The Journal of Veterinary Medical Science

FULL PAPER

Theriogenology

Effect of post artificial insemination treatment with two different progesterone intravaginal devices on conception and synchronization of the returning estrus in Japanese Black cows

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J. Vet. Med. Sci.
80(12): 1822–1828, 2018
doi: 10.1292/jvms.17-0094

Received: 22 February 2017
Accepted: 18 September 2018
Published online in J-STAGE: 18 October 2018

ABSTRACT. The objective of this study was to evaluate the effects of post artificial insemination (AI) treatment with intravaginal progesterone device (P4 device) on conception rate, synchronization of returning estrus and plasma P4 concentration in Japanese Black cows. Nineteen cows were treated with DIB (1.0 g P4) from Day 12 to 19 (Day 0=day of the first AI), 27 cows were treated with a CIDR (1.9 g P4) from Day 12 to 19, and 33 cows were not treated after the first AI (control). Estrous behavior was daily examined between Day 20 and 25, and cows returning to estrus were inseminated (the second AI). On Day 19, plasma P4 concentration was not different among DIB, CIDR and control groups. There was no significant difference in conception rate after the first AI among three groups (DIB: 63.2%, CIDR: 66.7% and control: 72.7%). In non-pregnant cows, there was no significant difference in the proportion of cows showed returning estrus between Day 20 and 25 (DIB: 57.1%, CIDR: 22.2% and control: 44.4%), and day of returning estrus was not synchronized. The overall conception rate after the first and second AI was not different among the groups. In conclusion, post-AI treatment with intravaginal devices containing 1.0 and 1.9 g P4 from Day 12 to 19 neither increased plasma P4 concentration nor improved fertility and synchronization of the returning estrus in Japanese Black cows.

KEY WORDS: CIDR, DIB, Japanese Black cow, progesterone, returning estrus

Improvement of conception rate after artificial insemination (AI) and detection of returning estrus of non-pregnant cows are fundamental in beef cattle production. Because of increased number of beef cattle per farm in Japan (http://www.maff.go.jp/j/tokei/kouhyou/tikusan/index.html#r), efficient methods to improve conception rate and detection of returning estrus after the AI are required. In dairy cows, insufficient concentration of progesterone (P4) in luteal phase after AI was related to poor embryo development [21]. To improve fertility of dairy cows, an intravaginal progesterone device (P4 device) has been used at various timing and duration after AI [2, 4, 17, 24, 25, 38]. In beef cows, information of effects of post-AI treatment with P4 device on fertility is limited. The treatment with a CIDR from Day 4–6 to 19 (Day 0=day of AI) (Modified Fast Back® program) was reported to increase conception rate of Japanese Black cows with repeat breeding [36]. However, the treatment with a P4 device from Day 5 to 14 [15] and from Day 7 to 20 [29] did not affect conception rate in beef cows. In dairy cattle, it has been reported that conception rate decreased by post-AI treatment with a P4 device from Day 4 to 9 [27] and from Day 2 to 9 [37], suggesting that P4 supplementation in early period has potential risk for deleterious effect on corpus luteum (CL) function.

Returning estrus of non-pregnant cows after AI is observed according to estrous cycle [13], whereas silent heat symptoms cause a failure of estrus detection [33]. Shift of feeding system to intensive farming and aging of farm labors make it difficult to detect estrus [33]. Timing of the returning estrus was shortened by luteal hypoplasia [26, 27] and prolonged by embryonic mortality [11]. In dairy cattle, treatment with a P4 device after AI from Day 13 to 20 [6] and from Day 12 to 19 [17] did not affect incidence

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of the returning estrus of non-pregnant dairy cows. In contrast, the returning estrus of non-pregnant dairy cow was synchronized from Day 14 to 21 [1], from Day 13–15 to 20–22 [3], from Day 14–17 to 21 [18] and from Day 17 to 22 [37] by post-AI treatment with P4 device. The returning estrus of non-pregnant beef cow was not synchronized by post-AI treatment from Day 5 to 14, but synchronized between Day 21 to 26 by the treatment from Day 14 to 21 [15]. In Japanese Black cows, dispersion for the timing of the returning estrus was reduced after treatment with a 1.9 g P4 device from Day 17 to 24 [14]. In general, estrus detection was performed from Day 20 to 21 based on estrus cycle in Japanese Black cows, so that more effective duration for post-AI treatment with P4 device to detect returning estrus is needed. Previous reports for post AI treatment with P4 devices mainly focused on the timing of treatment in dairy cattle [16, 31, 37]. There has been no report that measure plasma P4 concentration during post-AI treatment with P4 devices. Though several reports conducted post-AI treatment with CIDR (1.38 g P4) in dairy cattle, a CIDR (1.9 g P4) was previously used in Japanese Black cows [14, 36]. Insertion of P4 devices from Day 12–14 to 19–21 did not improve the conception rate in dairy cattle [3] and beef cattle [5, 15, 34]. However, devices which they used were CIDR (1.38 g P4) or second time used CIDR. Effects of DIB (1.0 g P4) and CIDR (1.9 g P4) have not been compared in both dairy and beef cattle. Effects of post-AI treatment from Day 12–14 to 19–21 with these P4 devices on conception rate and returning estrus have not been clarified.

