—Research Note—

Relationships between the Conception Rate of Estrus Synchronization Using Estradiol Benzoate and CIDR (Progesterone) and Other Parameters in Holstein Lactating Dairy Cows

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Abstract. The purpose of this study was to determine the relationships between conception rate and other parameters before estrus synchronization with a Controlled Internal Drug Release Device (CIDR) and estradiol benzoate (EB). In the estrus synchronization program, animals were injected with 2 mg EB and then received a CIDR. Seven days later, the CIDR was removed and the animals were given an injection of Prostaglandin F_{2α}. Twenty-four hours later, they received an injection of 1 mg EB, and they were artificially inseminated 24 h after that. This program was applied to 258 Holstein cows in Tohoku-machi (Aomori, Japan). Blood was collected at the beginning of the program, and the conception rate was determined about 40 days after insemination. The relationships among conception rate, blood biochemical values, age, body condition score and days in milk were statistically analyzed to determine better conditions for cow conception. The conception rate of the cows in the high progesterone group (more than 1 ng/ml, P₄+) was significantly higher than that of the low progesterone group (less than 1 ng/ml, P₄-; 47.9% vs. 28.6% P<0.01). In the P₄- groups, the serum phospholipid level was significantly higher in the conception group than in the non-conception group, and the same tendency was seen in the P₄+ groups. Blood urea nitrogen (BUN), albumin (Alb), and total cholesterol (TChol) were significantly higher in the conception group compared with the non-conception group, but no with P₄ was observed. We concluded that 1) the conception rate of the P₄- group was remarkably low, that 2) the low conception rate and low P₄ level was related to a low PL level and that 3) BUN, Alb and TChol were higher in the conception group, although no relation with P₄ was found.

Key words: Conception rate, Controlled Internal Drug Release Device (CIDR), Estrus synchronization program, Holstein cow

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Decline of the conception rate and extension of the calving interval in the dairy cow resulted in economic losses. In recent years, decline of the conception rate has become a serious problem [1, 2]. One problem related to this is low fertilization resulting from an unsuitable nutrition status after calving. Some researchers have shown that low energy intake or body condition score (BCS) causes decreased conception rates [3, 4]. Milking requires expenditure of energy, and if the animal's BCS is low, increase of the negative energy balance (NEB) causes a negative influence on breeding function.

Another problem is that the estrus signs become weak and AI timing is unsuitable [5]. To solve these problems, some estrus synchronization programs, such as ovsynch, heatsynch [6], quicksynch [7] and modified programs using a Controlled Internal Drug Release (CIDR) have been developed. In this study, a synchronizing method that utilizes EB and a CIDR was used to clarify the influence of nutrition and internal secretion state at the time of synchronization start.

Materials and Methods

Animals

A total of 258 lactating Holstein dairy cows from 13 herds in Tohoku-machi (Aomori, Japan) were used in the present study. Mean days in milk (DIM), BCS (on a scale from 1 to 5) and age were 123.7 ± 74.8 days, 2.6 ± 0.4 and 4.2 ± 1.8 years, respectively.

Breeding program and pregnancy diagnosis

The breeding program was a CIDR short program widely used in Japan. The estrus synchronization program, which used a CIDR (CIDR-B; InterAg, Hamilton, New Zealand) and estradiol benzoate (EB; Ginandoul; Sankyo, Tokyo, Japan), is shown in Fig. 1. The cows were injected intramuscularly with EB 2 mg, and a CIDR was intravaginally inserted and left in place on Day 0. On Day 7, the CIDR was removed, and 25 mg prostaglandin F_{2α} (PGF_{2α}; Pronalgon F; Pharmacia, Tokyo, Japan) was administered intramuscularly. On Day 9, the cows were administered 1 mg EB to bring them into heat, and AI was performed on Day 10. Forty days after AI, pregnancy was determined by rectal palpation.

Blood biochemistry

Blood samples were collected at the beginning of the breeding program and centrifuged to separate the plasma. Total protein (TP), glutamate oxaloacetate transaminase (GOT), total cholesterol (TChol), blood urea nitrogen (BUN), calcium (Ca), inorganic phosphorus (iP), magnesium (Mg), phospholipid (PL) and albumin (Alb) levels in the plasma were measured using an AU 400 auto-analyzer (Olympus, Tokyo, Japan) at the Animal Hospital of Kitasato University. Progesterone (P_4) levels were measured by enzyme-linked immunosorbent assay (ELISA) in the Department of Clinical Veterinary Theriogenology of Kitasato University [8].

Data analysis

Based on P_4 concentration, the cows were divided into two groups as follows, 1) the P_{4+} group, which had P_4 levels of more than 1 ng/ml, and 2) the P_{4-} group, which had P_4 levels of less than

1 ng/ml. The conception rates of the P_{4+} and P_{4-} groups were calculated, and differences were evaluated using the Chi-square test.

The effects of P_4 (P_{4+} and P_{4-} groups) and conception (conception and non-conception groups) on blood biochemistry values, age, DIM and BCS were assessed using two-way analysis of variance (ANOVA) and a post hoc test (Turkey's test). Statistical analyses were conducted using JSTAT for Windows (programmed by M Satoh; Vector, Tokyo, Japan).

Results

Of the 258 cows, 106 conceived, and the conception rate was 41.1%.

There were 167 cows in the P_{4+} group, and 80 of the cows conceived. There were 91 cows in the P_{4-} group, and 26 of the cows conceived. The χ -square tests revealed that the conception rate of the P_{4+} group (47.9%) was significantly higher than that of the P_{4-} group (28.6%; Table 1).

