THE DAIRY COWS HEAT ABILITY ASSESSMENT BY CHANGES OF PROGESTERONE CONCENTRATION AT POSTPARTUM

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Abstract. The objectives of the current study were to determine clinical value of changes in progesterone levels when evaluating cow's ability to estrus. Overall, 25 Lithuanian Holstein cows from one commercial dairy herd in Lithuania were studied. The progesterone level fluctuation (profile) in milk was detected with semi quantitative methods every five days, over the 20 to 90 DIM periods. Cows were monitored for estrus an hour before the morning milking, an hour after evening milking and 15 minutes after the noon milking. Cows in heat were identified by common clinical signs. Cows were divided into two groups of 25 animals: group 1– estrus was observed up to 90 days after calving, group 2 – estrus was not detected until 90 days after calving. Milk yield was collected and its composition determined during milking every 30 days starting on day 30 after calving.

The peak concentration of progesterone was observed on the average on the 30th, 45th, 70th and 90th day after calving. Progesterone concentrations varied in a similar way in both groups. In the group with estrus, the concentrations of progesterone (8.2 proc. $p<0.05$) and milk yield (5.6 % $p>0.05$) were higher. Statistical correlation between progesterone levels and cows yielding was not determined ($r=0.2$, $p>0.05$). Atypical (unrepresentative on estrus cycle) rise in progesterone levels was detected on the 70th day after calving. This might be associated with higher milk yield. Progesterone changes are indicative of cows ability to estrus. If elevated progesterone levels characteristic to estrus cycle are detected and reach maximal values, one might expect that cow's estrus will start on the 90th day after calving.

Keywords: progesterone, estrus cycle, cow

Introduction. Impaired progesterone metabolism is one of the main reasons for disturbed conception in cows (Hansen, 2011). The rise in progesterone concentration is associated with the rise in estrous activity and follicle formation. Progesterone concentration remains low (<2ng/ml) after calving and rises after first ovulation and the formation of corpus luteum. The first ovulation can occur on the 12th day. The increased progesterone concentration (>7ng) indicates the formation of corpus luteum. Another heat might be expected in about 17 days. According to A. J. H. Stronge et al. (2005), the optimal progesterone concentration for successful conception is 7–8 ng/l. G. E. Lamming and Darwash (1998) state that monitoring of progesterone concentration in milk is an objective and precise method to evaluate the function of ovaries after calving and to determine the exact time for insemination. Heifers have higher progesterone levels in comparison to milking cows (Rizos et al., 2010). This difference can be explained by the peculiarities of cow metabolism (Sangsritavong et al., 2000; Sangsritavong et al., 2002; Vasconcelos et al., 2003) and the formation of corpus luteum (Pretheeban et al., 2010). The reproductive qualities decrease with the increasing milk yield.(Lucy et al., 2001; Stronge et al., 2005). Low levels of progesterone might influence the conception (Wolfenson, 2006). However, P. Hansen (2011) reports that the fertility (conception rates) does not depend on the amount of milk. Other authors have noticed the negative correlation between the levels of progesterone and milk yield 15 days after the insemination (Wiltbank et al., 2001; Hommeida et al., 2004). Atypical fluctuations of progesterone levels do not depend on the age and lactation status of the cow (Peterson, 2007). Early ovulation and changes in progesterone concentration are associated with milk production and the duration of estrus cycle (Kafi et al., 2010). When cows are taken care of, they tend to go into heat 25–30 days after calving. During the first month after calving, about 18–30% of cows conceive again. Insemination is recommended during the second heat and for record-holders – during the third heat (Rajamahendran et. al., 1993).

The **aim of the study** is to determine the prognostic value of changes in progesterone levels in respect to reproductive function of postpartum cows in heat and not in heat 90 days after calving.

Methods and materials. The study was conducted in 2010 in a dairy farm according to the Law on the Care, Keeping and Use of Animals, No. 8-500 of the Republic of Lithuania (legislated in November 6, 1997;„Valstybės žinios“, 28/11/1997, N0. 108).

Twenty five Lithuanian Holstein dairy cows in the third and fourth lactation, weighing 6500–7500 kg, were included in the study. Milk progesterone concentrations were measured every 5 days 20–90 days after calving. Cows were inspected before the morning milking, 1 hour after the evening milking and 15 minutes after the noon milking to determine the presence of estrus. Cows in heat were determined by the presence of standing reflex. Cows were divided into two groups: 1 group – 12 cows, which had estrus 3 times in 90 days after calving, and 2 group – 13 cows, which did not gave estrus until the 90th day after calving. Milk yield measurement was started 30 days after calving and was performed every 30 days. The composition of the milk was evaluated in the same intervals. Middle portions of morning milk were evaluated by SE "Pieno tyrimai" according to standard methodology. The concentration of milk lipids, proteins and lactose were determined.

