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Characteristics of dairy cows with a pronounced reduction in first milk yield after estrus onset

Keisuke Koyama[1,*], Takeshi Koyama[1], Yoshitaka Matsui[1], Masahito Sugimoto[1], Naohito Kusakari[1], Ikuo Osaka[1] and Masashi Nagano[2]

[1] Konsen Agricultural Experiment Station, Hokkaido Research Organization, Nakashibetsu, Hokkaido 086–1135, Japan
[2] Laboratory of Theriogenology, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita-ku Kita 18 Nishi 9, Sapporo 060–0818, Japan

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Abstract

Some dairy cows display a pronounced reduction in first milk yield after estrus onset. The objective of this study was to evaluate the usefulness of the pronounced reduction in milk yield as an indicator of the optimal time for artificial insemination (AI). We confirmed that the frequency of a pronounced reduction in first milk yield after estrus onset in our dairy cows was 21.6%. We subsequently examined the differences in estrus intensity, feed intake, the timings of ovulation and fertility between cows with (cows with milk reduction) and without (cows without milk reduction) a pronounced reduction in first milk yield after estrus onset. Compared to cows without milk reduction, the percentage of cows that were milked actually during estrus tended to be higher in cows with milk reduction. However, the dry matter intake 1 day before the first milking after estrus onset was similar in both the groups of cows. In cows with milk reduction, the percentage of cows that ovulated 22–24 hr after the first milking following estrus onset was higher than that in cows without milk reduction. The pregnancy rate in cows with milk reduction that were inseminated 3–8 hr after milking during which the pronounced reduction in milk yield was exhibited was significantly higher than that in cows with milk reduction inseminated during other periods. These results indicate that the optimal time for AI can be narrowed by detecting a pronounced reduction in first milk yield after estrus onset.

Key Words: Artificial insemination; Dairy cow; Estrus; Milk yield; Ovulation

Introduction

A reduction in milk yield is one of the changes related to estrus in dairy cows[15]. In particular, the first milk yield after estrus onset is reduced[8]. However, a few cows display a pronounced reduction in first milk yield after estrus onset, which has been defined as $\geq$25% reduction compared with milk yield during diestrus, and this characteristic has a low sensitivity (29%) as an indicator of estrus[18]. In contrast, a large number of healthy cows that display a pronounced reduction in milk yield initiates estrus immediately before milking, and

*Corresponding author: K. Koyama, Konsen Agricultural Experiment Station, Hokkaido Research Organization, Nakashibetsu 086–1135, Japan
Phone: +81–153–72–2004. Fax: +81–153–73–5329. E-mail: koyama-keisuke@hro.or.jp
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the positive predictive value of a pronounced reduction in milk yield for identifying estrus is high (100%)[18]. In a tie-stall barn, it is difficult to detect the timing for artificial insemination (AI) to achieve a high pregnancy rate in dairy cows, because it is difficult to observe standing behavior and detect a distinct increase in the number of steps at estrus by pedometer[19]. If a pronounced reduction in first milk yield after estrus onset is related to ovulation time and fertility, this characteristic could be useful to determine the timing for AI, especially in a tie-stall barn. However, differences in the characteristics between cows with and without a pronounced reduction in first milk yield after estrus onset are unclear.

The mechanism of a pronounced reduction in first milk yield after estrus onset remains unclear, but two hypotheses have been proposed. First, the reduction in milk yield after estrus onset may be a result of reduced milk ejection because of a high 17β-estradiol (E_2) level at the time of milking[18]. The administration of E_2 interferes with the milk-ejection reflex and decreases the milk yield in rats[3], and serum E_2 concentrations correlate with estrus duration[10]. The plasma E_2 level peaks at the time of the luteinizing hormone (LH) surge, which occurs around estrus onset, and subsequently declines[2]. Therefore, the duration of estrus may be longer and the interval between estrus onset and milking may be shorter in cows with the reduction in milk yield after estrus onset than in cows without it. Second, the reduction in milk yield may be caused by a temporary reduction in feed intake during estrus[9] because rumination and feeding time decrease[5,14]. Therefore, cows with the reduction in milk yield after estrus onset may face a temporary deficiency of energy at estrus.

