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The Therapeutic Effects of a Progesterone-Releasing Intravaginal Device (PRID) with Attached Estradiol Capsule on Ovarian Quiescence and Cystic Ovarian Disease in Postpartum Dairy Cows

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Abstract. The objective of this study was to evaluate the effects of a progesterone-releasing intravaginal device (PRID) containing an estradiol benzoate capsule on ovarian dysfunction, including ovarian quiescence, follicular cyst (FC) and luteal cyst or cystic corpus luteum (LC/CCL), in postpartum dairy cows. These ovarian dysfunctions were examined by palpation per rectum relative to plasma progesterone status. The results of clinical examination and hormone assay determined ovarian quiescence in 13 cows, FC in 15 cows and LC/CCL in 7 cows. These cows were treated with PRID for 12 d and then clinical examination was performed. After PRID removal, the proportion of cows exhibiting estrous signs within 7 d and confirmed formation of CL within 7–14 d (markedly effective) were 69.2 % (n=9) for ovarian quiescence, 46.7 % (n=7) for FC, and 28.6 % (2 cows) for LC/CCL. Two cows (15.4 %) in ovarian quiescence, 5 cows (33.3%) with FC and 4 cows (57.1 %) with LC/CCL did not exhibit estrous signs but were recognized as having formed CL within 12–16 d after removal of PRID (effective). These results suggest that treatments of PRID with estradiol benzoate for 12 d have therapeutic efficacy on ovarian dysfunction including ovarian quiescence, FC and LC/CCL in postpartum dairy cows.

Key words: PRID, Ovarian quiescence, Follicular cyst, Luteal cyst or cystic corpus luteum, Dairy cow (J. Reprod. Dev. 50: 341–348, 2004)

Ovarian quiescence and ovarian cyst occur frequently during the postpartum period in dairy cows, and are serious types of ovarian dysfunction causing infertility and delay of rebreeding, resulting in economic loss to farmers [1, 2]. Ovarian quiescence is confirmed by the absence of functional ovulatory follicles and luteal tissues, and blood progesterone concentrations below 1 ng/ml in the postpartum period. This condition is a type of anestrus that occurs during the

postpartum period and is affected by various factors such as the season, nutrition, suckling conditions and breed [2, 3]. Ovarian cysts are characterized by the persistent existence of enlarged anovulatory follicles in postpartum dairy cows. The clinical diagnosis of ovarian cyst is based on the reproductive case history, palpation via rectum of a smooth, fluid-filled structure greater than 25 mm in diameter persisting for 10 days or more, and absence of CL on the surface of the ovary [4–6].

Some previous studies have suggested that

progesterone-releasing devices have a therapeutic effect on ovarian quiescence and ovarian cysts [7–10]. In those studies, the progesterone-releasing device was used in combination with GnRH, hCG or PGF_{2α} treatment. In addition, a single insertion of a PRID (progesterone-releasing intravaginal device) with or without an attached estradiol capsule has been used for the therapeutic treatment of ovarian quiescence and follicular cyst (FC) [11, 12]. It has been known that estradiol given with PRID has both a luteolytic role as well as a key role in synchronizing follicular waves [13, 14].

Until recently, some reports have shown that it is difficult to discriminate between ovarian cyst and cystic corpus luteum (CCL) by rectal palpation [13, 15, 16]. The accuracy of the diagnosis of FC and luteal cyst (LC) by palpation via rectum has been reported to be 58 to 68% [9, 17]. LC and CCL have a thicker cystic wall and are usually associated with higher peripheral concentrations of progesterone compared with FC [16, 18]. Therefore, PRID might be used in cows with LC or CCL in the clinical field, when the primary diagnosis of ovarian function was based on by rectal palpation.

