—Original Article—

Effect of CIDR 12 to 19 Days After AI on Detection of Returning Estrus and Conception Rate in Dairy Cows

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Abstract. Detection of returning estrus in dairy cows after AI and re-insemination without delay are important in shortening the calving to conception interval. The objectives of this study were to show the effectiveness of CIDR insertion 12 to 19 days after AI on returning estrus and shortening the calving to conception interval in dairy cows. Seventy-nine dairy cows from two commercial dairy farms were synchronized for first postpartum estrus using a CIDR-Heatsynch protocol, and 76 cows (96.2%) showed estrus signs within 2 days after EB injection and were inseminated. The cows were then divided randomly into two groups. Thirty-seven cows were treated with a CIDR from 12 to 19 days after AI (CIDR group), while the other 39 cows were not treated and served as a control group. Milk samples were collected twice weekly from one week before the commencement of the CIDR-Heatsynch protocol until 7 to 9 days after removal of device. Detection rates of returning estrus 20 to 25 days after AI (within 6 days after removal of the device) were 30.4% in the CIDR group and 47.6% in the control group. According to the progesterone profiles, almost half of the non-pregnant cows that did not show estrus 20 to 25 days after AI had high progesterone concentrations from days 20 to 25, 59.1% in the CIDR group and 50.0% in control group. The calving interval was not significantly different between the CIDR (162 ± 50 days) and control groups (151 ± 40 days). In conclusion, CIDR insertion 12 to 19 days after AI did not increase the detection rate of returning estrus. As a consequence, there was no effect of the CIDR treatment on the calving to conception interval.

Key words: Calving interval, CIDR, Re-synchronization, Synchronization

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The first AI conception rate in dairy cows has recently been reported to be 20 to 40% [1–4]. Cows that do not conceive after first AI should be re-inseminated without delay to minimize the calving to conception interval. It is, therefore, of paramount importance that returning estrus of cows failing to conceive is detected efficiently and re-insemination is conducted after detection of estrus.

However, the returning estrus detection and re-insemination rates 20 to 25 days after AI have been reported to be about 28.0% in non-pregnant cows [5, 6].

The rate of detection of returning estrus would be substantially improved if returning estrus could be synchronized 20 to 25 days after AI. Recently, some studies showed that intra-vaginal insertion of a CIDR 14 to 21 days after AI increased the return rate of non-pregnant cows [7]. Other studies, however, have failed to show any improvement [6, 8]. More recently, a study described that addition of estradiol benzoate one day after CIDR removal increased the rate of returning to estrus [9].

We assumed that CIDR insertion during a period from 12 to 19 days after AI may better synchronize returning estrus to around 21 days after AI than CIDR insertion 14 to 21 days after AI. In most cows, luteolysis commences 17 to 18 days after estrus [10], and the dominant follicle starts to grow to maturity [11]. CIDR insertion

12 to 19 days after AI may not interfere with that phenomenon and may still facilitate non-pregnant cows returning to estrus 20 to 25 days after AI due to supplementation of progesterone during the mid and late luteal phase.

The objectives of this study were, therefore, to examine whether CIDR insertion 12 to 19 days after AI is effective in improving the returning estrus detection rate 20 to 25 days after AI and shortening the interval from calving to conception.

Materials and Methods

Location, cows and housing

A total of 79 lactating Holstein Frisian cows from two dairy herds were used in this study. The experiment was conducted during the period of June 2006 to November 2007.

One herd (U farm) was located in Hokkaido, Japan, which is in northern Japan, and the other herd (T farm) was in Tottori, which is in western Japan. Cows were housed in free stall barns and milked twice daily. The herds consisted of 110 and 150 Holstein cows with an annual rolling herd average of 9,500 and 12,000 kg per cow per lactation, respectively.

The cows used in this experiment were in 63 to 107 days in lactation. The mean ($\pm$ SD) days postpartum was 82 ± 10.5 days. Parity was 1.9 ± 1.0 on average (mean $\pm$ SD) and ranged from one to four. The cows were clinically examined for the presence of pyometra, endometritis, urovagina and any other reproductive abnormalities before the experiment. Only cows with a BCS >2.5 [12] and having no reproductive disorders were used for the

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Fig. 1. Protocol for re-synchronization of returning estrus in dairy cows after synchronization of first postpartum estrus with CIDR-Heatsynch. GnRH: gonadotropin-releasing hormone, PGF_{2α}: prostaglandin F_{2α}, EB: estradiol-benzoate, Estrus & AI: cows showing estrus and artificial insemination, d0: day of start of Heatsynch, (d0): day of AI.

experiment.

