Regulating Estrus and Therapy of Repeat-Breeder and Anestrous Holstein Heifers Using Progesterone Releasing Intravaginal Devices (PRIDs)

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ABSTRACT. Fourty repeat-breeder and anestrous Holstein heifers were treated with one of four methods using progesterone releasing intravaginal devices (PRIDs), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue and synthetic gonadotropin releasing hormone (GnRH analogue). A PRID was inserted into the vagina for 7 or 12 days and a single injection of $PGF_{2\alpha}$ analogue (500 μ g) was administered one day before PRID removal. On the use of 12-day insertion, the estradiol-benzoate (E.B.) capsule containing 10 mg estradiol was excluded from PRID. The 12-day treatment of PRID without the E.B. capsule and a $PGF_{2\alpha}$ analogue showed encouraging results in both estrus incidence (10/10; 100%) and the subsequent fertility (6/10; 60.0%) following fixed-time artificial insemination with frozen semen. Conception rate in heifers treated with two injections of $PGF_{2\alpha}$ analogue 11 days apart was disappointed (3/10; 30.0%). The treatment of GnRH analogue (200 μ g) and $PGF_{2\alpha}$ analogue 9 days apart has showed a high conception rate (6/7; 85.7%) as well as the 12-days PRID treatment (9/10; 90.0%), when re-insemination in heifers returned to estrus within 24 days after treatment was involved. The present study indicates that the use of PRID combined with a $PGF_{2\alpha}$ analogue is more effective for regulating the estrous cycle and the subsequent fertility of repeat-breeder and anestrous heifers than the two-injection regime of $PGF_{2\alpha}$ analogue, and that the E.B. capsule may be unnecessary when a $PGF_{2\alpha}$ analogue was administered one day before PRID removal.—**KEY WORDS:** PRID, $PGF_{2\alpha}$ analogue, repeat-breeder, heifer.

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A vaginal coil (progesterone releasing intravaginal devices: PRIDs) has been widely used for synchronization of estrus and ovulation in cattle and has been found the efficiency in regulating the estrous cycle and in obtaining an acceptable fertility [2, 3, 13, 16, 17]. However, studies on the use of such a progestin in cattle with reproductive disorders have been lacking. Effect of PRID has been affected by the insertion period of PRID and the use of the capsule containing 10 mg estradiol-benzoate (E.B.) attached with PRID [3, 5, 17]. Our previous report [4] showed a possibility of regulating estrus and of improving fertility in repeat-breeder beef cattle when PRID was inserted for 12 days without the E.B. capsule and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was given one day before PRID removal. Roche [14], Smith [16], and Smith *et al.* [17] have stated that the E.B. capsule is necessary

if the period of PRID insertion is shortened (7–10 days) instead of a longer period (12–14 days).

The objectives of the present study were to compare two methods for the use of PRID (7-day insertion with E.B. capsule and 12-day insertion without E.B. capsule) on estrus regulation and the subsequent fertility in repeat-breeder and anestrous Holstein heifers, and to compare the effect of PRID with the two-injection regime of $PGF_{2\alpha}$ analogue and a combined treatment with synthetic gonadotropin releasing hormone (GnRH analogue) plus a $PGF_{2\alpha}$ analogue.

MATERIALS AND METHODS

Animals: The present study was conducted during September to November, 1984 at Tawa Field Station, Shibechema-cho, Hokkai-

Fig. 1. Time-schedule of treatments in the study. Figures in the upper and under of the line show days from commencing treatment and after treatment, respectively.
*indicates the days of blood collection.

do, Japan. Forty Holstein heifers were used for the experiment. The age and body weight of the animals were 24.1 ± 3.1 (mean $\pm$ S.D.) in months and 461 ± 74.0 (mean $\pm$ S.D.) kg, respectively. The animals were grazing on a pasture of 28.1 hectares during the study. Thirty-two of 40 heifers were cyclic, but had not conceived by 2–8 (mean 3.54) artificial insemination for about 5 months before commencing the experiment, and the remaining 8 heifers were in the anestrus due to unknown reasons.