In most cases of ovarian quiescence and FC, PRID insertion induces estrus and ovulation within 7 days after treatment. However, in the case of LC or CCL, the efficacy of PRID insertion is not clear. Currently, PRID has not yet been approved for use in cows in Japan. The present study was designed to evaluate the therapeutic effects and progesterone profiles after a single treatment with a PRID containing an estradiol benzoate capsule on ovarian quiescence and cystic ovarian diseases, including FC, LC or CCL in postpartum dairy cows.

Materials and Methods

General

This study was carried out during the period of October 1997 to May 1999 at 30 commercial dairy farms in Chiba Prefecture in Japan. Postpartum dairy cows (Holstein), diagnosed tentatively as ovarian quiescence or cystic ovarian disease were used. They were from 2 to 8 years of age (mean $\pm$ SD: 4.2 ± 1.5 years) and 60 to 324 d postpartum (141.3 ± 69.8 d). Their body weights ranged from 475 to 700 kg (587 ± 54.8 kg) and the status of their body condition was fair or good at the time of treatment. All animals were maintained indoors using a tie stall system and were feed on concentrates with hay, silage and water ad libitum.

Diagnoses of ovarian quiescence and cystic ovarian disease

Cows were examined by experienced veterinary clinicians. The tentative diagnosis of ovarian dysfunction was based on the results of rectal palpation conducted at least twice for the determination of ovarian quiescence and three times for ovarian cyst at 5–7 days intervals. Ovarian quiescence (n=16) was diagnosed when there was neither functional or cyclic corpus luteum (CL), nor cystic follicular structure in ovaries. Cystic ovary (n=22) was initially diagnosed in cows which had a cystic structure greater than 25 mm in diameter and absence of a functional CL in ovaries examined by palpation via rectum, as described in a previous study [4, 6]. Final diagnoses were determined based on these observations together with the analyses of progesterone concentrations in blood samples. In 3 of 16 cows (18.2%) diagnosed as ovarian quiescence by rectal palpation, the progesterone concentration was relatively high (>1.0 ng/ml) in at least one blood sample collected before PRID treatment.

Table 1. Diagnosis of types of ovarian dysfunction, ovarian quiescence, follicular cyst (FC), and luteal cyst or cystic corpus luteum (LC/CCL), by means of palpation via the rectum compared to serum progesterone classification

Diagnosis by palpation	Progesterone concentration	Number of cows	Final diagnosis
Ovarian quiescence (n=16)	<1 ng/ml	13 (81.2)*	Ovarian quiescence
	≥ 1 ng/ml	3 (18.8)	Ovarian cyclic activity
Cystic ovary (n=22)	<1 ng/ml	15 (68.2)	FC
	≥ 1 ng/ml	7 (31.8)	LC/CCL

*Figures in brackets indicate per cent (%).

These cows were excluded from the experiments because the ovarian status was assumed to have cyclic activity (Table 1). Cows diagnosed with cystic ovary were finally classified into 2 groups (Table 1). In 15 of 22 cows, the plasma concentration of progesterone was lower than 1 ng/ml in all samples collected before PRID treatment, and these cows were diagnosed as FC. In the other cows, a progesterone concentration exceeding 1 ng/ml was found in at least one sample collected before PRID insertion. We considered that they had functional luteal tissue on the cystic structure of the ovary, and these cows were diagnosed as LC or CCL. However, it is difficult to distinguish CCL from LC clinically by palpation per rectum, when ovulation bulges do not exist on the surface of CCL. Thus, in the present study, they were grouped as LC or CCL (LC/CCL).

Treatment with PRID

Animals were treated with a progesterone-releasing intravaginal device with an attached capsule containing 10 mg of estradiol benzoate (PRID; CEVA SANTE ANIMALE S.A., France). The PRID was inserted into the vagina after examination of the ovary by rectal palpation (Day 0) and was left in the animals for 12 d. After removal of the PRID, signs of estrus were monitored twice daily by the herd owner/manager of each farm for 7 days. The formation of CL in each cow was carefully observed for 16 days by palpation via the rectum to evaluate the therapeutic effect of PRID.

