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—Original Article—

Comparison of Plasma Concentrations of Estradiol-17β and Progesterone, and Conception in Dairy Cows with Cystic Ovarian Diseases between Ovsynch and Ovsynch plus CIDR Timed AI Protocols

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Abstract. The objectives of this study were 1) to determine the effects of adding a CIDR to the Ovsynch protocol on plasma concentrations of estradiol-17β and progesterone and conception in dairy cows with cystic ovarian diseases and 2) to examine associations among the estradiol-17β and progesterone concentrations and conception. Cows were diagnosed as having cystic ovarian diseases if they were found to have a cystic follicle (diameter ≥25 mm) without a corpus luteum by two palpations per rectum with an interval for 7 to 14 days. They were treated with either the Ovsynch (GnRH on Day 0, $PGF_{2\alpha}$ on Day 7 and GnRH on Day 9, with AI on Day 10; n=15) or Ovsynch+CIDR protocol (Ovsynch protocol plus a CIDR from Day 0 to Day 7; n=23). Plasma estradiol-17β concentrations were determined on Days 0, 7 and 9, and plasma progesterone concentrations were determined on Days 0, 7, 9 and 17. The plasma estradiol-17β and progesterone concentrations at all of the days examined and conception rates did not differ significantly between the two timed AI protocols. The progesterone concentrations on Day 17 and conception rates were lower ($P<0.05$) for cows with low concentrations of estradiol-17β (<2 pg/ml) on Day 9 than for cows with high concentrations of estradiol-17β (≥2 pg/ml). The present study suggests that, in dairy cows with cystic ovarian diseases, addition of a CIDR to the Ovsynch protocol had no remarkable effects on plasma estradiol-17β and progesterone concentrations during and after the treatments or on conception after timed AI. This study indicates that the low plasma estradiol-17β concentration at the second administration of GnRH in the protocols can be a predictor for impaired luteal formation and lower likelihood of pregnancy in dairy cows with cystic ovarian diseases.

Key words: Cystic ovarian disease, Dairy cows, Estradiol-17β, Progesterone, Timed AI

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An ovarian cyst is a large follicular structure that failed to ovulate [1]. Bovine ovarian cysts are a serious cause of reproductive failure because they occur frequently and the affected cows are infertile [2]. They are classified as either follicular cysts, which are thin-walled and contain little or no luteal tissue in the cyst wall, or luteinized cysts, which are thick-walled and contain luteal tissue in the cyst wall [1–3]. It has been suggested that luteinized cysts diagnosed clinically may include a cystic corpus luteum (CL), which is a CL with a large fluid-filled central cavity, because it is difficult in some cases to distinguish between both types of cystic ovaries [4, 5].

The primary therapies for bovine follicular cysts include either use of GnRH analogue or exogenous progesterone [1, 2, 6], since an underlying mechanism in the development of follicular cysts involves a hypothalamic defect that does not respond to the increased estrogen level derived from the mature follicle and does not release GnRH [7–10]. On the other hand, cows with luteinized cysts can be successfully treated with $PGF_{2\alpha}$ when correctly diagnosed [1]. Recently, the Ovsynch protocol, which is an ovulation-synchronization protocol, or an estrus-synchronization protocol that uses a controlled internal drug releasing (CIDR) followed by $PGF_{2\alpha}$, has been applied to treat dairy cows with ovarian cysts in a few reports [11, 12]. It has been suggested in postpartum suckled beef cows that the addition of a CIDR to the conventional Ovsynch protocol (Ovsynch plus CIDR) increases plasma progesterone concentrations on the day of $PGF_{2\alpha}$ treatment, 7 days after the beginning of the Ovsynch, and induces a clear rise of estradiol-17β concentrations from the time of the $PGF_{2\alpha}$ treatment to the time of the second GnRH treatment, and that it improves conception rates after timed AI [13–17]. The CIDR-based timed AI protocols have been applied for also dairy cows with follicular cysts in acceptable conception rates [18, 19]. However, there have been no reports that have directly compared the Ovsynch and Ovsynch plus CIDR protocols in regard to the plasma concentrations of sex steroid hormones and conception in cows with cystic ovarian diseases. It is expected that addition of a CIDR to the Ovsynch protocol in cows with cystic ovarian diseases may increase progesterone concentrations at the time of $PGF_{2\alpha}$, may alter estradiol-17β concentrations at the time of the $PGF_{2\alpha}$ or the time of the second GnRH treatment, and then may improve conceptions following the timed AI.