Treatment: Time-schedule of the treatment was shown in Fig. 1. Thirty-two repeat-breeder and 8 anestrus heifers were randomly divided into four groups with 10 animals each. The PRID used in the present experiment was impregnated with 2.25 g progesterone in the silastic rubber (length 9.0 cm and diameter 4.0 cm) and a piece of nylon was attached to one end (Abbott Lab., Australia). For group I, a PRID with the capsule of 10 mg E.B. was inserted into the vagina with an aid of a special rod and was left for 7 days. On the 6th day after PRID

insertion, the animals were received a single intramuscular injection of 2 ml (500 μ g) PGF_{2α} analogue (Estrumate: Sumitomo Chemical Co., Japan). For group II, similarly in group I, a PRID without the E.B. capsule was inserted for 12 days and the PGF_{2α} analogue was administered on the 11th day after PRID insertion. For group III, two injections of the PGF_{2α} analogue were administered intramuscularly 11 days apart. For group IV, a 4 ml (200 μ g) of GnRH analogue (Conceral: Takeda Industrial Co., Japan) and the PGF_{2α} analogue were given 9 days apart.

Blood collection and progesterone measurement: As shown in Fig. 1, blood was collected from the caudal vein by 10 ml-heparinized vacutainers 7 times per animal in all groups. Plasma was separated immediately after collection by centrifugation at 1,600 g for 10 min and stored at -20°C until measurement of progesterone (P) concentrations. Plasma P concentrations were measured by a radioimmunoassay using P kits "Daiichi" (Daiichi Radioisotope Lab. Ltd., Japan). The mean recovery rate was 94.1% ($n=16$). The

Table 1. Estrus incidence of treated heifers

Group	I	II	III	IV
No. heifers	10	10	10	10
No. estrous heifers within 4 days after treatment (%) ^{a)}	8(80.0)	10(100)	8(80.0)	6(60.0)
No. heifers received >2 mountings by other heifers ^{b)} (%)	7(70.0)	8(80.0)	5(50.0)	4(40.0)
	15(75.0) ^{c)}		9(45.0) ^{c)}	

a) Not significant.

b) Not significant.

c) $0.05 < p < 0.10$.

intra-assay coefficient of variations within the study was 7.85% ($n=16$).

Artificial insemination (A.I.): After treatment, estrus was observed three times (0600, 1300 and 1700 hrs) with 1 hr period each, daily for 4 days. The interval between the cessation of treatment and the first sign ('standing heat') of estrus, and the time and rate of synchronizing estrus were investigated. A.I. was performed at 48, 72 and 96 hrs after treatment with frozen-thawed semen in a 0.5 ml straw made from a fertile bull in all heifers regardless of estrus incidence. Fertility expressed by plasma P concentrations (day 20–22), non-return (day 24) and pregnancy by rectal palpation (day 60–90) after the final A.I. (day 0).

Statistics: Data on estrus incidence and plasma P concentrations were subjected to analysis of variance, and differences were analysed by Student's t-test [19]. Data on fertility were compared by chi-square analysis.

RESULTS

Estrus incidence: Estrus incidence of treated heifers in each group was shown in Table 1. Estrus behavior was observed in 32 of 40 treated heifers. Twenty-four of the 32 estrous heifers showed more than two times

Fig. 2. Patterns of estrus incidence in treated heifers of each group. Slanting bars indicate heifers not received more than two mountings during the estrus observation.

of 'standing heat' during the observation period. There were no significant difference among the groups for the proportions of estrous heifers within 4 days after treatment (Table 1). The proportion of heifers stood to more than two mountings by other heifers during the observation period was higher in heifers treated with PRID (15/20; 75.0%, group I and II) than in non PRID-treated heifers (9/20; 45.0%, groups III and IV), although it was failed to reveal a significant difference ($0.05 < P < 0.10$). Pattern of estrus incidence in treated heifers of each group were shown in Fig. 2. All heifers in group II showed estrus and their estrus was precisely synchronized at the 2nd day after treatment.