Experimental design

A total of 79 cows including 28 cows at U farm and 51 cows at T farm were inseminated artificially with frozen thawed semen after synchronization of estrus with a CIDR-Heatsynch protocol. The cows were then randomly assigned into two groups for the re-synchronization experiment. The 51 cows at T farm consisted of 25 cows in the CIDR group and 26 cows in the control group. Likewise, the 28 cows at U farm included 12 cows in the CIDR group and 16 cows in the control group (Fig. 1).

Estrus synchronization treatment

The 79 cows at both farms received a CIDR containing 1.9 g of progesterone (EAZI-BREED CIDR®, Livestock Improvement Association of Japan, Tokyo, Japan) in the vagina, and 100 µg of GnRH analogue (Fertilin acetate, Conceral®, Schering-Plough Animal Health, Tokyo, Japan) was injected intramuscularly on the day of CIDR insertion. Seven days later, the CIDR was removed, and an intramuscular injection of 500 µg PGF_{2α}-analogue (Cloprostenol, Resipron® C, ASKA Pharmaceutical, Tokyo, Japan) was given. One mg estradiol benzoate (EB; Kawasaki Pharmaceuticals, Tokyo, Japan) was injected intramuscularly the day after PGF_{2α} treatment. The cows were observed for estrus within 2 days after EB injection and were inseminated artificially based on detection of estrus.

Synchronization of returning estrus

After insemination following the CIDR-Heatsynch protocol, the cows were divided randomly into two groups. A group of 37 cows were treated with a CIDR from 12 to 19 days after AI (CIDR group), while the other group of 39 cows was not treated and served as the control. The cows were observed for estrus 20 to 25 days

after first AI, and those detected in estrus were re-inseminated artificially (Fig. 1).

Milk sampling and progesterone ELISA

Milk samples were collected twice weekly (Monday and Thursday) during morning milking from one week before commencement of the protocol until 25 days after AI. About 10 ml of milk was collected in a plastic tube containing 15 mg potassium dichromate (Wako Pure Chemical Industries, Osaka, Japan), and was kept at 4 °C until assay. Progesterone concentrations in milk were determined by an enzyme immunoassay established and validated by Isobe *et al.* [13]. The intra and interassay coefficients of variation in high and low progesterone pooled milk samples were 7.1 ± 1.2 and $14.6 \pm 0.3\%$ ($n=6$) and 7.7 ± 1.2 and $14.6 \pm 0.3\%$ ($n=6$), respectively.

Detection of estrus, AI and pregnancy diagnosis

At U farm, signs of estrus were observed by the farmers around 0630 h in the morning and at around 1800 h in the afternoon when the cows were moving from the barn to the milking parlor. At T farm, tail paint (All - Weather® Paintstik® LA - CO Industries, Elk Grove, IL, USA) was used as a heat detection aid in addition to visual observation for standing and mounting. Additionally, bellying and decrease in milk yield were also considered to be signs of estrus. Estrus detection was performed three times a day by the farmers, from 0500 to 0900 h in the morning, around noon and from 1600 to 1900 h in the afternoon. Cows were inseminated artificially by a veterinarian at U farm and by the farmer at T farm with frozen thawed semen from proven Holstein sires. Pregnancy diagnosis by transrectal palpation was conducted 35 days after AI or later by veterinarians at both farms.

Definition of returning estrus based on progesterone profiles

The effects of treatment with a CIDR for re-synchronization were evaluated based on progesterone concentrations 17 to 19 days, 20 to 24 days and 26 to 28 days after AI. Progesterone concentrations below 5.0 ng/ml were considered low, indicating absence of a CL, while progesterone concentrations ≥ 5.0 ng/ml were referred as high, indicative of luteal activity [13]. Cows showing high progesterone concentrations during 17 to 19 days after AI and low progesterone concentrations during 20 to 25 days after AI were considered to have returned to estrus. Other cows showing high progesterone concentrations during 17 to 19 days after AI and also high progesterone concentrations 20 to 25 days after AI were considered not to have returned to estrus 20 to 25 days after AI.

Statistical analysis

The statistical analysis was carried out using the statistical package SPSS 12.0 for Windows (SPSS, Chicago, IL, USA). Estrus detection and conception rates were compared between the CIDR and control groups during the period of 20 to 25 days after AI by a Chi-Square test. A probability of $P < 0.05$ was considered statistically significant.

