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—Research Note—

Estrus Synchronization and Conception Rate after a Progesterone Releasing Intravaginal Device (PRID) Treatment from the Early Luteal Phase in Heifers

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Abstract. The objective of the present study was to evaluate estrus synchronization and conception rate after progesterone releasing intravaginal device (PRID) treatment from the early luteal phase in the presence or absence of estradiol benzoate (EB) in heifers. Heifers (n=11) were assigned randomly to two treatments; insertion of a PRID containing 1.55 g progesterone with a capsule attached including 10 mg EB (P+EB; n=6) and the PRID withdrawn the EB capsule (P-EB; n=5). The PRID was inserted into the vagina on Day 2 of the estrous cycle (Day 0 was the day of ovulation) and was left for 12 days. The proportion of heifers exhibiting standing estrus within 3 days after PRID removal was 83.3% (5/6) for the P+EB group, and 80.0% (4/5) for the P-EB group, respectively. Conception rate by artificial insemination on synchronized estrus was 80.0% (4/5) in the P+EB group, and 100% (4/4) in the P-EB treatment group, respectively. These results suggest that a PRID treatment from 2 days after ovulation for 12 days in the presence or absence of EB has an effect on the synchronization of estrus and produces a beneficial conception rate in heifers.

Key words: Early luteal phase, Estradiol benzoate, Estrus, Heifers, PRID

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Control of the estrous cycle in cow herds is an important technique in farm management to improve reproductive performance. There are two methods for synchronization of estrus; one is to utilize gestagen to mimic the endocrine environment of the luteal phase, and the other is to utilize prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to induce regression of the corpus luteum. In a recent study, Pursley *et al*. [1] has established an ovulation synchronization protocol (Ovsynch) by controlling the follicular wave in lactating dairy cows.

A progesterone intravaginal device treatment has been in use for many years as a method of controlling the estrous cycle in cows. There is a general agreement that almost all cows treated with the progesterone device intravaginally for 12 days exhibit estrus within several days after device removal. However, some studies have reported that the timing of hormonal treatment influences the effectiveness of the device. For example, progesterone device treatment decreased estrous exhibition when the treatment was started from the early luteal phase [2]. Several researches have revealed that an immediate reduction of progesterone levels in circulation followed by device removal is a main determinant of induction of estrus within 3 days after device removal.

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Sreenan *et al.* [2] and Macmillan *et al.* [3] mentioned that the day of device removal fell on the functional stage of the corpus luteum (CL) during the normal estrous cycle if the device was implanted for 10 or 12 days from the early luteal phase. In this case, it is possible that progesterone secretion from the functional CL inhibits the occurrence of estrus. Moreover, it is reported that supplemental administration of estradiol benzoate (EB) at the start of progesterone treatment improved the induction rate of estrus and conception rate in cows [4]. In view of this finding, it is necessary to clarify the advantageous role of EB treatment in cows treated with a progesterone intravaginal device from the early luteal phase.

The aim of the present study was to evaluate the beneficial effects of progesterone treatment from the early luteal phase in heifers. Our protocol was to insert a progesterone releasing intravaginal device (PRID) for 12 days, starting 2 days after ovulation. The effects of the PRID were also evaluated for the induction rate of estrus and conception rate in the presence or absence of EB.

Materials and Methods

Eleven cyclic Holstein heifers maintained at a farm in Saitama Prefecture, Japan, were used. The experiment was conducted during the period from December to March. Their heifers were from 14 to 18 months of age. Their body weights ranged from 324 to 411 kg and the status of the body condition was good at the time of treatment. All animals were maintained in a paddock. They were fed 1.5 to 2.0 kg of concentrates, and they had free access to a low-moisture silage of orchardgrass or Italian ryegerass and fresh water.

Animals were divided into two groups, the P+EB (n=6) and P-EB (n=5) groups. The P+EB group received a progesterone releasing intravaginal device with a capsule attached containing 10 mg of estradiol benzoate (PRID®; CEVA SANTE ANIMALE SA, France). The P-EB group received a PRID® withdrawn the EB capsule. The PRID was inserted into the vagina on Day 2 (Day 0=the day of ovulation as determined by rectal palpation) and was left in the animals for 12 days. After removal of the PRID, standing estrus was monitored twice daily by visual observation. Artificial insemination (AI) was carried out within 12 h after detection of standing estrus (i.e., AM/PM rule). In this study, the estrous cycle was considered to be synchronized when the length of the estrous cycle was shortened and standing estrus was observed within 3 days after PRID removal. Pregnancy diagnosis was conducted using per rectum palpation of the uterus between 50 and 60 days after AI.