Therefore, in the present study, to improve the reproductive performance of Japanese Black cows by the post-AI treatment, we studied the effects of the post-AI treatment with two different P4 devices from Day 12 to 19 on conception rate, synchronization of returning estrus and plasma P4 concentration.

MATERIALS AND METHODS

Animals

Field trial was conducted during April 2012 to May 2013, and three commercial farms in Shimane prefecture, Japan (two farms in location A and a farm in location B). The herds consisted of 54 and 100 cows at each farm in location A, and 42 cows at a farm in location B. A total of 79 suckled Japanese Black cows between 43 and 145 days postpartum (DPP) (71.7 ± 23.4 days, mean ± SD) was used in this study (age: 5.4 ± 2.6 years old): 10 and 48 cows in location A and 21 cows in location B. Parity of cows was 3.9 ± 2.2. The cows were subjected to standard management practices in each location, housed in tied stall, provided with fresh water, and fed with hay and concentrate. The cows were clinically normal, and their body condition score (1 to 5 scale with 0.25 increment points, BCS) was 3.0 ± 0.3.

Experimental design

In location A, estrus of cows (n=58) was visually monitored for standing estrus, mounting activities and mucus discharge. The cows were inseminated approximately 12 hr after onset of the estrus by on-farm technicians (the first AI). Cows at a farm in location B (n=21) were inseminated after Ovsynch protocol [10]. The cows were injected with 100 μg GnRH analogue (fertirelin acetate; Conceral™, Schering-Plough Animal Health KK, Tokyo, Japan) on 10 days before AI, followed by 25 mg dinoprost (PG; Pronalgon®, Pfizer Japan, Nagoya, Japan) on 3 days before AI, and 100 μg GnRH analogue on day before AI.

On Day 12 (Day 0=day of the first AI), reproductive organs of cows were examined by rectal palpation and transrectal ultrasonography using a real-time B-mode scanner with a 7.5 MHz linear array transducer (Tringa linear, Esaotepie medical, Maastricht, The Netherlands). Cows with a CL larger than 1 cm in diameter (n=79) were randomly divided into three groups: 1) DIB group in which cows were treated with a P4 device containing 1.0 g P4 (DIB, Kyoritsu Seiyaku Co., Tokyo, Japan) in vagina from Day 12 to 19 (n=19), 2) CIDR group in which cows were treated with a P4 device containing 1.9 g P4 (Eazibreed™ CIDR®, Livestock Improvement Association of Japan, Inc., Tokyo, Japan) from Day 12 to 19 (n=27), or 3) control group in which cows were not treated (n=33). Blood samples of the cows on Day 19 were collected 3–5 min after the removal of P4 device and measured for plasma P4 concentration. Estrous behavior was daily examined between Day 20 and 40. Cows returning to estrus between Day 20 and 25 were inseminated (the second AI), and those returning to the estrus between Day 26 and 40 were classified as delayed estrus. Pregnancy diagnosis was carried out by transrectal ultrasonography on Days 40, 60 and 80.

P4 assay

We collected blood samples from 18 cows in DIB group, 22 cows in CIDR group and 27 cows in control. Twelve cows were excluded from P4 assay because of accidental excitement of cows at blood sampling. The samples were collected from jugular vein into heparin-treated 1.5 m*l* tubes (Fuji Film Medical Co., Ltd, Tokyo, Japan), placed on ice for approximately 1 to 6 hr, and then centrifuged at 1,500 g for 10 min. Plasma was decanted into the tubes and stored at −20°C until analysis. Plasma P4 concentrations were measured by enzyme immunoassay (Spotchem Vidas SV-5010, Sysmex bioMerieux, Tokyo, Japan). The inter-assay coefficient of variation was on average 10%. The measurement range was 0.25–80 *ng*/m*l*. The cross-reactivities are follows: progesterone 100%, 20α-hydroxyprogesterone 0.03%, 6β-hydroxyprogesterone 0.29%, 16α-hydroxyprogesterone 0.20%, 17α-hydroxyprogesterone 1.18%, desoxycorticosterone 1.15%, corticosterone 0.09%, testosterone 0.01%, estron 0.01% and estradiol <0.01.

