FULL PAPER ***Theriogenology***

Clinical Response of Ovarian Cysts in Dairy Cows after PRID Treatment

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ABSTRACT. We investigated the therapeutic effects of a progesterone releasing intravaginal device (PRID) on cystic ovarian disease (COD) and reproduction performance of cows. The possible influence of PRID on metabolic and/or health status was also examined. A total of 40 Holstein-Friesian cattle, with ovarian cystic structures, ≥2.5 cm in diameter, persisting for more than 7–14 days, without a corpus luteum (CL) were used for the study. PRID or placebos were inserted into the vagina for 12 days. Five animals lost the intravaginal device before removal and one was culled. Based on plasma progesterone concentration on the day of treatment, 20 (17 PRID and 3 placebos) of the remaining 34 cows had follicular cysts (progesterone ≤1 *n*g/m*l*) and 14 (10 PRID and 4 placebos) had luteal cysts (progesterone >1 *n*g/m*l*). Fourteen (82%) of the PRID-treated follicular cystic cows responded with formation of a CL within 14 days after treatment, and an overall conception rate of 53.8%. Likewise, 70% of the treated luteal cystic cows responded with CL formation and 71.4% conception rate. No significant differences were observed in hematocrit (Ht), white blood cell count and serum levels of glucose, blood urea nitrogen, aspartate aminotransferase, and alanine aminotransferase, between the day of PRID insertion and removal, in animals with follicular and luteal cysts. PRID treatment resulted in ovulation 2–4 days later and formation of a CL in cows that recovered.

KEY WORDS: cattle, cystic ovary, PRID.

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Cystic Ovarian Disease (COD) remains an important cause of the extension of the interval from calving to conception and thus a serious cause of infertility in dairy cows. Many treatment strategies have been used to resolve the cystic condition. These include manual rupture, dexamethasone, progesterone, GnRH, hCG, and $PGF_{2\alpha}$ [5, 14].

GnRH is currently the treatment of choice for undifferentiated COD [5, 32]. LH is released in response to GnRH resulting in luteinization of the cystic structure(s) or ovulation of follicles present at the time of treatment. Most cows that respond positively to GnRH are in estrus between 18 and 23 days after treatment [35]. To shorten the period from GnRH treatment to estrus, $PGF_{2\alpha}$ can be used 9–14 days after GnRH [1, 5, 15]. A new breeding protocol GnRH/$PGF_{2\alpha}$/GnRH (Ovsynch) has also been used to treat COD [2]. $PGF_{2\alpha}$ alone is the treatment of choice for luteal cysts. Cows with luteal cysts respond to treatment with $PGF_{2\alpha}$ by regression of the cyst, development of follicles and estrus in 2–5 days.

The use of progesterone for the treatment of COD has been well documented and is known to relieve COD [13, 23]. Progesterone may be administered by injection, intravaginal devices [19] or ear implants [17]. A progesterone releasing intravaginal device (PRID, Sanofi Sante Animal Health, France) has been used for estrus synchronization, treatment of postpartum anestrus, COD and other functional abnormalities in dairy cattle [12]. PRID has also been used for cases of COD that are not responsive to GnRH [24, 34]. Nanda *et al.* [25] reported that progesterone treatment appeared to heal endocrine lesions prevailing in cows with follicular cysts, after LH response to oestradiol was restored following treatment with PRID for 7 days.

Although PRID has been used to treat COD, its exact mode of action is not known very well. Dolezel *et al.* [7], using ultrasonography monitored ovaries during treatment with PRID, and found that cysts either did not respond, underwent luteinization, enlarged or disappeared, showing that PRID caused regression or luteinization of the cysts that responded to treatment. The proposed mode of action is through steroid hormone negative feedback on LH and FSH release; cysts are terminated and a new follicle wave starts [28]. This results in the presence of an estrogen active dominant follicle that ovulates after termination of treatment. This study aimed to evaluate the therapeutic effects of PRID on COD, safety and reproduction performance of cows after treatment. To evaluate safety, the possible influence of PRID on metabolic and/or health status was examined. Although some studies have reported the use of PRID in treatment of inactive ovaries, repeat breeding, and estrus synchronization of cattle [9, 10] there is still a need to investigate the therapeutic effects of PRID on cystic ovaries.