Evaluation of efficacy

The therapeutic effects of PRID treatment were classified according to the estrous signs and ovarian responses into markedly effective (ME), effective (E) and not effective (NE) as follows. ME: estrous signs appeared within 7 d after removal of PRID, and the formation of CL (detected by rectal palpation) and an increase in the plasma concentration of progesterone (≥ 1 ng/ml) were recognized between 7 and 14 d after PRID removal. E: estrous signs were not exhibited within 7 d after PRID removal, but the development of CL was confirmed by rectal examination and an increase in plasma concentration of progesterone (≥ 1 ng/ml) between 12 and 16 d after PRID removal. NE: there was neither clear estrous sign nor development of

CL in ovaries during the evaluation period.

Blood sampling and hormone assay

Blood samples were collected via jugular venipuncture to determine the ovarian steroid profiles prior to PRID insertion (Day -14 and Day -7 for cystic ovarian disease; Day -7 for ovarian quiescence), during PRID treatment (Day 0, Day 2–3, Day 12) and after the removal of the PRID (Day 14–15, Day 25–26, Day 31–32 and Day 46–47). Samples were collected in heparinized tubes and placed on ice. Within 1 h of collection, the samples were centrifuged and the plasma was stored at -20°C until assayed for the concentrations of progesterone and estradiol- 17β by a procedure previously described [19].

The sensitivities of the assays were 0.12 pg/ml for estradiol- 17β and 14.5 pg/ml for progesterone. The intra- and inter-assay coefficients of variation were 3.6 % and 20.7 % for estradiol- 17β and 8.7 % and 15 % for progesterone, respectively.

Statistical analyses

Data were analyzed by analysis of variance (ANOVA) using the StatView computer program (StatView 4.5, Abacus Concepts Inc., Berkeley, CAL, 1995) to determine the significance of differences among therapeutic effects. During PRID treatment, the significance of differences of the mean progesterone concentrations for each diagnostic group was tested using a paired *t*-test.

Results

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

Ovarian quiescence

The therapeutic effects of PRID treatment on ovarian quiescence are summarized in Table 2. The number of cows in the ME, E and NE groups was 9, 2 and 2, respectively. The plasma progesterone profiles of these groups are shown in Fig. 1. All of the groups with ovarian quiescence showed similar progesterone profiles until the day of PRID removal (Fig. 1, left 3 panels). The plasma progesterone profile after PRID insertion was characterized by an initial increase followed by a gradual decrease and by an immediate decline to

Table 2. Therapeutic effect of PRID on ovarian dysfunction

Diagnosis	Efficacy of PRID		
	ME	E	NE
Ovarian quiescence (n=13)	9 ¹⁾ (69.2) ²⁾	2 (15.4)	2 (15.4)
FC ³⁾ (n=15)	7 (46.7)	5 (33.3)	3 (20.0)
LC/CCL ⁴⁾ (n=7)	2 (28.6)	4 (57.1)	1 (14.3)

ME: markedly effective, E: effective, NE: not effective.

¹⁾Number of cows. ²⁾Figures in brackets indicate per cent (%). ³⁾Follicular cyst. ⁴⁾Luteal cyst or cystic corpus luteum.

Fig. 1. Representative patterns of progesterone concentration in cows from the ovarian quiescence (left panels) group, follicular cyst (FC, middle panels) group, and luteal cyst or cystic corpus luteum (LC/CCL, right panels) group classified according to the three therapeutic responses, markedly effective (ME, upper), effective (E, middle) and not effective (NE, bottom). The dotted bars indicate the duration (Days 0–12) of PRID treatment.

the baseline after the withdrawal of the PRID. The concentrations of progesterone on Day 2–3 and on Day 12 were significantly increased during the period of PRID insertion ($p < 0.01$) compared to those on Day 0 before PRID treatment (Table 3). After removal of the PRID, the progesterone levels in the ME and E groups varied with time similarly to those in the normal estrous cycle (Fig. 1, #IQ4

and #GQ3), but not in the NE group (Fig. 1, #GQ1).

