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Original article

Relationship between in-line milk progesterone before and after artificial insemination and fertility outcomes in dairy cows

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Abstract

The aim of this study was to evaluate the possibility to predict outcomes of artificial insemination (AI) in dairy cows based on in-line milk progesterone (P4) concentration. The research was carried out on the herd of loose housing 245 dairy cows of 2-4 lactations, with average milk yielding 11.000 kg per cow. Milk sampling, measuring, and recording of milk P4 concentration was carried out using the Herd Navigator (HN). The grouping was performed according to the following three indices: the first by reproductive condition – pregnant or not pregnant after AI, the second by P4 concentration from day 20 before AI to day 20 after AI, and the third by P4 concentration at AI time. There was a significant difference in P4 concentration in the group of pregnant cows from day 15 to day 9 before AI, and it was by 18.3% higher compared to that in the group of non-pregnant cows in the said period ($p < 0.01$). The milk P4 concentrations began to differ mostly from day 10 after AI. At that time, the average P4 concentration in the group of pregnant dairy cows was by 36.8% higher compared to that in the group of non-pregnant cows ($p < 0.01$). A statistically significant difference between the ratio of the cows with high, medium, and low P4 concentration on days 20-16 before AI ($p < 0.01$) was determined. The highest number of cows with up to 2-3 ng/ml P4 concentration became pregnant at the AI time.

In-line milk P4 records captured on day 10-15 before AI can be used to predict the proper for reproduction period. By P4 concentrations on day 10 after AI, the ratio of pregnant cows in herd can be assessed.

Key words: in-line milk progesterone, cow reproductive performance

Introduction

Correct identification of cow's fertility status is important to further optimize reproductive performance in dairy cows and consequently enhance farm profitability (Inchaisri et al. 2010). In order to minimize fertility-related losses, it is essential to obtain a complete image of a cow's reproduction status as soon and accurately as possible (Adriaens et al. 2018). Milk progesterone (P4) is widely accepted as a useful parameter to obtain a complete and direct image of a cow's reproduction status (Martin et al. 2013, Adriaens et al. 2017) and data could be successfully used in the development of methods for reduction of postpartum (*pp*) anoestrus (Yotov et al. 2013). To the best of our knowledge, only a few studies have attempted to characterize heritable endocrine fertility traits in dairy cows from in-line milk P4 records (Tenghe et al. 2015, Tyndall et al. 2017). A fully automated in-line P4 analyzer has recently become commercially available to monitor real-time ovarian activity, in addition to estimating time of estrus in cycling cows and pregnancy, based on P4 profiles (Mottram 2016, Yu and Maeda 2017). This technique is applied using sophisticated computing algorithms and can be performed on-farm (von Leesen et al. 2013). With the use of Herd Navigator (HN), cow performance and milk P4 concentration can be easily monitored on a real-time basis, and factors contributing to improper reproductive management can be identified and corrected to improve farm performance (Blom et al. 2015). The concentration of milk P4, which is determined by HN, can vary between 0 to 50 ng/ml. In HN, the threshold value between the follicular phase and the luteal phase is set to 4 ng/ml (Friggens et al. 2008). Cows with pregnancy loss between day 1 after artificial insemination (AI) and calving had significantly lower milk P4 concentrations at days 10, 21 and 30 after AI (Nyman et al. 2018). According to McCoy et al. (2006), when milk P4 concentration are low (<3 ng/ml), such cows are susceptible to delayed ovulation. Roelofs et al. (2006) reported that when the P4 concentration is 5.0 ng/mL, it is possible to anticipate the ovulation time. The cows showing low milk P4 concentration on day 7 (Bisinotto et al. 2013) or the day 15 of ovulation preceding AI are less fertile than those with the greater milk P4 concentration preceding AI (Folman et al. 1973). The increasing milk P4 concentration following ovulation are the factors associated with improved fertility (Bruinje et al. 2017).

The aim of this study is to explore the ability to predict the outcomes before and after AI by in-line milk P4 concentration in high yielding productivity of dairy cows.

Materials and Methods

The research was carried out on the herd with 450 loose housing dairy cows. There were selected 245 cows of 2-4 lactation, with average milk yield of 11.000 kg per standard lactation. Milk yield of the cows was calculated using the herd management software of the integrated DeLaval DelPro™ 4.2 dairy farm management system. All cows were clinically healthy. P4 concentration was monitored using HN (DeLaval International, Tumba, Sweden). Milk sampling, measuring, and recording of milk P4 concentration was performed using the HN. Feed ration throughout the year at the same time was balanced according to the physiological needs of the researched cows. Feeding took place every day, at 06:00 a.m. and 6:00 p.m. The cows were milked two times per day – at 05:00 a.m. and 05:00 p.m.