MATERIALS AND METHODS

Animals: The experiment was conducted at three veterinary clinics, covering a total of over 30 farms in Hokkaido,

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Japan. A total of 40 Holstein-Friesian cattle diagnosed with cystic ovaries, aged between 2 and 17 years (5.4 ± 2.8 mean ± SD), were used for the study. COD was diagnosed by palpation per rectum concurrently with plasma progesterone determination. It was defined as the presence of one or more cystic structures, ≥ 2.5 cm in diameter, on one or both ovaries, which persisted for over 7 days in the absence of a corpus luteum (CL). Cows included in the study were ≥ 40 days postpartum. For confirmation of diagnosis, blood samples for progesterone determination were collected twice; at the time of initial diagnosis and 7–14 days later. The progesterone concentration had to be ≤ 1.0 *n*g/m*l* for follicular cysts and > 1.0 *n*g/m*l* for luteal cysts on both occasions, or at the time of PRID insertion with the presence of cysts and no CL.

Most farmers kept their animals in either stanchions or freestall. In addition to silage, hay and concentrates, the animals were allowed to graze during spring, summer and autumn. The average milk yield per cow on the day of cyst diagnosis was 29.4 ± 8.2 kg (mean ± SD). Calving was throughout the year, and breeding started after 60 days postpartum. Cows were artificially inseminated.

The cows were in fair or good body condition (body condition score 2.75–3.25 on a 1–5 scale), healthy, had no history of other reproductive problems and no treatment with other hormones within the last 2 months. The animals calved between March 1997 and October 1998 and at the time of treatment were between 40 and 300 (105 ± 12, mean ± SE) days postpartum.

Treatment protocol: Following diagnosis, a PRID containing 1.55 g of progesterone and a 10 mg estradiol capsule, or placebo (a stainless steel coil covered with an inert silicon elastomer like PRID, without progesterone or estradiol capsule) was inserted into the vagina by hand, after disinfecting the surgeons' arms and vulva area, and left in place for 12 days. On the day of PRID removal, examination of the reproductive tract was carried out by palpation per rectum. Within 7 days after PRID removal, the cows were observed for estrus and artificial insemination (AI) was carried out after detection of estrus. Formation of CL was checked by palpation per rectum and plasma progesterone measurement 7–14 days after PRID removal in cows observed in estrus and 12–14 days after treatment in cows not observed in estrus. As part of the examination of the reproductive tract, visual examination of the external genitalia and vaginoscopic examination of the vagina and external cervical orifice were conducted. Pregnancy diagnosis was carried out 35 days or more after AI by palpation per rectum.

Blood sampling and analysis: Blood samples were collected from the coccygeal vessels into tubes containing heparin for plasma and plain tubes for serum. The samples were collected once, 2–3 hr or more after feeding on the days of initial diagnosis, PRID insertion, PRID removal and CL check. After collection, blood samples for serum were stored at 4°C for 20–24 hr and then centrifuged at 1,700 × g for 15 min. Serum was collected, and either stored at –20°C for less than a month or immediately analyzed for glucose (GLU), blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Heparinized blood samples were centrifuged at 1,700 × g for 15 min, and plasma was collected and stored at –20°C until assayed for progesterone. Heparinized blood samples were also used to determine hematocrit (Ht) and total white blood cell count (WBC). GLU, BUN, AST, ALT, Ht and WBC were determined twice, on the day of insertion and on removal of PRID or placebo.

Plasma concentrations of progesterone were determined after diethyl ether extraction using a double antibody-enzyme immunoassay kit (Kambegawa Laboratory, Tokyo, Japan) on microtiter plates coated with second antibody. The assay used anti-rabbit IgG goat antibody coated on the plates, progesterone-3- carboxymethyl oximino-horse radish peroxidase as a tracer and rabbit anti progesterone-3 (E)-carboxymethyl oximino-BSA IgG. The inter- and intra-assay coefficients of variation were 11.2% and 6.5%, respectively, at a concentration of 1.7 *n*g/m*l*.

To determine the Ht, heparinized blood was aspirated in a capillary tube, centrifuged for 5 min at 6,800 × g and read on a Ht scale. WBC, GLU, BUN, AST and ALT were determined using biochemical auto-analyzers, SE9000 Sysmex (Tokyo, Japan) for WBC and Hitachi 736 (Tokyo, Japan) for GLU, BUN, AST and ALT.