The study aimed to evaluate the usefulness of a pronounced reduction in first milk yield after estrus onset as an indicator of optimal time for AI. First, we confirmed the frequency of a pronounced reduction in first milk yield after estrus onset in our dairy cows. Next, we examined the differences in estrus intensity, feed intake, the timings of estrus onset and ovulation, and fertility between cows with and without a pronounced reduction in first milk yield after estrus onset.

Materials and Methods

Animals: We used 124 postpartum lactating Holstein cows housed at the Konsen Agricultural Experiment Station, Hokkaido, Japan. The cows were clinically healthy and exhibited natural estrus from 50 to 221 days in milk (DIM) from June 2011 to October 2016. They were housed in a free-stall barn under the normal management program of the Konsen Agricultural Experiment Station and fed a total mixed ration (TMR) diet consisting of grass silage, corn silage, and concentrate. The cows had free access to water and a salt-based mineral supplement. Each feeder was equipped with a validated electronic monitoring system (Insentec, B.V., Marknesse, the Netherlands) for measuring the feed intake. The TMR intake was measured from the weight of residual TMR in the feeder and was automatically recorded[4]. The dry matter content of TMR was analyzed once a week. Cows were milked twice a day from 09 : 00 to 11 : 00 hr and from 19 : 00 to 21 : 00 hr in a herringbone milking parlor, and the milking time and milk yield were automatically recorded at each milking.

The animals in the present study were maintained and cared in accordance with the practices outlined in the Guide for the care and use of agricultural animals in agricultural research and teaching[6].

Detection of estrus: The onset and end of estrus times were detected using a pedometer (Gyuho, Comtec, Miyazaki, Japan). The pedometer was attached to the front leg, and the number of steps per hour was recorded using a computer. The onset and end of estrus times were defined as previously described[17]. If the number of steps

during 1 hr exceeded a threshold, i.e., the mean plus two times the standard deviation of the number of steps in 1 hr recorded over the same time during the previous 10 days, it was defined as an increase in the number of steps. If this increase occurred for two consecutive hours, the animal was defined to be in estrus. The time of estrus onset was defined as the time of the first detection of an increase in the number of steps. The end of estrus was defined as no increase in the number of steps over two consecutive hours. The time of the end of estrus was defined as the last detection of an increase in the number of steps. To confirm that the detected estrus was true estrus, transrectal ultrasonography with a 5.0-MHz linear transducer (HS-101V, Honda Electronics, Toyohashi, Japan) was used to confirm ovulation and formation of corpus luteum 1–2 and 7–10 days after estrus onset, respectively.

Detection of ovulation and artificial insemination: The ovaries of the cows were examined using transrectal ultrasonography every 6 hr from estrus onset until ovulation was detected. The time of ovulation was considered to be 3 hr before the disappearance of a dominant follicle greater than 10 mm internal diameter. The cows were inseminated with unsorted frozen–thawed semen from 9 bulls after estrus onset by 10 skilled technicians to evaluate the relationship between a pronounced reduction in first milk yield after estrus onset and fertility. The time of AI was from 10 : 00 to 12 : 00 hr or 13 : 00 to 17 : 00 hr according to the a.m.–p.m. rule[11]. Pregnancy was diagnosed using transrectal ultrasonography 60 days after AI on the basis of the detection of an embryo with a beating heart.