Identification of estrus, AI, and examination of pregnancy rate

Estrus was determined by milk P4 decrease at AI time on 60-70 DIM. Once the HN software detects the decrease of milk P4 down to <5 ng/ml, a notification on the occurrence of estrus is recorded. It is assumed that the occurrence of estrus has been determined precisely when the milk P4 concentration decreases again and the range between the decreases is 20-21 day. AIs were performed within the 16-18 hour onset of estrus. The pregnancies were examined with an ultrasonic scanner (Digital Diagnostic Ultrasound Devices (model - HG 9300) from Caresono Technology Co., Ltd., at the frequency of 6.5 MHz, using a linear rectal transducer, 35 days after the AI.

Grouping

Grouping was performed according to the following three criteria: the first – by reproductive condition (pregnant or not pregnant), the second – by milk P4 concentration from day 20 before AI until AI, and the third – by milk P4 concentration at AI time. The first: the cows that became pregnant after AI – the group of pregnant cows (n=165) and those that did not become pregnant after AI – the group of non-pregnant cows (n=80). Milk P4 concentration was recorded 20 days before AI and 20 days after AI. The second: the following three groups by the concentration of milk P4: <5 ng/ml – low P4, 5-10 ng/ml – medium P4, >10 ng/ml – high P4 concentration 20 days before AI. The ratio of the cows with different P4 concentration on different day was calculated. The third: the following four groups by the concentration of milk P4: <2 ng/ml, 2-3 ng/ml, 3-4 ng/ml, >4 ng/ml concentration. The sam-

Table 1. Milk P4 concentration in pregnant and non-pregnant cows before AI.

Days	Pregnant		Non-pregnant	
	M	SE	M	SE
- 20	4.940	0.517	3.506	0.577
- 19	7.524	0.944	5.737	1.479
- 18	8.588	0.892	8.784	1.211
- 17	9.069	0.846	6.603	1.019
- 16	10.520	0.838	11.471	1.378
- 15	11.295	0.909	8.520	1.139
- 14	13.922*	1.093	10.426*	1.294
- 13	14.001	0.934	11.984	1.199
- 12	15.461	0.708	13.538	1.029
- 11	18.221*	0.602	14.707*	1.004
- 10	18.733**	0.638	14.417**	1.117
- 9	18.089	0.558	16.735	0.845
- 8	16.643	0.553	16.530	0.939
- 7	11.700*	0.601	13.923*	0.909
- 6	9.651	0.793	12.246	1.052
- 5	15.834	1.069	15.844	1.140
- 4	17.901	1.048	17.200	0.994
- 3	18.308	0.866	17.021	0.974
- 2	14.700	1.226	14.899	1.339
- 1	11.181	0.617	9.897	0.633
AI time	2.937	0.058	2.863	0.083

*p<0.05 **p<0.01

ples of milk for the identification of P4 were taken from day 20 before AI, at the time of AI, and until day 20 after AI.

Statistical analysis

The data were analyzed using the SPSS (the Statistical Package for the Social Sciences, 20.0) tool. The findings were provided as average values and standard errors. Pearson correlation and Chi-square were calculated to define the statistical differences among the groups. The differences in mean values of normal distributed traits were analyzed by the Student *t* test.

Results

P4 concentration had an increase with the equation in both groups until day 3 before AI. In both groups of the researched cows, the increase in milk P4 concentration takes place according to the following linear regression: in the group of pregnant cows $y=0.223x+10.367$ $R^2=0.0898$; and the group of non-pregnant cows $-y=0.3181x+8.2552$ $R^2=0.1998$. The average milk P4 concentration in the group of pregnant cows was by 7.8% higher compared to that in the group

of non-pregnant cows from day 20 before AI until AI ($p>0.05$) (Table 1).

There was a significant difference in milk P4 concentration in the group of pregnant cows from day 15 to day 9 before AI, and it was by 18.3% higher compared to that in the group of non-pregnant cows in the said period ($p<0.01$). The highest difference was on day 10 before AI. The average milk P4 concentration in pregnant dairy cows was by 23.0% higher compared to P4 concentration in the group of non-pregnant cows on day 10 before AI ($p<0.01$).