The concentrations of estradiol-17 β were lower than 5 pg/ml before PRID treatment in all animals, and showed similar changes to the progesterone concentrations during the PRID insertion (i.e., an initial increase (mean $\pm$ SEM: 9.3 ± 2.1 pg/ml) followed by a decline). After PRID removal, the time-dependent changes in the concentrations of

Table 3. Plasma concentrations (ng/ml) of progesterone during the period of PRID insertion in ovarian dysfunction such as ovarian quiescence, follicular cyst (FC), and luteal cyst or cystic corpus luteum (LC/CCL)¹⁾

Diagnosis	Day 0 ²⁾	Day 2–3	Day 12
Ovarian quiescence (n=13)	0.3 ± 0.07	3.8 ± 0.42 ^{ab}	2.1 ± 0.2 ^a
FC (n=15)	0.3 ± 0.06	4.5 ± 0.44 ^{ab}	2.4 ± 0.17 ^a
LC/CCL (n=7)	10.0 ± 2.93	12.4 ± 2.77 ^c	2.6 ± 1.21

¹⁾Data are presented as mean ± SEM. ²⁾Indicates the day of PRID insertion. ^aIndicates significant differences from Day 0 ($P < 0.01$). ^bIndicates significant differences from Day 12 ($P < 0.01$). ^cIndicates significant differences from Day 12 ($P < 0.05$).

estradiol-17 β were similar to those in the normal estrous cycle in the ME and E groups, but not in the NE group.

Cystic ovarian disease

The therapeutic effects of PRID treatment in the FC group are summarized in Table 2. Of the 15 cows, 7 cows were classified as ME, 5 cows as E and 3 cows as NE. All cows of these groups showed similar progesterone patterns until the day of PRID removal (Fig. 1, middle 3 panels). The concentrations of progesterone on Day 2–3 and on Day 12 were significantly increased after PRID insertion ($p < 0.01$) compared to those on day 0 before PRID treatment (Table 3). After withdrawal of the PRID, the ME and E groups showed similar time-dependent progesterone changes to those seen in the normal estrous cycle (Fig. 1, #HC1 and #CC3), whereas the progesterone concentration in the NE group remained at the base line level after PRID removal (Fig. 1, #HC2).

The therapeutic responses of LC/CCL to PRID treatment are also presented in Table 2. A total of 7 cows with LC/CCL showed the following therapeutic effects: 2 were in the ME group, 4 in the E group and 1 in the NE group. These 3 groups exhibited variable concentrations of progesterone greater than 1 ng/ml before and during PRID insertion (Fig. 1, right 3 panels). The progesterone levels tended to increase after PRID insertion, but there was no significant difference ($P > 0.05$) between Day 0 and Day 2–3 (Table 3). Then the concentration of progesterone decreased on Day 12. There was a significant difference ($P < 0.05$) between Day 2–3 and Day 12. After withdrawal of the PRID, the progesterone concentrations decreased immediately after removal of the PRID in the ME and E groups (Fig. 1, #FC1 and #IC6), whereas the

cows with NE exhibited a delayed decline of the progesterone levels (Fig. 1, #DC5). Cyclic changes in the progesterone concentrations were found after the removal of the PRID in all animals of the LC/CCL group.

The circulating concentrations of estradiol-17 β were greater than 10 pg/ml in the FC group and 3 pg/ml in the LC/CCL group before PRID treatment. After PRID removal, the time-dependent changes of the estradiol-17 β concentration were similar to those in the normal estrous cycle in the ME and E groups, but the concentrations were greater than 12 pg/ml in the FC group and were various in the LC/CCL group until the end of experiment in the NE group.