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The present study was performed to determine the effects of adding a CIDR to the conventional Ovsynch protocol on plasma concentrations of estradiol-17β and progesterone and conception in dairy cows with cystic ovarian diseases. Also, this study explored any associations among the estradiol-17β and progesterone concentrations and conception in the cows treated with the two protocols.

Materials and Methods

Animals

This study was conducted from April 2004 to July 2005 on 11 commercial dairy farms in the Tanba area of Kyoto Prefecture, Japan. Nine dairy farms used a tie-stall style, and the remaining two farms used a non-tie style (one used a free-barn and the other used free-stalls). Thirty-eight lactating Holstein cows diagnosed with ovarian cystic diseases (age, 63.5 ± 4.5 months; days postpartum, 204.1 ± 20.2 days; mean ± SEM) were used for this study. Diagnosis of the ovarian cystic diseases was performed by palpation of the ovaries per rectum twice with an interval for 7 to 14 days. The criteria for the ovarian cystic diseases was presence of a cystic follicle (diameter ≥25 mm) without a CL in a cow at both palpations. The palpations were performed by a single experienced veterinarian. The ovarian cystic diseases of the cows were further classified, according to the plasma progesterone concentrations of the cows on the day of the second rectal palpation (Day 0; start of the protocol), into follicular cysts (<0.5 ng/ml, n=18) or luteinized cysts/cystic CLs (≥0.5 ng/ml, n=20).

Treatments and sample collection

The cows with cystic ovarian diseases were randomly assigned into two groups, i.e., the Ovsynch group and Ovsynch+CIDR group. The ovulation synchronization protocols were started at the time of the second rectal palpation of the ovaries when the cystic ovarian diseases were diagnosed. The cows in the Ovsynch group (n=15; follicular cysts n=7, luteinized cysts/cystic CLs n=8) received the standard Ovsynch protocol, namely, 100 µg of a GnRH analogue (fertirelin acetate; Conceral™, Intervet Schering-Plough Animal Health, Tokyo, Japan) intramuscularly (im) on Day 0 (start of the protocol), followed by 25 mg of a $PGF_{2\alpha}$ (tromethamine dinoprost; Pronalgon F™, Pfizer Japan, Tokyo, Japan) im on Day 7 and 100 μg fertirelin acetate im on Day 9 (in the afternoon). Timed AI took place on the morning of Day 10, approximately 20 h after the second GnRH treatment. The cows in the Ovsynch+CIDR group (n=23; follicular cysts n=11, luteinized cysts/cystic CLs n=12) received an intravaginal CIDR containing 1.9 g of progesterone (EAZI-BREED™; Livestock Improvement Association of Japan, Tokyo, Japan) for 7 days, starting on Day 0, in addition to the standard Ovsynch protocol. Approximately 55 days after AI, pregnancy was diagnosed by palpation of fetal membranes per rectum.

Blood samples were collected on Days 0, 7, 9 and 17 from all cows in both treatment groups. Blood was collected from the coccygeal vein into heparinized vacutainers and was centrifuged at 800 × *g* for 20 min at 4 C. The plasma was separated and stored at –30 C prior to hormone assays.

Hormone assays

The concentration of estradiol-17β in blood plasma was measured by RIA as described previously [13–16, 20]. The concentration of progesterone in blood plasma was measured by RIA using the processed standard curve as described previously [13–16, 20]. Anti-estradiol-17β-6-BSA serum (GDN no. 244) and anti-progesterone-11-BSA serum (GDN no. 337) were used for the estradiol-17β and progesterone assays, respectively. The sensitivity of the estradiol-17β and progesterone assays were determined to be 0.20 pg/ml and 78 pg/ml, respectively, and the intra-assay CVs were 17.4% (n=4) and 10.5% (n=6), respectively. All of the plasma samples for each hormone were measured within an assay.