Statistical analysis

The effect of locations and herd on conception rate was evaluated by univariate logistic regression analysis. In each group and all cows, multivariate logistic regression analysis was used to determine the impact of the following factors: parity, DPP, BCS,

Table 1. Reproductive status of Japanese Black cows after the first and second AI

Reproductive status of cows a)	Number (%) of cows in each treatment			P c)
	DIB b)	CIDR b)	Control	
The first AI				
Inseminated on Day 0	19	27	33	
Pregnant after the first AI	12 (63.2)	18 (66.7)	24 (72.7)	>0.05 (NS)
Non-pregnant after the first AI	7 (36.8)	9 (33.3)	9 (27.3)	>0.05 (NS)
Returning to estrus between Day 20 and 25	4 (57.1)	2 (22.2)	4 (44.4)	>0.05 (NS)
The second AI				
Inseminated on day of returning estrus	4	2	4	
Pregnant after the second AI	3 (75.0)	2 (100.0)	1 (25.0)	>0.05 (NS)
Overall pregnant	15 (78.9)	20 (74.1)	25 (75.8)	>0.05 (NS)

$P<0.05$ was considered significantly different. a) Pregnancy diagnosis on Days 40, 60 and 80. b) Treated with a DIB or CIDR from Day 12 to 19. c) *P*-values among each treatment. NS: Not significantly different.

CL diameter, season and type of estrus. Conception rates and proportion of cows returning to estrus between Day 20 and 25 were analyzed by Fisher's exact test. Plasma P4 concentrations among groups and between pregnant and non-pregnant cows in each group were compared by Kruskal-Wallis test and Wilcoxon rank sum test, respectively. Differences with $P<0.05$ were considered significant. Analysis of 2 × 3 Fisher's exact test was performed with IBM SPSS statistics ver. 23.0 software (IBM Corporation, Armonk, NY, U.S.A.). The other data were analyzed using Ekuseru-Toukei 2008 software for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

Conception rate on Day 40 in locations A and B was as follows: DIB group, 60.0% (9/15) vs. 75.0% (3/4); CIDR group, 73.7% (14/19) vs. 50.0% (4/8); control group, 79.2% (19/24) vs. 55.6% (5/9). There was no significant difference in conception rate between locations A and B in each group. Using univariate logistic regression analysis, it was indicated that neither herd nor location accounted for conception rate in each group. Since number of cows in each experiment group was small, data from locations A and B were pooled. Combined data of conception after the first AI showed that 63.2, 66.7 and 72.7% of cows were pregnant in DIB, CIDR and control groups, respectively, and were not significantly different among the groups (Table 1). Table 2 shows models accounting for parity, DPP, CL diameter, BCS, season and type of estrus in each group and all cows by multivariate logistic regression analysis. In CIDR group, parity showed significant association with conception rate (OR=0.41, $P=0.04$). No significant interactions were found in DIB and control groups. In non-pregnant cows, 57.1 (4/7), 22.2 (2/9) and 44.4% (4/9) returned to estrus between Day 20 and 25 in DIB, CIDR and control groups, respectively (Table 1). There was no significant difference in returning rate to estrus between Day 20 and 25 among the groups. Returning estrus in DIB, CIDR and control groups was detected during Day 22–23, Day 22–25 and Day 21–25, respectively (Fig. 1). Day of the returning estrus was not synchronized during a period from Day 20 to 25 in all groups. Incidence of delayed estrus was not different among the groups (DIB: 14.3%, CIDR: 33.3%, control: 11.1%). The conception rates after the second AI were 75.0 (3/4), 100.0 (2/2) and 25.0% (1/4) in DIB, CIDR and control groups, respectively (Table 1). Overall conception rates after the first and second AI were 78.9, 74.1 and 75.8% in DIB, CIDR and control groups, respectively, and were not significantly different among the groups. Pregnancy loss was not detected between Day 40 and 80 in all groups.

