely than influence by its own performance and genetics.

There was no significant impact of milk yield on CLA. In scientific studies influence of milk yield has been diversely discussed. Patton et al. (2007) could not determine a phenotypic relation between milk yield and CLA. Wathes et al. (2007) found that cows with a CLA on d 42 post partum claim a higher peak of milk yield than cows with a CLA on d 24. A significant genetic correlation between predicted milk yield and natural logarithm of CLA could be detected by Royal et al. (2002). Pollott and Coffey (2008) compared two genetic lines regarding CLA, the selection line derived from the highest genetic merit bulls, the control line maintained at average of genetic merit. Control line cows showed first luteal activity 6 days before selection line cows.

Cows with a milk protein content ≤3.5 % presented delayed first luteal activity. Patton et al. (2007) and Windig et al. (2008) confirm a significant influence of milk protein concentration on first luteal activity. Cows are relatively inefficient at converting dietary crude protein into a usable N source because of fermentative activity of microbial population in the rumen. Important for protein synthesis is a well-adjusted relation between microbial and undegradable protein. Reduced milk protein often reflects insufficient ratios and/or a negative energy balance.

Concerning influence of parity on CLA own results correspond with results of Wathes et al. (2007) who also detected a significantly later CLA for primiparous cows. Primiparous cows are still in growth. Physiological stress due to milk production and continuous growth could be a reason for delayed onset of luteal activity of primiparous cows.

Cows with assisted calving or dystocia showed delayed CLA. In the study of Royal et al. (2002) influence of metritis and dystocia could be proved as significant. Furthermore, no influence of placental retention on first luteal activity could be determined (Royal et al., 2002).

According to Wathes et al. (2007) cows with a lower BCS in week 7 post partum showed a higher probability for a longer interval until the CLA. Loss of BFT from first to second milk recording can be explained by mobilization of body fat due to a negative energy balance.

Results of Patton et al. (2007) and Stürmer (2010) indicate that a high dry matter intake during early lactation was connected with an early onset of ovarian cyclical activity. In this observation cows of the category ≥0 mm back fat mobilization can be assumed to have a higher compensation of negative energy balance due to a higher feed intake. This is reflected in a short interval until CLA.

In conclusion, a genetic relationship between milk yield and fertility could be detected in other studies (Royal et al., 2002; Pollot and Coffey, 2008; Windig et al., 2008), but phenotypic impact of milk yield on fertility cannot be confirmed by own results regarding CLA. Phenotypical milk yield only tends to have an influence on CLA. A severe or even a moderate negative energy balance after calving is more detrimental for cyclical activity shown by parameters milk protein content and change in BFT.

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