Plasma progesterone concentrations on Day 17 were regarded as high (equivalent to cows with functional luteal tissue; ≥0.5 ng/ml) or low (equivalent to cows without functional luteal tissue; <0.5 ng/ml). The threshold value of 0.5 ng/ml for progesterone was based on the mean minus one SD of the concentrations on Day 17 in Holstein cows in the present study that were subsequently diagnosed as being pregnant. The threshold value of 0.5 ng/ml for progesterone concentrations in blood in cows has been used previously [21–23]. Plasma estradiol-17β concentrations on Day 9 were defined as high (≥2 pg/ml) or low (<2 pg/ml). The threshold value of 2 pg/mL for estradiol-17β was based on the mean minus one SD of the concentrations on Day 9 (when large, preovulatory follicles were expected to have been present) in Holstein cows in the present study that were subsequently diagnosed as being pregnant, as described previously [14].

Statistical analyses

The conception rate was defined as the number of pregnant cows divided by that of treated cows. Differences of conception rates between two groups were examined with the Chi-square test (SPSS version 18.0J; SPSS Japan, Tokyo, Japan). Differences of age and postpartum interval between two groups were analyzed by *t* test (SPSS version 18.0J).

For the concentrations of estradiol-17β and progesterone, the effects of treatment and day, and the treatment by day interaction, were evaluated by repeated-measures ANOVA, using the Generalized Estimating Equations procedure (SPSS version 18.0J). Differences in mean concentrations between the treatment groups on specific days, and between days in each treatment group were analyzed by least significant difference. Differences were taken as significant when $P<0.05$, and a 'tendency' denotes $0.05 \le P \le 0.1$.

Data for plasma estradiol-17β on Day 9 and progesterone on Day 17 in cows with cystic ovarian diseases in both of treatment groups were combined and analyzed to examine associations among both steroids concentrations and conception, because the steroids concentrations did not differ between the treatment groups.

Results

No significant differences were found between the Ovsynch and Ovsynch+CIDR groups in the age of the cow (Ovsynch 64.0 ± 5.7 *vs*. Ovsynch+CIDR 63.2 ± 6.6 months) or postpartum interval (Ovsynch 224.9 ± 29.8 *vs*. Ovsynch+CIDR 188.5 ± 19.7 days) at the start of this experiment. No significant differences were observed between the Ovsynch and Ovsynch+CIDR groups in the age and postpartum interval at the start of this experiment when the cystic ovarian diseases were classified into follicular cysts or luteinized cysts/cystic CLs (data not shown).

The conception rates of the cows with cystic ovarian diseases did not differ (P>0.1) between the Ovsynch (46.7%, 7/15) and Ovsynch+CIDR (43.5%, 10/23) groups. Also, when the cows with cystic ovarian diseases were classified into follicular cysts or luteinized cysts/cystic CLs, there were no differences (P>0.1) between the Ovsynch (follicular cysts 42.9%, 3/7; luteinized cysts/cystic CLs 50%, 4/8) and Ovsynch+CIDR (follicular cysts 45.5%, 5/11; luteinized cysts/cystic CLs 41.7%, 5/12) groups. The conception rates did not differed (P>0.1) between the younger (<60 months; 57.9%, 11/19) and older (≥60 months; 31.6%, 6/19) cows, between the cows with shorter (<180 days; 43.8%, 7/16) and longer (≥180 days; 45.5%, 10/22) postpartum intervals or between the tie-stall (38.9%, 7/18) and non-tie styles (50.0%, 10/20).

In comparison of the plasma estradiol-17β concentrations between the two protocols, there was an effect of day (P<0.05), but the effects of treatment and treatment by day interaction were not significant in both the cows with follicular cysts and luteinized cysts/cystic CLs. The estradiol-17β concentrations at all the days examined did not differ significantly between the Ovsynch and Ovsynch+CIDR groups in both types of cystic ovary. In cows with follicular cysts (Fig. 1, upper panel), the estradiol-17β concentrations decreased (P<0.05) from Day 0 to Day 7, but did not change (P>0.1) during the same period in cows with luteinized cysts/cystic CLs (Fig. 1, lower panel). The estradiol-17β concentrations increased (P<0.005) from Day 7 to Day 9 in the Ovsynch+CIDR group, but did not change significantly during the same period in the Ovsynch group in both types of cystic ovary.