Plasma P4 concentration on Day 19 of pregnant and non-pregnant cows is shown in Table 3. Plasma P4 concentrations of pregnant cows were approximately 14 *ng*/m*l*. There was no significant difference in plasma P4 concentrations among DIB, CIDR and control groups in pregnant, non-pregnant and total cows. Plasma P4 concentrations of non-pregnant cows in DIB and control groups were low compared to that of pregnant cows ($P<0.01$).

DISCUSSION

The present study showed that post-AI treatment with intravaginal devices containing 1.0 and 1.9 g P4 from Day 12 to 19 did not affect fertility and returning estrus in Japanese Black cows. As previously reported in Japanese Black cows [23, 39] and dairy cattle [30], methods to perform AI (natural estrus vs Ovsynch protocol) had no effect on conception rate. In dairy and beef cows, the intravaginal devices containing 0.95 g (CIDR-B), 1.38 g (CIDR), 1.55 g (progesterone releasing intravaginal device: PRID) and 1.9 g P4 (CIDR) were used for post-AI treatment, and these devices increased plasma and milk P4 concentration [7, 20, 24, 28]. Previously used devices were also used for the treatment, but their P4 doses were not clear [6, 16, 37]. A CIDR containing 1.86 g P4 was reported to lose 1.06 g P4 after 15-days intravaginal insertion [18]. Since P4 dose of DIB (1.0 g P4) is lower than CIDR (1.9 g P4), there is a potential risk for a lack in plasma P4 concentration during the treatment period. Because non-pregnant cows exhibited a decline in plasma P4 concentration after Day 15 in dairy cows [22], measurement of plasma P4 concentration on Day 19 is efficient to compare the effect among groups. This study indicated that post-AI treatment with DIB (1.0 g P4) nor

Table 2. Multivariate logistic regression model for odds of pregnancy for parity, days post partum (DPP), CL diameter, BCS and season in DIB, CIDR and control groups

Group	Variable	Class	n	Odds ratio	95% confidence interval		*P*
DIB [a)]	Parity	Continuous	19	0.71	0.23	2.14	0.54
	DPP	Continuous	19	1.05	0.97	1.14	0.23
	CL diameter	Continuous	19	6.88	0.19	253.03	0.29
	BCS	Continuous	19	1.85	0.00	784.46	0.84
	Season	0 (Winter)	3				
		1 (Spring)	5	1.04	0.02	54.86	0.99
		2 (Summer)	5	0.52	0.02	16.19	0.71
		3 (Autumn)	6	0.33	0.01	9.58	0.52
	Type of estrus	0 (Natural)	15				
		1 (Ovsynch)	4	3.09	0.01	>999.99	0.46
CIDR [a)]	Parity	Continuous	27	0.41	0.17	0.95	0.04*
	DPP	Continuous	27	0.92	0.82	1.04	0.17
	CL diameter	Continuous	27	24.80	0.07	>999.99	0.28
	BCS	Continuous	27	0.06	<0.001	12.15	0.30
	Season	0 (Winter)	9				
		1 (Spring)	6	4.17	0.11	155.38	0.44
		2 (Summer)	8	193.33	0.03	>999.99	0.25
		3 (Autumn)	4	1.76	0.03	117.49	0.79
	Type of estrus	0 (Natural)	19				
		1 (Ovsynch)	8	5.74	0.05	727.37	0.48
Control	Parity	Continuous	33	0.96	0.60	1.52	0.86
	DPP	Continuous	33	0.99	0.96	1.03	0.74
	CL diameter	Continuous	33	3.22	0.47	22.35	0.24
	BCS	Continuous	33	3.16	0.10	99.76	0.51
	Season	0 (Winter)	10				
		1 (Spring)	7	2.97	0.19	46.29	0.44
		2 (Summer)	8	1.94	0.17	22.11	0.59
		3 (Autumn)	8	6.63	0.40	109.83	0.19
	Type of estrus	0 (Natural)	24				
		1 (Ovsynch)	9	0.15	0.01	1.77	0.13
All cows	Parity	Continuous	79	0.77	0.59	1.01	0.06
	DPP	Continuous	79	1.00	0.97	1.02	0.70
	CL diameter	Continuous	79	2.58	0.84	7.90	0.10
	BCS	Continuous	79	0.83	0.12	5.77	0.85
	Season	0 (Winter)	22				
		1 (Spring)	18	1.94	0.44	8.65	0.38
		2 (Summer)	21	2.73	0.60	12.54	0.20
		3 (Autumn)	18	1.86	0.44	7.95	0.40
	Type of estrus	0 (Natural)	58				
		1 (Ovsynch)	21	0.62	0.18	2.15	0.45
	Treatment	0 (CIDR)	27				
		1 (DIB)	19	0.41	0.10	1.79	0.24
		2 (Control)	33	1.18	0.35	3.99	0.78

a) Treated with a DIB or CIDR from Day 12 to 19. *$P<0.05$.