The increase in milk P4 concentration after AI was observed in both groups of cows, where in the group of pregnant cows $y=1.1848x+1.423$ $R^2=0.9261$; and in the group of non-pregnant cows $y=0.7647x+1.2742$ $R^2=0.9615$. Significantly different milk P4 concentration after AI was observed in the groups of pregnant and non-pregnant cows in the period from day 7 to day 20 after AI (34.7%) ($p<0.01$) (Table 2). The major change in milk P4 concentrations was observed from day 10 after AI. At that time, the average milk P4 concentration in the group of pregnant dairy cows was by 36.8% higher compared to that in the group of non-pregnant cows ($p<0.01$). In the period from day 10 to day 20 after AI, the average milk P4 concentration in the group

Table 2. Milk P4 concentration in pregnant and non-pregnant cows after AI.

Days	Pregnant cows		Non-pregnant cows	
	M	SE	M	SE
AI time	2.934	0.058	2.863	0.083
+ 1	3.960	0.477	3.349	0.609
+ 2	5.215	0.824	3.157	0.732
+ 3	3.642	1.588	4.566	1.462
+ 4	6.431	1.014	4.495	0.815
+ 5	7.878*	0.274	5.562*	0.940
+ 6	7.280	0.487	5.721	0.790
+ 7	8.042**	0.643	4.520**	0.664
+ 8	11.228	1.106	8.719	1.834
+ 9	15.266**	0.604	9.239**	1.288
+ 10	18.088**	0.560	11.432**	1.309
+ 11	17.904**	0.532	11.073**	0.991
+ 12	19.185**	0.497	10.891**	1.142
+ 13	20.643**	0.425	11.883**	1.394
+ 14	21.493**	0.353	13.630**	1.281
+ 15	21.690**	0.317	14.122**	1.023
+ 16	22.186**	0.285	14.753**	1.023
+ 17	22.277**	0.281	15.289**	1.231
+ 18	22.801**	0.227	15.754**	1.345
+ 19	22.762**	0.231	16.429**	1.162
+ 20	22.527**	0.305	15.969**	1.335

* $p < 0.05$ ** $p < 0.01$

of pregnant dairy cows was by 35.4% higher compared to that in the group of non-pregnant cows ($p < 0.01$).

A statistically significant difference between the ratio of cows with high, medium, and low milk P4 concentration at any time of day before AI ($p < 0.01$) was established. On day 16-20 before AI (Fig. 1), the number of cows with low milk P4 concentration was by 22.8% ($p < 0.01$) higher compared with that with medium P4 concentration. By day 11-15 before AI, the number of cows with low milk P4 concentration decreased by 33.8% ($p > 0.05$). The highest number of cows with high milk P4 concentration was observed on day 6-10 before AI. The number of cows with medium milk P4 concentration differed by 14.4% ($p > 0.05$) between day 11-15 and day 1-5 before AI.

The average concentration of milk P4 in pregnant and non-pregnant cows during AI mounted to 2.68 ± 0.048 ng/ml. The highest number of cows with the milk P4 concentration of 2-3 ng/ml became pregnant during AI (Fig. 2). The number of pregnant cows with the milk P4 concentration of < 2 ng/ml, compared with those with the milk P4 concentration of 2-3 ng/ml, was lower by 14.8% ($p < 0.01$); compared with the cows with the milk P4 concentration of 3-4 ng/ml, it was lower by 4.4% ($p < 0.01$); and compared with those with

the milk P4 concentration of > 4 ng/ml, it was lower by 12.3% ($p < 0.01$).

Discussion

About half of the dairy cows develop at least one *pp* health disorder (Santos et al. 2010). The *pp* disorders is another factor related to the milk production that alters reproductive physiology which is the metabolic clearance rate of steroid hormones (Wiltbank et al. 2006). There is strong evidence that both excessive and insufficient P4 concentrations at specific time points are negatively associated with pregnancy results (Lonergan et al. 2016). Milk P4 concentrations during both cycles preceding and following insemination affect embryo survival.

A number of studies have reported lower milk P4 concentrations in higher yielding cows and suggested increased metabolic clearance of P4 as the principle cause of luteal inadequacy (Wiltbank et al. 2014a). Luteal P4 is essential for the preparation of the uterus and oocyte before breeding, as well as for the maintenance of an optimal uterine environment and for supporting the uterus to develop the embryo/fetus during