Evaluation of treatment: Positive ovarian response to treatment or recovery was defined as occurrence of estrus within 7 days and formation of a CL 7–14 days after PRID removal. Those animals in which estrus was not detected but CL was palpated per rectum 12–14 days after PRID removal were considered to have responded to the treatment as well. A negative ovarian response or no recovery was defined as absence of both estrus within 7 days and CL 14 days after PRID removal or estrus within 7 days but no CL 14 days after PRID removal.

Statistical analysis: Fisher's exact test was used to test for the difference between two sample populations. For normally distributed data, Student's *t*-test or its modification, the Welch *t*-test, was used to test for the difference between two sample means. The Mann-Whitney U-test was used for not normally distributed data. Confidence interval analysis was used to confirm the results of *t*-test and Mann-Whitney U-test. Differences were considered to be significant at $p<0.05$ unless stated.

RESULTS

Recovery and reproductive performance after PRID treatment: Of the 40 animals with COD, 5 lost the intravaginal device during the 12-day treatment period, and one animal was culled. These six cows were therefore, excluded from further analysis. The other 34 animals were divided to two groups, 20 with follicular cysts, of which 17 were treated with PRID and 3 with placebos and 14 with luteal cysts, of which 10 were treated with PRID and 4 with placebos.

Recovery and reproductive performance after PRID treat-

Table 1. Recovery and reproductive performance after PRID treatment in cows with ovarian cysts

	Follicular Cysts	Luteal Cysts	Total
Number of Cows	17	10	27
Recovery[a)] (%)	14(82.3)	7(70.0)	21(77.8)
Days to estrus (mean ± SD)	2.6 ± 1.3	3.1 ± 1.2	2.8 ± 1.4
Inseminated	13[b)](76.5)	7(70.0)	20(74.1)
1st AI conception (%)	3(23.1)	1(14.3)	4(20.0)
2nd AI conception (%)	2(20.0)	4(60.0)	6(37.5)
Overall conception (%)	7(53.8)	5(71.4)	12(60.0)
Days to conception (mean ± SD)	39.3 ± 23.4	30.2 ± 16.5	35.5 ± 19.8

a) Cows showing heat within 7 days and CL palpated within 14 days after PRID removal.
b) One cow was culled after recovering.

ment is summarized in Table 1. Of the 17 treated cows with follicular cysts, 14 cows expressed estrus within 7 days with formation of CL within 14 days after PRID removal. Thus, the recovery rate after PRID treatment in cows with follicular cysts was 82.3%. Of the 10 cows with luteal cysts, 7 showed estrus within 7 days and had a CL within 14 days of PRID removal. The recovery rate was 70%. The recovery rate in all cows regardless of category, follicular or luteal cysts was 77.8%.

Of the three control cows with follicular cysts, 2 had CL by 14 days after placebo removal, even though only one expressed estrus within 7 days. The third cow did not recover spontaneously and still had cysts 34 days after placebo removal. Of the 4 control cows with luteal cysts, only 1 cow had CL, 14 days after placebo removal, but no heat was observed. In this cow the cyst was still present on the day of placebo removal, but was not detected 14 days later.

There was no significant difference in recovery rate between cows with follicular and luteal cysts, and their respective controls. However, overall there was a significant difference (χ^2 = 3.11, 1df, p<0.1) in recovery rate, between treated and control cystic cows (77.8% versus 42.8%).

In most of the cows that responded to treatment a dominant follicle was palpated on the day of PRID removal. After estrus, ovulation and formation of CL, cystic structures were still present in 9 of 14 cows with follicular cysts, and in 3 of 7 cows with luteal cysts. The intervals from PRID removal to heat were 2.6 ± 1.3 and 3.1 ± 1.2 days (Mean ± SD) for follicular and luteal cysts, respectively.

In some cows, there was a little pus in the vagina, which had no foul smell, on removal of PRID, and required no treatment.

First and second service conception rates after treatment, in cows that recovered from follicular cysts, were 23.1 and 20.0%, respectively, and the overall conception rate was 53.8%. Mean interval in days between treatment and conception was 39.3 ± 23.7 (SD). In cows that recovered from luteal cysts, the second service conception rate was 60.0% and the overall conception rate was 71.4%. Mean interval in days between treatment and conception was 30.2 ± 16.5 (SD). The number of services per conception ranged from 1 to 3 (mean 1.83).