Blood samples were collected via jugular venipuncture to determine the ovarian steroid profiles on Days 0, 2, 3, 4, 6, 8, 10, 12 and 14, and daily from Day 15 to the following day of ovulation. Samples were collected in heparinized syringes and placed on ice. Within 30 min of collection, the samples were centrifuged at 3000 rpm for 15 min at 4 C, and the plasma was stored at −20 C until assay for the concentrations of progesterone and estradiol–17β by a procedure previously described [5].

The sensitivities of the assays were 0.02 ng/ml for progesterone and 0.38 pg/ml for estradiol-17β. The intra- and inter-assay coefficients of variation were 10.1% and 28.0% for progesterone and 23.8% and 21.1% for estradiol-17β, respectively.

During PRID treatment, the significance of differences of the mean progesterone and estradiol-17β concentrations for each group was tested using the Student's t-test. Conception rate was analyzed by Fisher's exact probability test. $P<0.05$ was considered to be statistically significant.

Results

In all heifers, the PRID remained in the vagina until removal, and no vaginitis was found clinically throughout the experiment.

Occurrence of estrus

The occurrence of estrus after PRID removal is summarized in Table 1. The proportion of heifers exhibiting standing estrus within 3 days after PRID removal in the P+EB and P-EB groups were 83.3% and 80.0%, respectively. The inter-ovulatory interval in these heifers were 17 or 18 days. The remaining two heifers, No. 35 in the P+EB group and No. 26 in the P-EB group, exhibited estrus on days 5 and 7 after PRID removal, respectively. The inter-ovulatory interval in these heifers were 20 and 22 days, respectively.

Table 1. The occurence of estrus after PRID removal

Group	Day after PRID removal						
	1	2	3	4	5	6	7
P+EB (n=6)	0[1)]	2	3	0	1	0	0
P-EB (n=5)	0	3	1	0	0	0	1
total	0	5	4	0	1	0	1

[1)]The number of heifers.

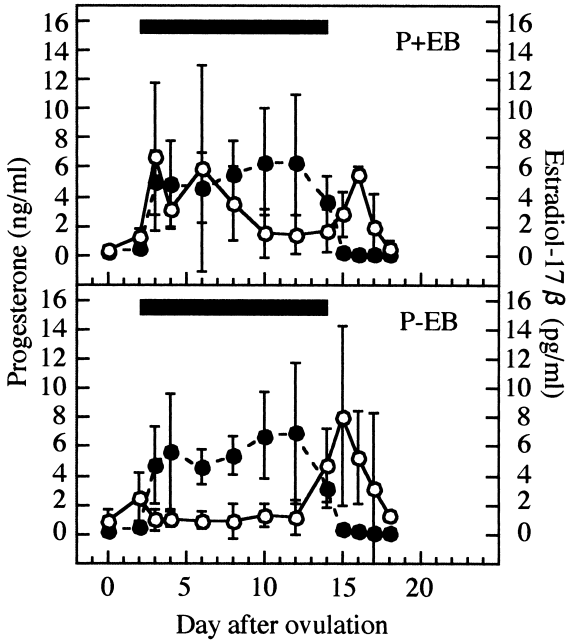

Fig. 1. Daily mean plasma progesterone (filled circles) and estradiol-17β (open circles) concentrations in heifers exhibiting estrus within 3 days after PRID removal for P+EB (upper panel, n=5) and P-EB (bottom panel, n=4), respectively. Error bars represent SD.
: Period of PRID insertion.

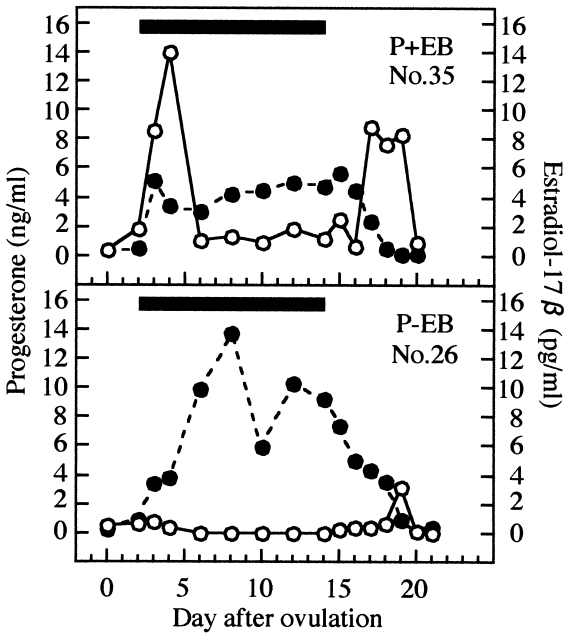

Fig. 2. Plasma progesterone (filled circles) and estradiol-17β (open circles) concentrations in a heifer (No. 35) from the P+EB (upper panel) group and in a heifer (No. 26) from the P-EB (bottom panel) group. These heifers (Nos. 35 and 26) exhibited estrus on days 5 and 7 days after PRID removal, respectively.
: Period of PRID insertion.