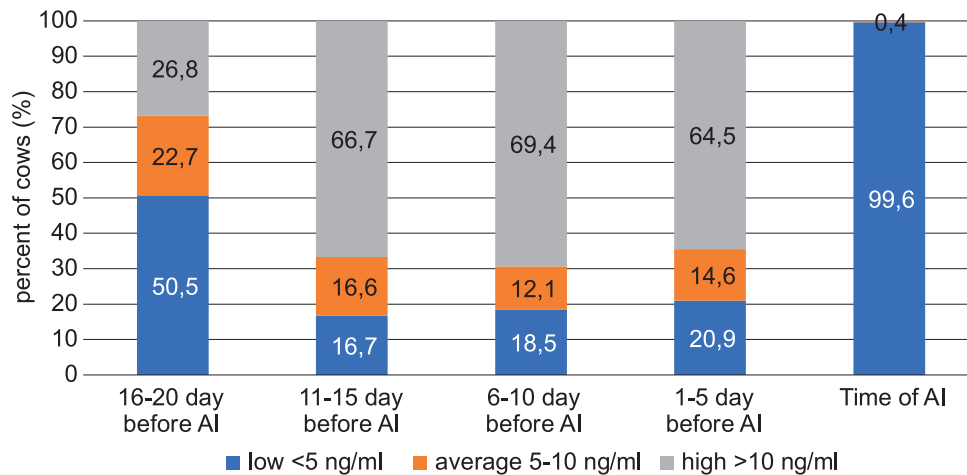


Fig. 1. The ratio of cow number according to P4 concentration depending on time before AI.

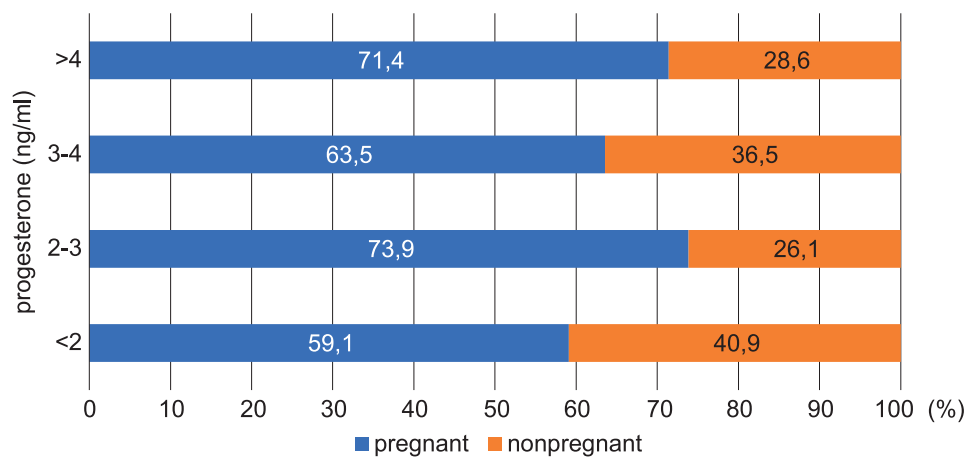


Fig. 2. Cows reproduction status depending on milk P4 concentration at the AI.

gestation in the cow (Karen et al. 2014). Embryo development and embryo survival principle is controlled by P4 (Mann and Lamming 1999). The in-line milk P4 analysis system makes the assumption in determining the relation between P4 and the outcomes of AI.

The change of milk P4 concentration before AI is determined by physiological alteration of estrus cycle. The concentration of milk P4 before AI time is very important on fertilization and pregnancy maintenance. Based on in-line P4 profiles, greater P4 concentration near time of AI (d-2 in multiparous and d5 in primiparous cows) and lesser P4 beyond d10 were negatively associated with a successful pregnancy (Bruinjé et al. 2017, Bruinjé et al. 2019). According to the study data, P4 concentration was higher in the group of pregnant cows from day 15 to day 10 before AI. It is important to note that increased P4 concentration during day 15-10 before AI is associated with a capability for sexual cycle (Diskin et al. 2012). It can be that lower P4 concentration during the luteal phase preceding AI might have caused altered luteolytic signal resulting in premature luteolysis in the subsequent

luteal phase post AI, as previously reported (Cerri et al. 2011). In the groups of pregnant and non-pregnant cows, the decrease in milk P4 was observed from day 10 before AI. However, in the group of pregnant cows, this change is obvious. A temporarily slight increase in milk P4 was observed in both groups on day 6 before AI. In the groups of pregnant and non-pregnant cows, the second decrease in P4 was observed 3 days before AI, but the indices are the same for both groups. The P4 concentration was highest (5–12 ng/ml) on the fourth day prior to estrus, then they progressively decreased to a nearly nondetectable concentration on the day prior to estrus (Henricks et al. 1971, Inskeep 2004). The concentration of P4 reached a peak of 6-7 ng/ml in mid-cycle, then they began to fall 4 days before estrus, to the values of about 1 ng/ml at estrus and remained low for the further 4 days (Smith et al. 1979, Bisinotto et al. 2013).