Plasma progesterone profile: In the cows that had a positive response after treatment, on day of PRID removal, 7 (41.0%) and 6 (60.0%) cows with follicular and luteal cysts, respectively, had progesterone concentrations ≤ 1.0 *n*g/m*l*. The mean ± (SE) progesterone concentration in these cows was 1.38 ± 0.16 *n*g/m*l* and ranged from 0.9–3.1 *n*g/m*l*, on the day of PRID removal. At the time of detection of CL (day 19 to 26), all the cows that had a positive response after PRID treatment had plasma progesterone concentrations >1.0 *n*g/m*l*, thus confirming the diagnosis of an active CL.

In the cows that had a negative response after treatment, on day of PRID removal, 2 (12.0%) and 1 (10.0%) cows with follicular and luteal cysts, respectively, progesterone concentrations were ≤ 1.0 *n*g/m*l*. At the time of CL detection (day 19 to 26), all the cows that had a negative response had plasma progesterone concentrations ≤ 1.0 *n*g/m*l*. In both cows with follicular and luteal cysts, there was no significant difference in plasma progesterone profile on the day of PRID removal between recovered and non-recovered cows. There was also no significant difference in plasma progesterone at the day of PRID removal between cows that had recovered from follicular and luteal cysts.

Blood indices: Blood indices are summarized in Table 2. For both follicular and luteal cysts, there were no significant changes in all of the criteria between the day of PRID insertion and PRID removal. No significant differences were observed in these metabolic indices between cows that recovered after treatment and those that did not. The mean for all the blood responses remained within the normal range in cattle.

DISCUSSION

The results of the present study confirm that follicular and luteal cysts can be effectively treated using PRID. PRID treatment resulted in ovulation 2–4 days later and formation of a CL. The recovery rates observed in this study are comparable to other studies [16, 24]. Since the recovery rates of follicular and luteal cysts after PRID treatment did not differ in this study, PRID can be effectively used for treating cows with COD without differentiating follicular

Table 2. Blood indices in cows that recovered after PRID treatment

	Follicular cysts (Mean ± SE) (n=11)		Luteal Cysts (Mean ± SE) (n=6)	
	PRID IN	PRID OUT	PRID IN	PRID OUT
Ht(%)	28.5 ± 0.8	27.76 ± 1.03	32.16 ± 2.05	31.23 ± 1.01
WBC ($10^3/mm^3$)	8.09 ± 1.09	5.94 ± 0.392	7.95 ± 0.80	6.87 ± 0.87
GLU (mg/d*l*)	64.75 ± 1.93	64.75 ± 2.14	62.83 ± 2.98	64.5 ± 3.7
BUN (mg/d*l*)	13.18 ± 1.37	15.03 ± 1.28	19.28 ± 1.92	17.96 ± 1.96
AST (IU/*l*)	80.67 ± 4.83	73.18 ± 2.75	81.83 ± 4.27	84.5 ± 4.5
ALT (IU/*l*)	27.08 ± 3.74	23.36 ± 3.60	82.83 ± 3.38	27.5 ± 1.5

from luteal cysts. When luteal cysts are differentiated from follicular cysts, $PGF_{2\alpha}$ is the treatment of choice [12].

Due to the small number of control animals used in the present study, effects of PRID on follicular and luteal cysts could not be shown by differences in recovery rates with their respective controls. However when combined, the treated group showed a higher recovery rate than the control group ($p<0.1$), indicating the effectiveness of PRID treatment.

The number of days from treatment to recovery i.e. estrus within 7 days and CL within 14 days of PRID removal, is similar to a previous report [24]. The low first service conception rate after treatment, could be attributed to the ovulation of aged oocytes from persistent follicles which may have developed in a low progesterone environment during the 12-day PRID treatment [29, 31]. This was confirmed by a slightly higher second service conception rate. Lower fertility after administration of progesterone is consistent with other studies [26]. Inadequate sperm transport and retarded embryo development, due to long term treatment may have contributed to the low first service conception rate [33]. The overall conception rate after 1–3 services observed in this study, after treatment of follicular cysts, 53.8% was considered under our field conditions to be satisfactory and was similar to 50.0% [8], but lower than that observed in other studies after PRID treatment, 88.0% [24] and 56.0% on first AI [16].