Experimental design: In the analyses 1 and 2, we performed analysis based on the dataset of 217 estrus events detected in 105 cows from June 2011 to October 2015. In the analysis 1, we investigated milk yield per milking event and the percentage of cows displaying a pronounced reduction in milk yield during the periestrus period (day −5 to 5; day 0 = the first milking after estrus onset) in 217 estrus events. A pronounced reduction in milk yield was defined as a reduction to ≤75% compared with the mean of the yield per milking collected during the previous week[7]. In the analysis 2, we compared the characteristics between 47 estrus events with a pronounced reduction in first milk yield after estrus onset (hereafter referred to as cows with milk reduction) and the 170 estrus events without it (hereafter referred to as cows without milk reduction), including parity, DIM, level of milk yield, milk yield per milking during the periestrus period, timing of estrus onset, estrus duration, interval between estrus onset and milking, occurrence of estrus during milking, dry matter intake (DMI) value 1 day before the first milking after estrus onset, and the number of steps per hour during estrus. The level of milk yield was defined as the mean daily milk yield during the 7 days before estrus onset. In the analysis 3, the timing of ovulation and fertility were analyzed in 154 and 175 estrus events detected in 88 and 108 cows from August 2011 to October 2016, respectively. The 63 estrus events in which we did not detect the time of ovulation at 6-hr interval were eliminated from analysis of the timing of ovulation. The 29 estrus events in which we performed AI after 150 DIM or more than 4 times after calving were eliminated from analysis of fertility.

Statistical analysis: In the analyses 1 and 2, generalized linear mixed models (GLMM) were used. In the analysis 1, we set the milk yield per milking event as the response variable and the date of milking as the explanatory variable. For post hoc multiple comparison of GLMM, we used Tukey's HSD test. In the analysis 2, we set parity, DIM, level of milk yield, milk yield per milking during the periestrus period, timing of estrus onset, estrus duration, interval between estrus onset and milking, occurrence of estrus during milking, DMI value 1 day before the first milking after estrus onset, and the number of

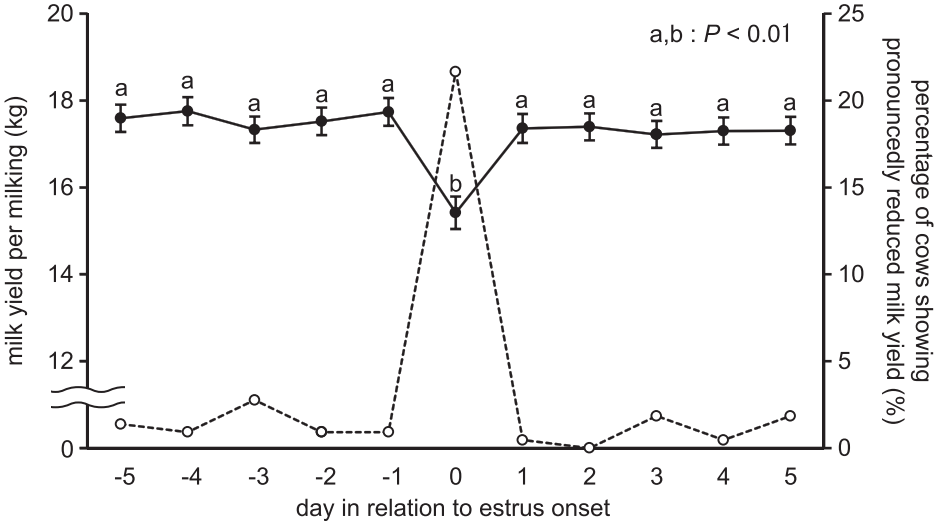

Fig. 1. Milk yield per milking event and the percentage of cows displaying a pronounced reduction in milk yield during the periestrus period. Solid line shows milk yield per milking event and dashed line shows the percentage of cows displaying a pronounced reduction in milk yield in 217 estrus events. Day 0 indicates the first milking after estrus onset and other days indicate the milk yields of 24 hr interval. Error bars represent mean ± standard error. **a, b** Different letters indicate significant difference ($P < 0.01$).

steps per hour during estrus as the response variable and occurrence of a pronounced reduction in first milk yield after estrus onset as the explanatory variable. The cow ID was set as a random effect in each GLMM. In the analyses 1–3, Chi-square test was used for analysis of the sensitivities of a pronounced reduction in milk yield for identifying estrus (the analysis 1), the percentage of cows that were milked during a particular period after estrus onset (the analysis 2) and that ovulated during a particular period after the first milking following estrus onset, and pregnancy rate (the analysis 3). In the analysis 2 and 3, Yates-corrected chi-square test for comparison between groups and the method of Benjamini and Hochberg[2] after chi-square test for multiple comparisons within the group was used. A P-value < 0.05 was considered statistically significant. All analyses were performed using the R software version 3.2.1 for Windows[13].