Plasma progesterone and estradiol-17β

The profiles of plasma progesterone and estradiol-17β in the heifers exhibiting estrus within 3 days after PRID removal are shown in Fig. 1. Plasma progesterone concentrations increased markedly after PRID insertion and were maintained at a level between 2 and 15 ng/ml until PRID removal in both groups. There were no significant differences in the plasma concentrations of progesterone throughout the experiment between the P+EB and P-EB groups. The concentrations of estradiol-17β on the day following PRID insertion in the P+EB group were significantly higher than in the P-EB group (7.1 ± 4.5 pg/ml vs 1.0 ± 0.6 pg/ml, $P<0.05$). In these heifers, concentrations of progesterone decreased immediately after PRID removal and were less than 1.0 ng/ml on the day following PRID removal. In the remaining two heifers (Nos. 35 and 26), which did not exhibit estrus within 3 days after PRID removal, progesterone concentrations were maintained at a level greater than 2 ng/ml until 3 to 4 days after PRID removal (Fig.2). The concentrations of progesterone in No. 35 and No. 26 declined to a level less than 1.0 ng/ml on Day 18 and Day 19, respectively. All heifers showed peak

estradiol-17β concentrations on the day of standing estrus.

Conception rate

Conception rate for the first AI during synchronized estrus after PRID removal was 80.0% in the P+EB group heifers (4/5) and 100% in the P-EB group heifers (4/4) exhibiting estrus within 3 days. There was no significant difference in conception rate between the P+EB and P-EB groups. The other two heifers (Nos. 35 and 26) were also pregnant by the first AI after PRID removal.

Discussion

In the present study, the PRID was inserted on Day 2 and then removed on Day 14. Day 14 is the functional stage of CL during the normal estrous cycle in cattle, and the CL has the ability to vigorously secrete progesterone at this stage. However, plasma progesterone concentrations decreased immediately to a level less than 1.0 ng/ml after PRID removal in almost all heifers in both groups (P+EB, 83.3%; P-EB, 80.0%), and then a preovulatory increase in plasma estradiol-17β concentrations occurred. Estrus was detected within 3 days after PRID removal in the heifers, and the conception rate was 80.0% and 100%, respectively. In the clinical field, the convergence of estrus within 3–4 days after treatment is required for the success of estrus synchronization. From a clinical point of view, therefore, the present results suggest that a PRID treatment from Day 2 of the estrous cycle for 12 days has beneficial effects for synchronization of estrus. In contrast to the present findings, Sreenan *et al*. [2] have reported that the rate of estrus synchronization decreased in cows treated with intravaginal progesterone impregnated pessaries (sponges) from the early luteal phase. The reason for the disagreement between their findings and our present results is unclear, although it may be related to differences in the progesterone device (PRID vs pessary).

Several studies have reported that exogenous progesterone treatment in the early luteal phase inhibited CL development in cows [6, 7]. A supraphysiological increase of progesterone levels in the early luteal phase caused the release of luteolytic $PGF_{2\alpha}$ in the early stages of the luteal phase in cows [8]. A reduction of plasma progesterone concentrations on the day following PRID removal was observed in the present study, suggesting that the CL had already lost the ability to secrete progesterone on the day of PRID removal. Consequently, the present findings lead us to speculate that PRID treatment from the early luteal phase induced early regression of the CL. On the other hand, it is possible that the CL was not influenced by the PRID treatment in the two heifers (Nos. 35 and 26) that did not exhibit estrus within 3 days after PRID removal.

In the present study, it was not clear if an EB treatment combined with a PRID from the early luteal phase had a beneficial effect. Macmillan *et al*. [3] reported that a controlled intravaginal drug releasing device (CIDR) treatment for 12 days from the early luteal phase in the presence or absence of estrogen administration at CIDR insertion reduced the length of the estrous cycle. In contrast, Sreenan *et al*. [2] reported that progesterone alone did not affect the length of the estrous cycle or function of the corpus luteum, whereas CL development was inhibited when estradiol was given on the day of progesterone treatment. In this regard, further studies are needed to determine the characteristics of ovarian structures as well as the endocrine profiles after PRID treatment in the presence or absence of estrogen treatment.

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