CIDR (1.9 g P4) was not able to increase plasma P4 levels for Japanese Black cows on Day 19 irrespective of pregnancy status (pregnant or non-pregnant). This result also showed the effect of DIB treatment on plasma P4 concentration was similar to CIDR treatment in Japanese Black cows. This study coincided with the previous report that 7-days treatment with DIB or CIDR for estrus synchronization resulted in similar mean plasma P4 concentration in dairy cows [32], although different P4 doses of CIDR affected plasma P4 concentration in CIDR-synch protocol [19]. In this study, though P4 values on Day 19 were significantly higher in pregnant cows than in non-pregnant cows in DIB and control group, it did not differ in CIDR group. However, information about plasma P4 concentration during post AI treatment with DIB and CIDR is limited in this study. Further study needs to be performed to determine impact of post AI treatment with P4 devices on plasma P4 concentration in Japanese Black cows.

In this study, treatment with DIB and CIDR from Day 12 to 19 did not affect the conception rate in Japanese Black cows. This result is similar with the previous studies in which the treatment with P4 device from Day 12–15 to 17–22 has been evaluated in dairy cows [6, 8, 9, 12, 17]. However, the post-AI treatment with P4 device from Day 10–14 to 17–21 increased [1, 31] but

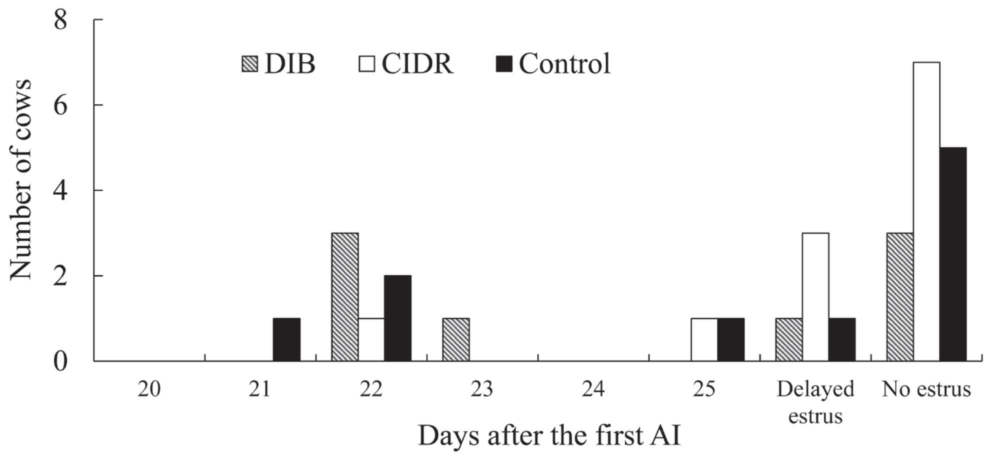

Fig. 1. Distribution of non-pregnant Japanese Black cows returning to estrus between Day 20 and 25. Cows were treated with a DIB or CIDR from Day 12 to 19 after the first AI. Delayed estrus: cows returning to estrus between Day 26 and 40. No estrus: cows returning to estrus on Day 41 or later, or without returning estrus. Day 0=day of the first AI.

Table 3. Plasma P4 concentration in Japanese Black cows on Day 19 (Day 0=day of the first AI)

Reproductive status of cows after the first AI [c]	Plasma P4 concentration (*ng*/m*l*) of cows in each treatment (n)			P [e]
	DIB [d]	CIDR [d]	Control	
Pregnant	14.1 ± 4.2 [a] (11)	13.9 ± 4.8 (17)	13.3 ± 5.5 [a] (19)	>0.05 (NS)
Non-pregnant	7.2 ± 4.9 [b] (7)	10.8 ± 5.4 (5)	5.4 ± 5.6 [b] (8)	>0.05 (NS)
Total	11.4 ± 5.5 (18)	13.2 ± 5.0 (22)	11.0 ± 6.6 (27)	>0.05 (NS)

Mean ± S.D. a–b) Different letters indicate significant differences between pregnant and non-pregnant cows in each treatment ($P<0.05$). c) Pregnancy diagnosis on Days 40, 60 and 80. d) Treated with a DIB or CIDR from Day 12 to 19. e) P-values among each treatment. NS: Not significantly different.