Table 2. Plasma progesterone concentrations (Mean $\pm$ S.D.: ng/ml) in treated heifers

	-7	-5	-1	0 ^{a)}	+3 ^{b)}	+12 ^{c)}	+24
Group I (n=10)	5.13 $\pm$ 4.23	7.83 $\pm$ 3.58	6.42 $\pm$ 2.58	2.48* $\pm$ 1.08	0.27* $\pm$ 0.17	5.41 $\pm$ 1.10	5.27 $\pm$ 3.68
Group II (n=10)	-12	-10	-1	0 ^{a)}	+3 ^{b)}	+12 ^{c)}	+24
	6.24 $\pm$ 3.62	7.34 $\pm$ 2.14	5.43 $\pm$ 4.18	1.59** $\pm$ 0.31	0.48 $\pm$ 0.16	4.55 $\pm$ 0.93	3.82 $\pm$ 2.69
Group III (n=10)	F-PG ^{d)}	F+2 ^{e)}	F+10	S-PG ^{f)}	+3 ^{b)}	+12 ^{c)}	+24
	5.56 $\pm$ 4.91	2.33 $\pm$ 2.63	5.55 $\pm$ 2.50	5.47 $\pm$ 3.34	0.84** $\pm$ 0.32	5.67 $\pm$ 3.10	5.77 $\pm$ 4.88
Group IV (n=10)	GnRH	+2 ^{e)}	+10	PG	+3 ^{b)}	+12 ^{c)}	+24
	4.60 $\pm$ 3.97	3.70 $\pm$ 2.12	7.13 $\pm$ 4.69	6.13 $\pm$ 4.72	0.79** $\pm$ 0.85	4.50 $\pm$ 1.83	3.91 $\pm$ 3.04

a) The day of PRID removal.

b) The day of the second A.I.

c) Twelve days after treatment.

d) At the first injection of PGF_{2 α} analogue.

e) Two days after the injection.

f) At the second injection of PGF_{2 α} analogue.*vs.**: Figures in the same column are significantly different ($p<0.05$).

Table 3. Results of fertility in treated heifers

Group	I	II	III	IV
No. heifers	10	10	10	10
Conception to fixed-time A.I.:				
Plasma P ^{a)}	7	9	6	6
N.R. ^{b)}	7	8	6	6
R.P. ^{c)}	4 (40.0)	6 (60.0)	3 (30.0)	5 (50.0)
Conception to A.I. within 24 days	4/8 ^{d)} (50.0)	9/10 ^{d)} (90.0)*	4/9 ^{d)} (44.4)*	6/7 ^{d)} (85.7)

a) Heifers with >1 ng/ml of plasma progesterone concentration at 20–22 days after A.I. were considered as conceived.

b) Non-return to estrus until 24 days after A.I.

c) Pregnancy examined 60–90 days after A.I. by rectal palpation.

d) No. of heifers available for rectal palpation 60–90 days after A.I.

* $P<0.05$.

The times between the cessation of treatment and the onset of estrus ('standing heat') were 51.0 ± 10.2 ($n=7$), 44.6 ± 2.8 ($n=8$), $57.1\pm$

13.0 ($n=5$), and 91.7 ± 17.6 ($n=4$) hrs for groups I to IV, respectively. The time of group IV was significantly ($p<0.05$) prolonged as compared with other groups.

Plasma progesterone concentration: Mean concentrations of plasma P throughout the study were shown in Table 2. The plasma P concentration at PRID removal in group II (1.59 ± 0.31 ng/ml) was significantly ($P<0.05$) lower than that of group I (2.48 ± 1.08 ng/ml). In all heifers except two and one heifers in groups III and IV, respectively, the plasma concentrations were less than 1 ng/ml at the time of the second A.I. The mean concentration of plasma P at the second A.I. in group I (0.27 ± 0.17 ng/ml) was significantly ($P<0.05$) lower than those of groups III (0.84 ± 0.32 ng/ml) and IV (0.79 ± 0.85 ng/ml).