GnRH has been widely used for the treatment of undifferentiated COD. The recovery rates after PRID treatment observed in this study, are similar to those observed after GnRH, 52.0 % [24] and 70.0–80.0 % [5]. Nanda *et al.* [24] also reported that there was no difference in recovery rates between GnRH and PRID treated cystic cows. The conception rate after PRID treatment was also similar to those reported in other studies after treatment with GnRH [6, 24]. This implies that PRID is as effective as GnRH in the treatment of COD.

It was observed in this study that in about 62.5% of follicular cysts and 50.0% of luteal cysts treated with PRID, cysts were still palpable though considerably reduced in size 14 days after PRID removal and that the animals had ovulated with subsequent formation of CL. Jeffcoate and Ayeliffe [12], using ultrasound also observed that cyst regression was largely complete 10 days after PRID treatment. Therefore, when a cyst is diagnosed in the presence of CL, it is most likely not functionally active.

The mechanism by which the cow recovers from COD after PRID treatment is not well known. Progesterone suppresses basal [11] and estradiol induced [23] LH release in cattle. It is proposed that negative feedback of progesterone on the hypothalamus and pituitary favours storage of gonadotropins over release. When the PRID is removed, LH and FSH are released promoting follicular growth, estrus and ovulation. Within 24 hr of PRID insertion, progesterone concentration increases, reducing both mean LH and pulse frequency through negative feedback on the hypothalamus and pituitary [3, 4, 17]. Thus LH is insufficient for maintaining the cyst and it undergoes functional regression and atresia. Cysts may also disappear or undergo functional luteinization during treatment [7]. With atresia of the cyst a new follicular wave begins, 4–5 days after PRID insertion resulting in the presence of a dominant follicle when treatment is terminated, with estrus and ovulation generally occurring 2–4 days later [4, 28]. The PRID used in this study had an estradiol capsule attached. Laurilla *et al.* [16] found no significant difference in recovery rate between COD cows treated with an intravaginal progesterone releasing device with an estradiol capsule and those with devices without the estradiol capsule. This suggests that the estradiol capsule in PRID may not be needed for treating COD. The mode of action of PRID in relieving luteal cysts is the same as described above. Progesterone from PRID supplements that from luteal cysts and together block LH pulse frequency leading to atresia of the cyst. As observed in earlier studies, cows with ovarian cysts fail to induce an LH surge in response to a dose of exogenous estradiol [27] and probably may have lost the ability to respond to the positive effects of estradiol [4]. Exogenous progesterone might play a role in restoring hypothalamus sensitivity to estradiol in cows with ovarian cysts. Progesterone concentrations ranged between 0.9 and 3.1 *n*g/m*l* in cows on the day of PRID removal. In 7 and 6 cows that recovered from follicular and luteal cysts, respectively, plasma progesterone concentrations were (1 *n*g/m*l* although they were expected to be higher, (1 *n*g/m*l*, as observed in some studies [21, 22]. As observed in other studies with PRID or CIDR, average plasma progesterone concentrations differ significantly in individual cows and are attributable to individual rates of metabolism and clearance of the absorbed progesterone, breed and size of animal [19]. Macmillan *et al.* [18]

observed that plasma progesterone concentrations fell rapidly after CIDR removal. Other studies observed similar progesterone concentrations at or near the end of progesterone treatment [8, 20, 30]. No significant difference in plasma progesterone on the day of PRID removal between cows that had recovered from follicular and luteal cysts, was observed in this study. This may suggest that, if a cyst is present at the time of PRID removal it is functionally inactive and not secreting progesterone. The period of PRID treatment of COD could be reduced to have a shorter interval between PRID insertion and recovery. However, progesterone treatment should be at least 9–10 days to allow functional termination of cysts [28]. Reduction of the treatment period might also help improve the first service conception rate.

In conclusion, PRID is an effective treatment for COD in dairy cattle. Treatment resulted in ovulation and formation of CL. In order to fully understand the mode of action of PRID in relieving COD, frequent monitoring of the ovaries using ultrasonography, coupled with LH, FSH, oestradiol and progesterone profiles will be necessary.

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