Results

Analysis 1: The mean of the first milk yield after estrus onset (day 0) was lowest during the periestrus period ($P < 0.01$, Fig. 1). The percentage of cows displaying a pronounced reduction in milk yield at the first milking after estrus onset (day 0), namely sensitivity of a pronounced reduction in milk yield for identifying estrus, was higher (21.6%, 47/217) than that on other periestrus days. In the milking during morning and evening, the sensitivities of a pronounced reduction in milk yield for identifying estrus were similar (22.9 and 19.8 %, respectively). In total, 25 cows (1.1%) displayed a pronounced reduction in milk yield after the 2170 milking events (217 cows × 10 days) during the periestrus period other than day 0. The positive predictive value of a pronounced reduction in milk yield for identifying estrus was 65.3% (47/72).

Analysis 2: The characteristics of cows with and without a pronounced reduction in first milk yield after estrus onset are shown in Table 1. DIM was higher in cows with milk reduction than in cows without milk reduction ($P < 0.05$). Compared with cows without milk reduction, first milk yield after estrus onset was lower in cows with milk reduction, but second milk yield after estrus onset was higher ($P < 0.01$).

Table 1. Characteristics of cows with and without a pronounced reduction in first milk yield after estrus onset

Items	cows with milk reduction*	cows without milk reduction*
n	47	170
Parity	2.7 ± 1.5	2.3 ± 1.4
DIM** (days)	104.1 ± 44.5[a]	87.7 ± 36.8[b]
Level of milk yield (kg/day)	34.0 ± 7.4	34.0 ± 6.8
Milk yield just before estrus onset (kg)	16.6 ± 4.6	16.5 ± 4.5
First milk yield after estrus onset (kg)	9.5 ± 4.3[b]	17.0 ± 4.6[a]
Second milk yield after estrus onset (kg)	20.9 ± 5.4[a]	16.5 ± 4.7[b]
Percentage of cows that started estrus by milking during morning (%)	64.1	59.6
Duration of estrus (hr)	10.2 ± 4.0	9.2 ± 3.5
Duration between estrus onset and milking (hr)	5.1 ± 2.6	5.6 ± 3.6
Percentage of cows in estrus during milking (%)	97.8[A]	86.5[B]
DMI*** for 1 day before the first milking after estrus onset (kg)	21.4 ± 3.7	21.1 ± 3.8
Number of steps per hour during estrus (steps/hr)	433.4 ± 129.1	421.9 ± 120.5

[a, b] Values with different superscripts within the same row are significantly different ($P < 0.05$).
[A, B] Values with different superscripts within the same row tend to be different ($P < 0.1$).
*cows with milk reduction: cows with a pronounced reduction in first milk yield after estrus onset
cows without milk reduction: cows without a pronounced reduction in first milk yield after estrus onset
**DIM: Days in milk ***DMI: Dry matter intake
Results are expressed as means ± standard deviation except the percentage data.

Compared to cows without milk reduction, the percentage of cows that were milked actually during estrus tended to be higher in cows with milk reduction ($P = 0.057$). DMI 1 day before the first milking after estrus onset and the number of steps per hour during estrus were similar in both the groups of cows. The distribution of the interval from estrus onset to milking is shown in Fig. 2. There were no differences in the percentages of cows of each time period between cows with and without milk reduction. In cows with milk reduction, the percentage of cows that were milked 4–6 hr after estrus onset showed highest value (44.7%), and the percentages of cows milked 1–3 and 7–9 hr after estrus onset were higher than that of cows milked 10–12 hr after estrus onset ($P < 0.01$). In cows without milk reduction, the percentage of cows milked 4–6 hr after estrus onset showed highest value (30.6%), and the percentages of cows milked 7–9 and 10–12 hr were similar.