Conception rate: Results of conception rates were shown in Table 3. Conception rates to fixed-time A.I. were not significantly different among the groups expressed by the

three methods. In each group, conception rates were decreased by 10–30% from 20–22 days to 60–90 days after the fixed-time A.I. In group II, both conception rates by the fixed-time A.I. (6/10; 60.0%) and including the re-insemination in heifers returned to estrus within 24 days after treatment (9/10; 90.0%) were higher than in other groups (Table 3). The overall conception rate in group II (90.0%) was significantly ($P < 0.05$) higher than that of group III (4/9; 44.4%). The combined treatment with GnRH and PGF_{2α} analogues (Group IV) also showed a comparable conception rate (6/7; 85.7%) with group II.

DISCUSSION

Among the treatment employed in the present study, a longer insertion (12 days) of PRID without the E.B. capsule and the injection of PGF_{2α} analogue one day before PRID removal showed an encouraging results in both regulating estrus and the subsequent fertility in heifers with the reproductive disorders. The short insertion (7 days) of PRID with the E.B. capsule was less effective than the longer insertion of PRID. This is in agreement with our previous report [4]. Furthermore, the use of PRID without the E.B. capsule and a PGF_{2α} analogue one day before PRID removal have provided an acceptable synchronization and conception rates to A.I. [1, 3]. Although estrogen from the E.B. capsule has been shown to regress the early *corpus luteum* at the initial stage of P treatment [10], the detailed function is not fully understood. It appears that estrogen from the E.B. capsule has attributed with luteolytic effects as well as stimulating the rate of absorption of P from PRID [16]. Table 2 showed that the plasma P concentration at PRID removal was significantly lowered in group II than that of group I, although both groups were administered the PGF_{2α} analogue one day before PRID removal. From the

fact, a short period of PRID insertion appears to be less sufficient to suppress the plasma P concentration at PRID removal than the longer period of PRID insertion. However, as compared with the non PRID-treated heifers (groups III and IV), the plasma P concentrations at the second A.I. in PRID-treated heifers (groups I and II) were greatly suppressed. Smith [16], Smith *et al.* [17], and Sprott *et al.* [18] emphasized the importance of E.B. capsule or additional estrogen treatment at PRID insertion for a short period (7–10 days), but an injection of PGF_{2α} or its analogue was not accompanied before or at the PRID removal in their studies. It appears from the present results and other reports [1, 3, 4] that the E.B. capsule may be unnecessary if PGF_{2α} or its analogue was administered one day before PRID removal. Folman *et al.* [3] showed that the pattern of estrus synchronization was more precise and conception rates were higher when dairy cows were treated with PRID omitting E.B. capsule and a PGF_{2α} analogue as compared with only PRID adding E.B. capsule. However, effect of the E.B. capsule should be further investigated in relation to insertion periods of PRID and the stage of the estrous cycle in animals treated with PRID [3, 5].

Although the two-injection of PGF_{2α} analogue have been successfully used for synchronizing estrus in dry cows and maiden heifers [9, 16], the present results by the two-injection regime of PGF_{2α} analogue 11 days apart have been disappointed. Reasons for the lowered conception rate observed after the two injections of PGF_{2α} analogue can not be precisely determined. However, the poor synchronization of estrus after the second injection likely reflected to the low conception, because A.I.s were performed on the time-basis. The influence of the stage of the estrous cycle in PGF_{2α} or its analogue to estrus incidence [7, 8, 12] and the conception rate [1] have been reported. In the present study, the stage of the estrous cycle at the