in another study decreased [3] conception rate in dairy cows. Though treatment with P4 from Day 5 to 9 increased embryo development and their interferon-τ production in dairy cows [21], the treatment with P4 device from Day 1–5 to 8–9 decreased conception rate [28, 37]. In dairy cows, plasma P4 concentration was similar on Day 8 and 10 in both pregnant and non-pregnant cows, while plasma P4 concentration from Day 12 to 17 was significantly higher in pregnant than non-pregnant cows [22]. A meta-analysis using a univariate binary random effects model showed no significant change in conception rate by the P4 treatment after Day 7 in cows [40]. The effect of post-AI treatment with P4 device was also influenced by parity (first and second lactation) [16, 37] and BCS (≤3.0, ≥3.25) [7] of dairy cows. Stevenson *et al.* [35] reported that the effect of post-AI treatment with P4 device depended on herd. In this study, we assessed the effect of location, parity, DPP, BCS, CL diameter and season on conception rate in each group. Location had no effect on conception rate in each group. Though parity in CIDR group is only a factor to reduce conception rate, it seems to be because of sample size and distribution of parity in each group. Total number of heifers and second-calf cows was 4 in CIDR group. Conception rate of heifers and second-calf cows was 100% (4/4) in CIDR group, though those in DIB group and in control group were 75.0% (6/8) and 72.7% (16/22), respectively. Further investigation with larger sample size is needed. It is conceived that because statistical variability of BCS was small in each group, BCS had no effect on conception rate in each group. In beef cows, the treatment with intravaginal devices containing various P4 doses from Day 13–14 to 20–21 did not affect conception rate [5, 15, 34]. In Japanese Black cows, the treatment with a device containing 1.9 g P4 from Day 17 to 24 did not affect conception rate [14]. By using intravaginal devices with 1.0 and 1.9 g P4, the present study confirmed that post-AI treatment with P4 device did not improve conception rate in Japanese Black cows as reported in other beef breeds. The post-AI treatment with P4 device might affect fertility in cows with some disorders in hypothalamo-hypophysial-gonadal axis, because the treatment improved reproductive status of repeat-breeder cows [36, 37].

In this study, returning rate to estrus of non-pregnant Japanese Black cows between Day 20 and 25 was not different among the groups, and day of the returning estrus was not synchronized during this period in each group. In dairy cows, the post-AI treatment with P4 devices from Day 12 to 19 neither affected returning rate nor synchronization of estrus in non-pregnant cows [17], while the treatment from Day 10–17 to 16–22 synchronized the returning estrus between Day 23 and 27 [3, 18, 37]. The returning estrus of non-pregnant beef cows was synchronized between Day 21 and 26 by the treatment with devices containing various P4 doses from Day 13–14 to 20–21 [5, 15, 34]. In Japanese Black cows, the returning estrus of non-pregnant cows after the treatment with a device containing 1.9 g P4 from Day 17 to 24 occurred on Day 28.7 ± 3.9 compared to Day 33.0 ± 26.9 in control group without any significant difference [14]. By using intravaginal devices with different P4 doses, the present study demonstrated that it did not affect the rate of returning estrus and synchronizing estrus in the post-AI treatment with P4 device from Day 12 to 19 in Japanese Black cows. For synchronization of the returning estrus, timing of P4 device removal should be further evaluated in Japanese Black cows. Additionally, Long *et al.* [17] reported that almost half of the non-pregnant dairy cows showed no returning estrus between Day 20 and 25 after P4 treatment and had a prolonged luteal phase. The presence of non-pregnant Japanese Black cows returning to estrus between Day 26 and 40 (delayed estrus) in this study might have been due to the occurrence of late embryonic mortality [11] and delayed luteolysis after P4 device removal. Incidence of late embryonic mortality should be further examined in Japanese Black cows.

In conclusion, post-AI treatment with both P4 devices (DIB and CIDR) from Day 12 to 19 neither increased plasma P4 concentration at device removal nor improved the fertility and synchronization of the returning estrus in Japanese Black cows. The doses of P4 (1.0 and 1.9 g) did not affect the reproductive performance in Japanese Black cows.

ACKNOWLEDGMENTS. The authors would like to thank owners and staffs of the farms for their outstanding cooperation. We thank Kyoritsu Seiyaku Co., Tokyo, Japan for providing DIB devices. We also thank Dr. Shambhu Shah for English proofreading of the manuscript and Mr. Hitoshi Oshiro, M.D. for support of statistical analysis.

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