The results of two-way ANOVA are shown in Table 2. TChol, PL, Alb and BUN were significantly higher in the conception groups ($P<0.05$), and the P_4 level had no significant effect. Interaction between the P_4 level and conception was only observed in relation to the PL level ($P<0.05$). The PL levels of the P_{4+} and conception, P_{4+} and non-conception, P_{4-} and conception and P_{4-} and non-conception groups were 270.7 ± 50.6 , 229.7 ± 32.1 , 218.0 ± 16.2 and 171.8 ± 48.1 mg/dl respectively. The Tukey multiple comparisons test showed that the PL levels of the P_{4+} and conception ($P<0.01$) and P_{4-} and conception groups ($P<0.05$) were significantly higher than that of the P_{4-} and non-conception group (Table 3). Therefore, the PL levels of the P_{4-} and non-conception

Fig. 1. Estrus synchronization program using a CIDR. Two mg EB and a CIDR were administered on Day 0. The CIDR was left in place and 25 mg $PGF_{2\alpha}$ was administered on Day 7. One mg EB was administered on Day 9, and AI was performed on Day 10.

Table 1. Numbers of cows and conception rates of each group

Group	Cows	Number of cows conceived	Conception rate (%)
P_{4-}	91	26	28.6 ^a
P_{4+}	167	80	47.9
total	258	106	41.1

^a $P<0.01$ for significant differences in conception rates between the P_{4-} and P_{4+} groups.

Table 2. Age, DIM, BCS and blood biochemistry values of the conception and non-conception cows

		Conception	Non-conception
Age	(year)	4.04 ± 1.73	4.36 ± 1.85
Mean days in milk (DIM)	(day)	131.6 ± 80.5	118.0 ± 70.2
Body condition score (BCS)		2.55 ± 0.40	2.55 ± 0.39
Total protein (TP)	(mg/dl)	7.53 ± 0.91	7.44 ± 0.90
Glutamate oxaloacetate transaminase (GOT)	(IU/l)	79.0 ± 20.1	76.8 ± 29.4
Total cholesterol (Tchol)	(mg/dl)	214.0 ± 50.3^a	201.8 ± 53.1
Blood urea nitrogen (BUN)	(mg/dl)	12.3 ± 3.6^a	11.8 ± 3.0
Calcium (Ca)	(mg/dl)	8.92 ± 0.76	8.81 ± 0.78
Inorganic phosphorus (iP)	(mg/dl)	5.84 ± 0.98	5.72 ± 1.05
Magnesium (Mg)	(mg/dl)	2.29 ± 0.22	2.29 ± 0.66
Phospholipid (PL)	(mg/dl)	$237.2 \pm 51.6^{a,b}$	219.3 ± 57.5
Albumin (Alb)	(g/dl)	3.60 ± 0.31^a	3.51 ± 0.35

^a $P<0.05$ for significant differences between the conception and non-conception groups.

^b There was interaction between the effects of P_4 level and conception ($P<0.05$).

Table 3. The PL levels (mg/dl) of the conception and non-conception cows in the P₄- and P₄+ groups

	Conception	Non-conception
P ₄ -	218.0 ± 16.2 ^a	171.8 ± 48.1
P ₄ +	270.7 ± 50.6 ^b	229.7 ± 32.1

^a Significantly higher than the P₄-non-conception groups (P<0.05).^b Significantly higher than the P₄-non-conception groups (P<0.01).

group was significantly lower than those of the P₄+ and conception and P₄+ and non-conception groups, but that of the P₄+ and non-conception group was not.

Discussion

In this study, we clearly showed that a low P₄ level at the start of this breeding program suppresses the conception rate. It has been reported that poor nutrition suppresses the activity of the ovaries and P₄ secretion and causes low conception rates [9]. Yamada [5] reported that the conception rate after the Ovsynch program is high in cows with a BCS of 3.75 during the dry period and 3.0 on the day of Ovsynch. In this study, the BCS at the start of the synchronization program was 2.55 regardless of conception or non-conception, and this lower BCS indicated that the nutritional status of the examined cows was lower than the optimal nutritional level for treatment with a fixed-time artificial insemination program.

The effects of P₄ and conception showed interaction on the PL level, and this suggested that there is a relationship between the levels of plasma PL and P₄ and the conception rate. Table 3 indicates that in low P₄ groups, PL of conception group is significantly higher than that of non-conception group, but not in high P₄ groups.

Some researchers have shown that there is a relationship between fat and ovarian activity. Ryan *et al.* [10, 11] indicated that a high fat diet causes increased frequency of luteal activity and alterations in specific aspects of ovarian steroidogenic potential. Other researchers have shown that PL activates protein kinase, which controls luteal cells [12, 13], and that lipoprotein, which is composed of PL, regulates P₄ production in luteal cells [14, 15].

Therefore, we hypothesized that a low plasma PL level inactivates the luteal body. In this study, some of the P₄- cows had insufficient levels of PL for activation of the luteal body, so the luteal body of the next cycle, which maintains pregnancy, was also inactive, causing a decline in the conception rate. This mechanism did not work in the P₄- cows that had sufficient levels of PL nor in the P₄+ cows and this may be the cause of the low conception rate in the P₄- group.

Interestingly, the P₄ level at the beginning of the program did not

affect Alb, BUN or TChol, although the conception rate decreased as their levels increased in the present study. Therefore, there may be other factors unrelated to luteal body activity and P₄ that reduce the conception rate, such as those related to Alb, BUN, TChol and probably PL. Further research is required to determine these mechanisms.

This study suggests that a low P₄ level at the start of the breeding program using a CIDR and EB caused the low conception rate and that this was related to a low PL level. It also suggested that BUN, Alb and TChol also affect the conception rate, regardless of the level of P₄.

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