Analysis 3: The distribution of the interval from the first milking after estrus onset to ovulation is shown in Fig. 3. The mean interval from the first milking after estrus onset to ovulation in cows with milk reduction was similar to that in cows without milk reduction (22.5 ± 3.7 hr vs 23.0 ± 5.3 hr, respectively). In cows with milk reduction, the percentage of cows that ovulated 22–24 hr after the first milking following estrus onset showed highest value (41.9%), and it was higher than that in cows without milk reduction (21.1%). In both the groups of cows, the percentages of cows that ovulated 16–18, 19–21, 22–24, 25–27 and 28–30 hr after the first milking following estrus onset were similar (6–19%).

The pregnancy rates of cows with and without a pronounced reduction in first milk yield after estrus onset are shown in Fig. 4. The pregnancy rates in cows without milk reduction were similar, regardless of AI timing. The pregnancy rate in cows with milk reduction that were inseminated 3–8 hr after milking was the highest (61.1%, 11/18) and significantly higher than that in cows during other periods (16.7%,

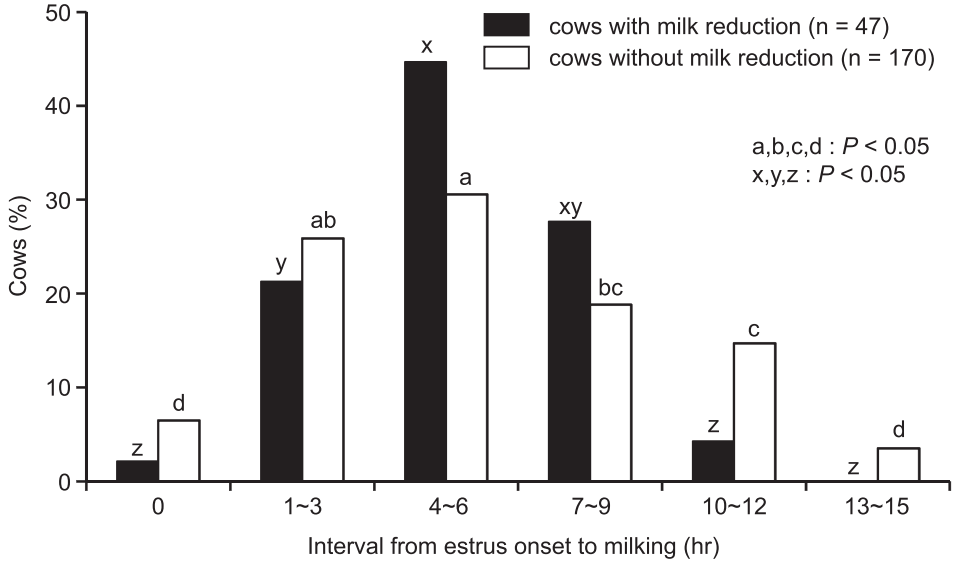

Fig. 2. Interval between estrus onset and milking in cows with and without a pronounced reduction in first milk yield after estrus onset. Cows with milk reduction: cows with a pronounced reduction in first milk yield after estrus onset. Cows without milk reduction: cows without a pronounced reduction in first milk yield after estrus onset. **a, b, c, d** Different letters indicate significant difference ($P < 0.05$). **x, y, z** Different letters indicate significant difference ($P < 0.05$).

2/12) ($P < 0.05$).