Discussion

The results of this study show that the clinical use of a progesterone-releasing device containing an estradiol benzoate capsule induces reliable therapeutic effects on various types of ovarian dysfunction such as ovarian quiescence and FC in postpartum cows. PRID plus estradiol treatment was clearly effective in treating ovarian dysfunction when the device was inserted for 12 days without supplemental treatment. Moreover, it seems that this treatment was able to induce ovulation within several days even in cases of LC or CCL. In previous reports, similar therapeutic effects on cystic ovarian disorders were found in groups treated with a PRID alone, GnRH alone and PGF_{2 α} alone or with combinations of these agents [9, 12, 20, 21]. Macmillan *et al.* [22] demonstrated that only 5–7 days of treatment with progesterone followed by estradiol injections could induce estrus and ovulation in anestrus cows. The present

results support the view that treatment with a PRID including an estradiol bolus alone provides sufficient therapeutic effects on cystic ovary and ovarian quiescence in dairy cows.

McLeod and Williams [21] as well as Jeffcoate and Ayliffe [12] reported the effectiveness of PRID attached with or without an estradiol capsule on FC in dairy cows. The present results demonstrate that PRID was also effective in 6 of 7 cows diagnosed as LC/CCL. Although the diagnostic information obtained in the present study was insufficient for discriminating between LC and CCL, 3 of these 7 cows seemed to have had CCL, because cyclic changes in their concentrations of progesterone were observed before the PRID treatment (e.g. Fig. 1, right panels, #FC1). PRID is known to be useful for the synchronization of estrus in cows. It also has been established that the inclusion of an estradiol benzoate capsule to PRID did not directly alter the progesterone secretion from the CL, but it affected the suppression of LH and FSH secretion [23–25]. Furthermore, the suppressed LH and FSH concentrations induced scant luteolytic effect [13]. In the present study, PRID treatment for CCL might have induced the regression of luteal tissue, followed by estrus and ovulation, through negative feedback action on gonadotropin secretion.

Cows treated with PRID showed significant increases in the circulating concentrations of progesterone compared with the pre-treatment levels in cases of ovarian quiescence and FC. The plasma concentrations of progesterone increased markedly to levels from 3 to 6 ng/ml in 2 to 3 days after intravaginal insertion of the PRID. The peak levels of progesterone during the period of PRID insertion were all within the physiological ranges that are seen in the luteal phase and are known to act on the hypothalamus and anterior pituitary gland to inhibit gonadotropin release [23, 24]. Some studies have suggested that the appearance of estrus within 7 days after PRID removal implies the effectiveness of the device, as shown by the ability of the two ovarian steroids to appropriately influence the hypothalamic-hypophyseal-gonadal axis [12, 23, 26].

Previous studies have supported the idea that concurrent treatment with progesterone and estradiol is required to terminate a current follicle wave and induce the emergence of a new wave 3–6 days later [14]. The administration of exogenous estradiol with progesterone suppresses the formation or decreases the diameter of the dominant follicle, presumably due to the suppression of FSH and LH via negative feedback action [14, 27]. Several lines of evidence suggest that dysfunction of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator and surge generator regulating the pulse and surge modes of gonadotropin secretion are contributing factors in the etiology of ovarian quiescence and FC, respectively [28–30]. It has been established that progesterone induces negative feedback effects on both generators. A plausible mechanism by which PRID might cure both ovarian quiescence and FC is that treatment with a PRID attached to a capsule containing estradiol benzoate induces the renewal of gonadotropin secretion by acting on the hypothalamic GnRH releasing generators, which in turn induces new follicular development and ovulation and the subsequent formation of corpus luteum, resulting in normal estrous cycles.

In conclusion, the present findings suggest that progesterone and estradiol benzoate treatment administered using a PRID have a high rate of therapeutic effectiveness in cows with reproductive disorders, such as ovarian quiescence, cystic ovary or cystic corpus luteum.

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