The development of corpus luteum (CL) causes an increase in P4 concentration after estrus. According to the study data, increased concentration of P4 was identified in both groups of pregnant and non-pregnant

cows in all study periods. However, the concentration of milk P4 was different in the groups of pregnant and non-pregnant cows in early time after estrus. Low P4 concentration during days 5-7 after AI was associated with low fertility in dairy cows (Strongeab et al. 2005). According to Yan et al. (2018), it is now well established that failure of the CL to produce sufficient P4 represents a major cause of early embryo loss and reproductive problems in the modern dairy cows. The average P4 concentration was lower in the group of non-pregnant cows by 27.1% until day 20 after AI ($p>0.05$). Decreasing P4 concentration after AI, which was found in non-pregnant cows on day 21 after AI, can also be explained by embryonic mortality (Nyman et al. 2018). On the other hand, suboptimal P4 support during the early luteal phase is likely to deleteriously affect embryo viability. It is possible to predict P4 during the early luteal phase based on earlier stage concentrations and thus identify the cows at risk of early embryo loss (McNeill et al. 2005). A slower than normal rise in P4 concentration and a lower total P4 concentration in the first 6 days after estrus were identified in low-fertility cows (Bage et al. 2002). Lower milk P4 concentration after AI could be a contributing factor to lower fertility (Garcia-Isperto and López-Gatius 2016). Milk P4 concentration in the groups of pregnant and non-pregnant cows begins to differ most significantly on day 10 after AI. This difference in P4 concentration can be explained by the developing of the CL.

Different cows showed different milk P4 concentration before AI. A statistically significant difference between the ratio of the cows with high, medium, and low milk P4 concentration at any time of day before AI was established. During estrus, in general all cows showed low P4 concentrations. However, 16-18% of the cows showed low milk P4 concentration on day 15-18 before estrus and 64% of the cows showed high milk P4 concentration on day 1-5 before estrus ($p>0.05$). The individual changes in milk P4 concentrations recorded by the in-line milk P4 analysis system determined the ratio of the cows with high, medium, and low milk P4 concentrations.

High P4 concentration at AI may lead to reduced fertilization rates, blocked ovulation or may affect the sperm transport (Wiltbank et al. 2014b). At the day of AI, P4 concentration is 1.82 ng/mL (Wilsdorf et al. 2016). According to the study data, most cows became pregnant when P4 decreased to 2-3 ng/mL during estrus. Humblot (2001) determined cows as pregnant if the P4 concentration value on day 0 (at AI) was $P4<3.5$ ng/mL, and on day 21 and 24 after AI it was $P4>5$ ng/mL.

The concentration of P4 in cows before AI varies according to the regularities of the sexual cycle (Barui

et al. 2015). According to the study data, low milk P4 concentration on day 15-10 before the expected time of AI suggests poor fertilization of cows. The concentration of milk P4 in cows is determined not only by physiological changes in milk P4, but also by different milk P4 profiles. Low milk P4 concentration during estrus is important for the fertilization ability of cows. Most cows did not fertilize when milk P4 concentration was 3-4 ng/ml. The group of pregnant cows had higher milk P4 concentration after AI than non-pregnant cows, but this difference was significant from day 10 after AI. Pregnant cows showed higher milk P4 concentrations than those that were later confirmed as non-pregnant immediately after AI. Bruinje et al. (2017) confirms the results of previous studies on the usefulness of in-line P4 before and after AI to predict the fertility outcomes of cows. Wider adoption of this precision technology would certainly improve our understanding of the factors affecting the reproductive physiology of the modern dairy cow, but it would also help to enhance fertility in dairy herds (Bruinje et al. 2017).

Conclusions

High milk P4 concentration on day 15-10 before the expected time of AI suggests possibilities conception of cows. In-line milk P4 records captured on day 15-10 before AI can be used to predict the right-for-reproduction period. Most cows became pregnant when milk P4 decreased to 2-3 ng/ml during estrus. Milk P4 concentration in the groups of pregnant and non-pregnant cows begins to differ most significantly 10 days after AI. According to milk P4 concentration on day 10 after AI, the ratio of pregnant cows in a herd can be assessed. These traits have variation and might help to define better breeding goals for improved fertility. The study revealed that measuring milk P4 concentration from in-line milk progesterone profiles can have positive effect on cow reproduction control. The HN has resulted in a reasonably accurate system. However, it should be noted that to validate this system, further extensive research work should to be carried out.

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