Discussion

In the present study, the mean of the first milk yield after estrus onset decreased, and the percentage of cows with milk reduction was 21.6%. Moreover, in cows with milk reduction, first milk yield after estrus onset was low, but second milk yield after estrus onset was high. These results are consistent with those of a previous study[8,18]. Then, there are few cows exhibited a pronounced reduction in milk yield during the periestrus period other than day 0. Therefore, it is reasonable to assume that "a pronounced reduction in milk yield" defined in the present study indicates the characteristic event after onset of estrus. However, the positive predictive value (65.3%) of a pronounced reduction in first milk yield after estrus onset for identifying estrus in this study was lower than that obtained in a previous study (100%)[18], which may be because of the level of milk production. The milk yield in the present study (approximately 34 kg/day) was higher than that reported previously (approximately 20 kg/day).

A high frequency of ovulation occurred 22–24 hr after milking during which the pronounced reduction in milk yield was exhibited. In contrast, cows without milk reduction showed no defined peak for the interval from the first milking after estrus onset to ovulation, probably because of the different distributions in the intervals from estrus onset to milking and/or the timing of the LH surge between the groups. The mean interval from the LH surge to ovulation is reportedly 24–28 hr in dairy cows, although the mean duration from estrus onset to ovulation is 24–43 hr[2].The present results suggest that the LH surge may occur immediately before milking during which the pronounced reduction in milk yield was exhibited and that a pronounced reduction in first milk yield after estrus onset is a useful predictor of ovulation time. The insemination–ovulation interval in lactating Holstein cows with a high probability of good quality embryos is 12–24 hr before ovulation[16]. Therefore, we could focus the optimal time for AI to achieve a high pregnancy rate in cows with

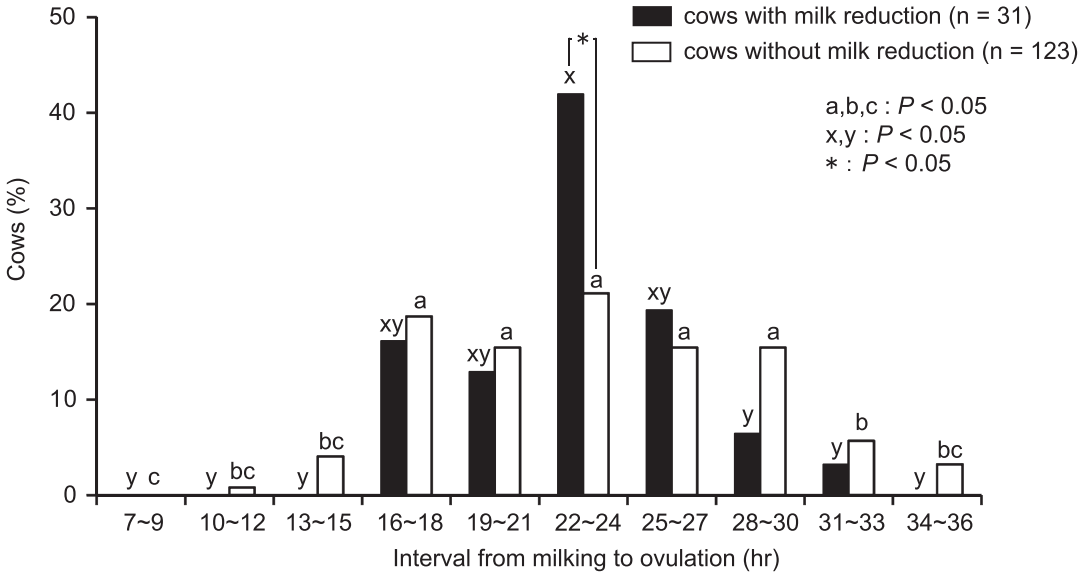

Fig. 3. Interval between the first milking after estrus onset and ovulation in cows with and without a pronounced reduction in first milk yield after estrus onset. Cows with milk reduction: cows with a pronounced reduction in first milk yield after estrus onset. Cows without milk reduction: cows without a pronounced reduction in first milk yield after estrus onset. **a,b,c** Different letters indicate significant difference ($P < 0.05$). **x,y** Different letters indicate significant difference ($P < 0.05$). * Asterisk indicates significant difference ($P < 0.05$).