Table 1. Effect of CIDR insertion 12 to 19 days after AI for re-synchronization of estrus and on reproductive performance in dairy cows

Item	CIDR group			Control group			Total
	U farm	T farm	Total	U farm	T farm	Total	
No. of cows synchronized for first AI	12	26	38	16	25	41	79
No. of cows with AI within 2 days after synchronization (%)	12 (100)	25 (96.2)	37 (97.4)	15 (93.8)	24 (96.0)	39 (95.1)	76 (96.2)
No. of cows pregnant after first AI (%)	5/12 (41.7)	9/25 (36.0)	14/37 (37.8)	8/15 (53.3)	10/24 (41.7)	18/39 (46.2)	32/76 (42.1)
No. of cows not pregnant after first AI (%)	7/12 (58.3)	16/26 (61.5)	23/38 (60.5)	8/16 (50.0)	13/25 (52.0)	21/41 (51.2)	44/79 (55.7)
No. of cows returning to estrus from day 20 to day 25	2	5	7	5	5	10	17
No. of cows resynchronized / cows not pregnant after first AI (%)	2/7 (28.6)	5/16 (31.3)	7/23 (30.4)	5/8 (62.5)	5/13 (38.5)	10/21 (47.6)	17/44 (38.6)
No. of cows that conceived at returning estrus within 6 days/ cows resynchronized (%)	1/2 (50.0)	1/5 (20.0)	2/7 (28.6)	5/5 (100)	2/5 (40.0)	7/10 (70.0)	9/17 (52.9)
No. of cows returning to estrus from day 26 to day 40	1	3	4	0	5	5	9
No. of cows returning to estrus from day 41 to day 50	1	2	3	0	0	0	3
No. of cows that conceived within 150 days (%)	3/7 (42.9)	5/16 (31.3)	8/23 (34.8)	4/8 (50.0)	5/13 (38.5)	9/21 (42.9)	17/44 (38.6)
No. of cows that conceived within 210 days (%)	5/7 (71.4)	9/16 (56.3)	14/23 (60.9)	6/8 (75.0)	9/13 (69.2)	15/21 (71.4)	29/44 (65.9)
Calving to conception interval (Mean $\pm$ SD; days)	159 $\pm$ 89	169 $\pm$ 48	162 $\pm$ 50	142 $\pm$ 42	164 $\pm$ 33	151 $\pm$ 40	158 $\pm$ 46

Table 2. Response to CIDR treatment based on progesterone profiles during re-synchronization of estrus in the CIDR-treated and control cows

Milk progesterone levels				Re-synchronization of estrus	No. of cows (%)	
17 to 19 days	20 to 25 days	26 to 28 days	CIDR group		Control group	
No. of cows not pregnant after first AI					23	21
No. of cows monitored for P4					22	18
H	L	H	Positive	5 (22.7)	7 (38.9)	
H	L	L	Positive	4 (18.2)	2 (11.1)	
H	H	L	Negative	2 (9.1)	1 (5.6)	
H	H	H	Negative	11 (50.0)	8 (44.4)	

H $\geq$ 5 ng/ml, L<5 ng/ml. 17 to 19 days: Before removal of CIDR, 20 to 25 days: 6 days after CIDR removal, 26 to 28 days: 7 to 9 days after CIDR removal.

Results

The estrus detection rates within 2 days after the CIDR-Heatsynch protocol for the first postpartum AI were 95.1% in control and 97.4% in CIDR groups. The first AI conception rates after synchronization in the control and CIDR groups were 46.2 and 37.8%, respectively. There was no difference in the non-pregnancy rates after first AI between U farm and T farm within both the CIDR and control groups. Likewise, the resynchronization rates of the two farms were not significantly different in both the CIDR and control groups (Table 1). Of the 23 cows (7 cows at U farm and 16 cows at T farm) not pregnant after the first AI in the CIDR group, 7 cows (30.4%) returned to estrus 20 to 25 days after AI, while 47.6% of 21 cows (7 cows at U farm and 14 cows at T farm) not conceiving after first AI in the control group returned to estrus. The conception rate after AI at returning estrus was 28.6% in the CIDR group, whereas it was 70.0% in the control group ($P<0.05$). The pregnancy rates within 150 days and 210 days postpartum in cows did

not conceive after first AI was not significantly different between the CIDR and control groups. There was no difference in the calving to conception intervals between the CIDR and control groups (Table 1).

The returning estrus rate 20 to 25 days after AI based on the progesterone profiles was 40.9% in the CIDR group and 50.0% in the control group, and 59.1% of the non-pregnant cows in the CIDR group and 50.0% of the non-pregnant cows in the control group showed high progesterone concentrations 20 to 25 days after AI, indicating a prolonged luteal phase (Table 2).