second injection of PGF_{2α} analogue was day 8–10 in 7 heifers responded by the first injection. Meanwhile, in heifers of group IV, the stage of the estrous cycle at the injection of PGF_{2α} analogue was day 6–7 if it was considered that the heifers had ovulated 2–3 days after the injection of GnRH analogue. Momont and Seguin [11] showed a high conception rate (73%) in heifers treated with a PGF_{2α} analogue on day 7 of the estrous cycle and only 21% in heifers treated on day 10 conceived after a single fixed-time A.I. With these respects, a combined treatment with GnRH and PGF_{2α} analogues 9 days apart has shown a promising results on fertility. It has been reported that over 80% of heifers ovulated after GnRH injection, although the luteal function has depressed earlier ('short cycle': 6–12 days) than the normal cycle [21]. In the present study, whether heifers treated with the GnRH analogue had ovulated was not determined by the presence of newly-formed *corpus luteum*. However, the plasma P concentrations were maintained at high level (6–7 ng/ml) until the injection time of PGF_{2α} analogue. Therefore, it was considered that all heifers in group IV presumably were at the luteal phase (day 6–7) when the PGF_{2α} analogue was administered, which would cause luteal regression followed by estrus incidence. In the present study, the estrus incidence in group IV significantly prolonged as compared with other treated groups. The interval (9 days) between GnRH and PGF_{2α} analogue injections may be one of reasons for the prolonged estrus incidence. A relationship between the interval of GnRH and PGF_{2α} analogue injections and the early regression of *corpus luteum* induced by the GnRH analogue would have likely influenced on the estrus synchronization and the subsequent fertility following the fixed-time A.I. As conception rates to A.I. within 24 days after present treatment were comparable to the PRID treatment (90.0% and 85.7% for groups II and IV, respectively), a combined

treatment with GnRH and PGF_{2α} analogues could be an alternative method for regulating the estrous cycle [15] and decreasing the incidence of postpartum anestrous cows [2].

A high embryonic loss has occurred at the early pregnancy probably due to P deficiency or subnormal uterine circumstances. However, fertility in the present study was acceptable, if it has considered that heifers used were repeat-breeders and in anestrous status and that A.I. was performed on a fixed-time basis. The conception rate (60%) of heifers with PRID omitting the E.B. capsule and a PGF_{2α} analogue one day before PRID removal was higher than the figures (30–33%) of repeat-breeders reported by Hawk [6] and Tanabe [20]. Furthermore, to simplify a labor and physical requirement involved in herd management, the precise synchronization of estrus and ovulation as shown in group II could offer a single A.I. on a time basis and eliminate the need for estrus detection. It has been also expected that the use of normal cycling heifers could improve conception rate following A.I. either at fixed-time basis or at observed estrus.

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要 約

黄体ホルモン放出陰内挿入器具 (PRID) によるリピートブリーダーおよび無発情牛の発情調整と治療: 福井 豊・小林正之・椿 実・菊地宣幸・小野 斉 (帯広畜産大学肉畜増殖学教室) ——ホルスタイン種 未経産牛 40 頭 (リピートブリーダー: 32頭, 無発情: 8頭) を4群 (各群10頭) にわけ, PRID, プロスタグランジン $F_{2\alpha}$ 類縁物質 ($PGF_{2\alpha}$ アナログ: エストラメイト, 500 μg), または性腺刺激ホルモン放出ホルモン類縁物質 (GnRH アナログ: コンセラル, 200 μg) を用いて発情調整および受胎性を検討した。PRID は7日間または12日間挿入し, 除去1日前に $PGF_{2\alpha}$ アナログを1回筋肉内注射した。PRID 12日間挿入群では, 10 mg エストラジオール・ベンゾエート (E. B.) が含まれるカプセルを除去した。PRID (-E. B.) 12日間挿入+ $PGF_{2\alpha}$ アナログで処置された10頭とも処置後2日目に発情を示し, 凍結精液による3回定時人工授精により6頭が受胎した。 $PGF_{2\alpha}$ アナログ2回注射 (11日間隔) 群では10頭中3頭のみが受胎した。また, GnRH アナログ+ $PGF_{2\alpha}$ アナログ注射 (9日間隔) 群では10頭中5頭が受胎した。処置後24日までの受胎は, PRID (-B. D.) 12日間+ $PGF_{2\alpha}$ アナログ群では10頭中9頭, GnRH アナログ+ $PGF_{2\alpha}$ アナログ群では7頭中6頭であった。以上の結果から, PRID (-E. B.) を12日間挿入し, 除去1日前に $PGF_{2\alpha}$ アナログを注射する方法は, $PGF_{2\alpha}$ アナログ2回注射より効果的であり, また, E. B. カプセルは不要と考えられた。