Fluctuations in milk progesterone levels were evaluated by Hormonost microlab (Germany), using biolab GmbH (Germany) reagents. Milk samples for the measurement of progesterone levels were obtained according to manufacturer's methodology – straight after milking into certain tubes covered with antibodies. Three drops of milk were mixed with 6 drops of dissolvent. The samples were incubated for 5 minutes at room temperature. After that, 3 drops of enzyme were added. After 3 minutes, the samples were flushed with water and 12 drops of substrate were added. The colour of this solution was evaluated with photo calorimeter. Statistical analysis was performed using SPSS for Windows 15 (SPSS Inc., Chicago, IL, USA). Analysis was performed using descriptive and one-way factorial (ANOVA) model and Spearman correlation matrix. The differences were determined by Student t-test. The data was statistically significant when $p < 0.05$.

Results and discussion. The progesterone concentration changes according to estrous cycle. When the cycle is physiological, progesterone concentration increases on the 4th day of the cycle, reaching the maximum value on the 8th day and remaining high until the 17th day (Wimpy et al., 1986). During estrus, progesterone concentration is minimal and increases after ovulation with the formation of corpus luteum. As shown in Fig. 1, the progesterone concentration in cows, which were in heat 90 days after calving, gradually increased and the first increase was identified on the 30th day (4.78±0.7 ng/ml.). The lowest progesterone concentration (3.61±0.3 ng/ml) was during estrus and was identified on the 35th day after calving. With the formation of corpus luteum the progesterone levels increased and the second increase was identified on the 45th day (5.43±0.9 ng/ml). If the conception did not occur, around the 17th day of the cycle corpus luteum degenerated and progesterone concentration decreased; on the 19th–23rd days of the cycle the cow entered the estrus. The estrus was identified on the 56th day after calving. According to the fluctuations in the progesterone levels, the next estrus and decrease in progesterone concentration was anticipated to occur on the 77th day after calving. From the 56th day to the 77th day, atypical progesterone changes were determined and the estrus was not observed. Some authors report, that the estrous cycle of the cows returns to normal on the 25th–30th days after calving (Båge *et al.*, 2002). According to M. Wiltbank et al (2006), high productivity cows return to normal physiological estrous cycle after calving later. The increased progesterone concentration is associated with more intensive steroid metabolism and completeness of estrous cycle. The highest progesterone concentration (6.57± 1.62 ng/ml) was detected on the 80th day after calving. Later, progesterone levels decreased and complete estrus was detected on the 90th day after calving. This may be a sign of complete return to normal cycle.

In dairy cattle *anestrus* period should not exceed 60 days (Mwaanga et al., 2000; Etherington et al., 1999). Low progesterone concentration can influence physiological processes before and after the conception. However, a relation between the longer anestrus period and fertility was not identified (Samarütel et al., 2008). K. Peterson and other authors (2006) report that atypical progesterone cycle is less frequent in older cows and depends on cattle breed. H. Dobson and others (2008) state, that progesterone concentration is 50% lower in cows with impaired bone structure. A decreased progesterone concentration is one of the reasons for impaired fertility (Stronge et al., 2005; Demetrio et al., 2007). As shown in Fig. 2, progesterone concentration had a tendency to increase during the study period in cows, which did not experience estrus during 90 days after calving. Significant decrease in progesterone concentration was observed on the 28 and 38th days after calving (2± 0.22 ng/ml and 2.4 ± 0.32 ng/ml), however there were no signs of estrus. The same as in the group where cows experienced estrus, progesterone concentration increased on the 30th–45th days after calving (3.0±02 and 3.8 ± 0.98 ng/ml.). On the 70th day after calving, progesterone concentration has increased significantly. In cows, which experienced estrus, these changes were not observed. The change in progesterone concentration, characteristic to estrus cycle, was observed from the 30th day after calving. Progesterone concentration was not associated with clinical signs of estrus in this group. J. Roelofs and other authors (2010) report, that usually first ovulation is not followed by estrus and around 50% of first estrus cycles last up to 10 days. M. McCoy and other authors (2006) state that if progesterone concentration is low (<3mg/ml), these cows have delayed ovulation and estrus passes without clinical signs. Around 10–40% of cows do not show any signs of estrus. Between first and second estrus after calving, the cycle lasts around 15 days (Custer et al., 1990). No significant difference in changes of progesterone concentration was observed in study groups. During the study period, progesterone concentration was 4.8% lower in cows, which did not experience estrus, when compared to cows that experienced estrus ($p<0.05$). First signs of estrus in this group were observed 90 days after calving. Then progesterone concentration in milk was the highest, when compared to all study period and constituted 89.4% ($p<0.05$) of progesterone concentration in cows, which experienced estrus before 90 days after calving.