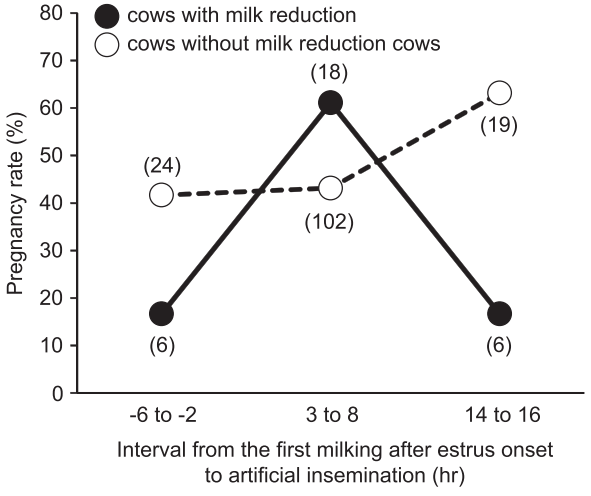

Fig. 4. Effects of interval from milking to artificial insemination on pregnancy rate of cows with and without a pronounced reduction in first milk yield after estrus onset. Numbers of cows inseminated are indicated in parentheses. Cows with milk reduction: cows with a pronounced reduction in first milk yield after estrus onset. Cows without milk reduction: cows without a pronounced reduction in first milk yield after estrus onset.

milk reduction on 0–10 h after the milking during which the pronounced reduction in milk yield was exhibited. In the present study, the pregnancy rate in cows with milk reduction was the highest when AI was performed 3–8 hr after milking during which the pronounced reduction in milk yield was exhibited. If the optimal time for AI in cows exhibited estrus before the evening milking is decided according to the a.m.–p.m. rule, AI would be performed the next morning. However, the next morning is too late in cows which exhibit the pronounced reduction in milk yield at the evening milking for AI to achieve a high pregnancy rate; therefore, AI should be performed as soon as possible within the same day. However, a limited number of cows were used to compare pregnancy rates in the present study; therefore further studies should be conducted to elucidate the relationship between a pronounced reduction in first milk yield after estrus onset and fertility.

In the present study, cows with milk reduction had a higher probability of being estrus during milking than cows without milk reduction. Low levels of oxytocin are released in the bloodstream after the initiation of milking during estrus compared with those during diestrus[1].

Moreover, in cows with milk reduction, first milk yield after estrus onset was low, but second milk yield after estrus onset was high. Therefore, inhibition of the milk–ejection reflex following the inhibition of oxytocin release from the pituitary gland may result in the pronounced reduction in milk yield during estrus followed by a compensatory increase at the next milking. On the other hand, DMI values 1 day before the first milking after estrus onset were similar, regardless of the pronounced reduction in milk yield. Pahl *et al*. [12] demonstrated that a reduced feed intake and rumination time during estrus do not cause a reduction in milk yield. Therefore, it is reasonable to assume that the temporary energy deficiency related to estrus does not affect the occurrence of a pronounced reduction in first milk yield after estrus onset and the reduction in milk yield after estrus onset is a result of reduced milk ejection rather than secretion.

DIM of cows with milk reduction was higher than those of cows without milk reduction in the present study. There are no reports regarding the relationship between a pronounced reduction in first milk yield after estrus onset and lactation stage. The speed of the milk flow peaks on approximately 50 DIM, followed by a gradual decline[20]. Cattle in the late stages of lactation may experience a pronounced reduction in first milk yield after estrus onset more easily because of their lower ability to eject milk than other cattle.

In conclusion, cows with milk reduction have a high probability of being in estrus during milking, but feed intake is similar, regardless of a pronounced reduction in first milk yield after estrus onset. More than 40% of cows ovulated 22–24 hr after milking during which the pronounced reduction in milk yield was exhibited, and it was assumed that the optimal time for AI to achieve a high pregnancy rate in cows with milk reduction was 0–10 h after the milking during which the pronounced reduction in milk yield was exhibited. Therefore, we recommend AI for cows immediately after detecting a pronounced reduction in first milk yield after estrus onset.

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