Discussion

In this study, the pregnancy rate after first AI at estrus synchronized by CIDR-Heatsynch was 42.1% (32/76), and this is similar to the results obtained by Ovsynch in dairy cows [14, 15]. Stevenson *et al.* [16] also reported that the conception rate in lactating cows after Ovsynch is 40% and that it is 50% after CIDR-Ovsynch, and

these are equivalent to the conception rate obtained in the present study.

Insertion of a CIDR from 12 to 19 days after AI did not increase the proportion of cows that were not pregnant and returned to estrus 20 to 25 days after AI. There were no differences in reproductive performance between the two farms, as indicated by similar non-pregnancy rates after first AI and no difference in resynchronization rate. Only 30.4% of the non-pregnant cows that were treated with a CIDR 12 to 19 days after AI returned to estrus 20 to 25 days after AI, while 47.6% of the non-pregnant cows in the control group showed returning estrus. These results are in agreement with those reported by El-Zarkouny and Stevenson, who found that treating dairy cows with CIDR devices 13 to 20 days after AI failed to increase the overall return rate to estrus in non-pregnant cows [6]. Chebel *et al.* [8] also reported that insertion of a CIDR 14 and 21 days after AI in dairy cows does not improve the re-insemination rate of non-pregnant cows 20 to 25 days after AI. On the other hand, some other studies [7, 9] have shown increased return rates to estrus in cows treated with a CIDR 13 to 20 days or 14 to 21 days after AI.

The rate of detection of returning estrus after infertile breeding is influenced by the occurrence of an extended luteal phase and the incidence of embryonic mortality. If the low return rate of cows after infertile insemination was due to silent estrus, the treatment with a CIDR in the luteal phase would have been more effective. The percentage of cows not returning to estrus 20 to 25 days after infertile insemination was 69.6% in the CIDR treated group and 52.4% in the control group. The authors expected that some of the cows that had silent estrus 20 to 25 days after first AI might come into estrus 41 to 50 days after the first AI. Only 3 (13.0%) of the 23 cows in the CIDR group showed estrus 41 to 50 days after first AI; none of the 21 control cows showed estrus 41 to 50 days after first AI. It seems that cows with silent estrus 20 to 25 days after AI still have silent estrus in the next cycle. According to the progesterone profiles, we found that almost half of the non-pregnant cows that did not show estrus during 20 to 25 days after AI had a prolonged luteal phase; more specifically, this occurred in 59.1% of the non-pregnant cows in the CIDR group and 50.0% of the non-pregnant cows in the control group (Table 2). We assumed that the extension of the luteal phase after AI is due mainly to embryonic mortality and delayed luteolysis. Alnimer *et al.* [7] reported that the prevalence of embryonic death during the period of day 28 to day 45 after AI was 11.9% in a group of lactating dairy cows that received a CIDR 14 to 19 days after AI and 17.5% in a non-treated group. Galvão *et al.* [17] showed that the prevalence of embryonic loss in lactating cows during the period of 27 to 41 days after AI is 15.3% to 18.1%.

The incidence of prolonged luteal phase in cows was higher than 50% in this study. Hommeida *et al.* [18] also reported a higher milk yield in cows with a prolonged luteal phase. A tendency towards an increase in the incidence of prolonged luteal phases of the estrus cycles has been observed in dairy cows [2, 19]. It has also been reported that high milk production and the resultant increase in the metabolic clearance rate cause a low plasma concentration of estradiol [20], which delays luteolysis [21]. The increased milk production has been associated with increased liver

blood flow and metabolic clearance of key steroid hormones [22]. El-Zarkouny and Stevenson reported that more than 62.0% of the non-pregnant cows returned to estrus later than 26 days after AI in the control and CIDR groups used in their study (CIDR inserted 13 to 20 days after AI) [6]. Another study also reported that approximately 20.0% of non-pregnant beef heifers returned to estrus later than 25 days after AI in a CIDR group (CIDR inserted 13 to 20 days after AI) and that approximately the rate was 22.0% in the control group [23]. Galon [24] reported that 10.9% of primiparous and 13.4% of multiparous cows had long estrus cycle lengths (25 to 36 days after AI), respectively. Bouchard and Du Tremblay showed that the interval between the first and second AIs is around 42 days and that the interval between the second and third AIs is 40 days according to lactation number in dairy cows in Quebec and Canada [25].

The pregnancy rates of cows that did not become pregnant after first insemination within 150 and 210 days postpartum of the CIDR-treated and control groups were not significantly different. These results are in agreement with the previous studies [5, 9].

In conclusion, the rate of detection of returning estrus after CIDR insertion 12 to 19 days after AI was not improved. As a consequence, there was no effect of the CIDR treatment on the calving to conception interval.

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