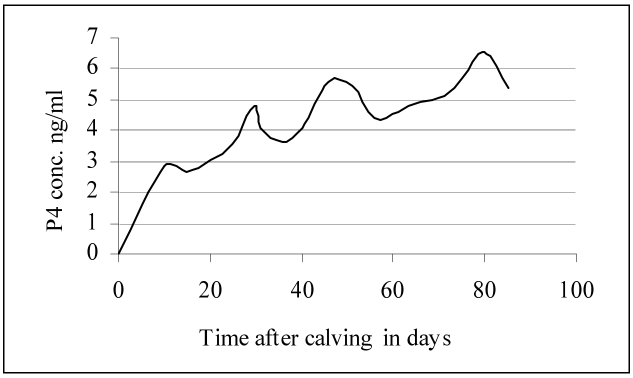

Fig. 1. **Changes in milk progesterone concentration in cows, which experienced estrus in 90 DM** (n=12)

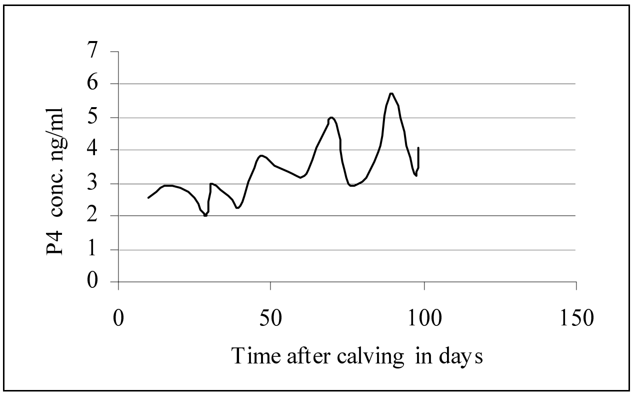

Fig. 2. **Changes in milk progesterone concentration in cows, which did not experience estrus in 90 DM** (n=13)

It is assumed that in early period after calving, cows have less energy and mature ovule is not produced and stereotypical behaviour, characteristic to estrus, is not promoted (Butler et al., 1981). S. Eicker and others (1996) report that there is no relation between increasing milk yield (until day 60) and reproduction. According to other authors, increasing milk yield (until day 80) prolongs service period and calving interval. As shown in Fig. 3, cows with heat had higher progesterone concentration (8.2%, p<0.05) and slightly higher milk yield (5.6%, p<0.05). No statistically significant relation between progesterone concentration and cow productivity was observed (r=0.2 p>0.05).

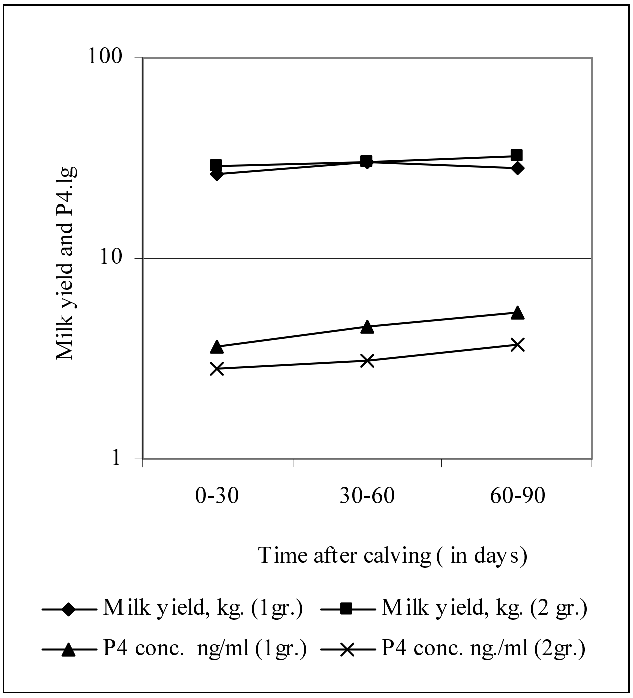

Fig. 3. **Changes in milk yield and progesterone concentration** (lg)

Cow's ability to estrus is associated with milk production. M. Wiltbank and others (2006) report that while productivity increases, it is difficult to inseminate the cow. Atypical progesterone concentration (not characteristic of estrus cycle) during the period of maximal yield and the absence of signs of estrus prove that the increase in milk yield during the first 40 days after calving has a negative impact on insemination index. It was assumed that this is associated with negative energy balance and changes in ovaries (deVries et al., 1998; deVries and Veerkamp, 2000; Winding et al., 2008).

We can determine the possibility of normal estrus by increased progesterone concentration, which is observed around the 90th day after calving.

The changes in progesterone concentration did not differ between the groups. One can judge about the reproductive function by progesterone changes when these changes are compared in all fresh milk samples. The increased progesterone concentration during the study period was associated with cow's ability to estrus.

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Received 8 April 2013
Accepted 2 February 2014