In comparison of the plasma progesterone concentrations between the two protocols, an effect of day was found (P<0.001), but the effects of treatment and treatment by day interaction were not significant in both the cows with follicular cysts and luteinized cysts/cystic CLs. The progesterone concentrations increased (P<0.001) from Day 0 to Day 7 in cows with follicular cysts (Fig. 2, upper panel) and in the Ovsynch+CIDR group of cows with luteinized cysts/cystic CLs (Fig. 2, lower panel), but did not change (P>0.1) during the same period in the Ovsynch group. The progesterone concentrations decreased (P<0.001) from Day 7 to Day 9 and then increased again (P<0.005) from Day 9 to Day 17 in both protocols in both types of cystic ovary.

Data for the plasma estradiol-17β and progesterone concentrations of both types of cystic diseases were combined, and the effects of pregnancy in the two protocols were examined. The plasma estradiol-17β and progesterone concentrations for pregnant cows did not differ between the protocols (P>0.1), and thus the data of both protocols for pregnant cows were combined and analyzed (Fig. 3). The data for the plasma estradiol-17β concentrations in the non-pregnant cows were analyzed separately for each protocol because an effect of protocol by day interaction was observed (P<0.001, Fig. 3 upper panel). The estradiol-17β concentrations on Day 9 were lower (P<0.05) for non-pregnant cows with Ovsynch than for pregnant cows and tended to be lower (P<0.1) in non-pregnant cows for Ovsynch than for Ovsynch+CIDR. The data for the

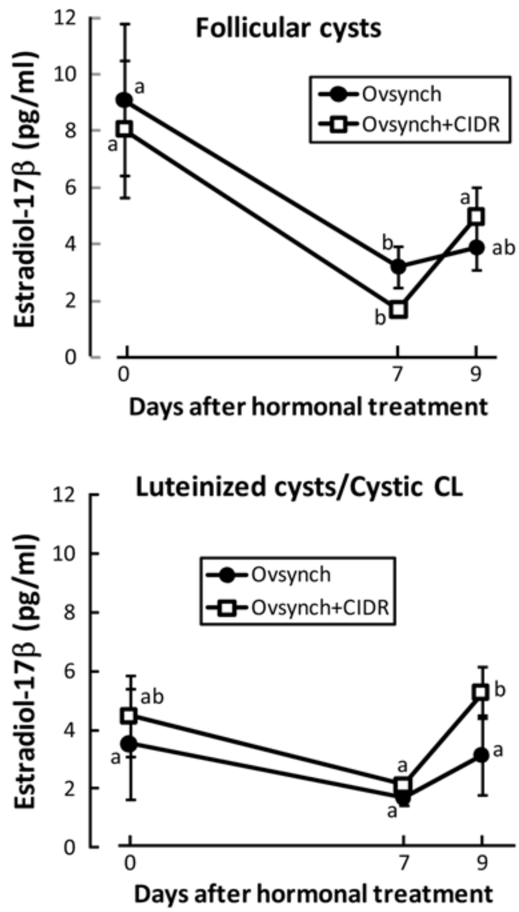

Fig. 1. Changes in plasma estradiol-17β concentrations in dairy cows with cystic ovarian diseases in the Ovsynch and Ovsynch+CIDR groups. The estradiol-17β concentrations are separately shown for follicular cysts (upper panel, Ovsynch; n=7, Ovsynch+CIDR; n=11) and luteinized cysts/cystic CLs (lower panel, Ovsynch; n= 8, Ovsynch+CIDR; n=12). Data are expressed as mean ± SEM. Values with different superscripts differ between days within a group (P<0.05).

plasma progesterone concentrations in the non-pregnant cows for both protocols were combined and analyzed because the effects of treatment and treatment by day interaction were not significant (Fig. 3, lower panel). The progesterone concentrations on Day 17 were lower (P<0.05) for non-pregnant cows than for pregnant cows.

The data for the plasma estradiol-17β and progesterone concentrations of both types of cystic diseases in both protocols were combined, and associations between both steroid concentrations and between steroid concentrations and pregnancy status were examined. Cows with a low estradiol concentration on Day 9 showed lower (P<0.05) plasma progesterone concentrations on Day 17 than cows with a high estradiol concentration (Fig. 4). The cows with a low estradiol concentration on Day 9 also showed a tendency for a higher rate of cows (P≤0.1) with a low progesterone concentration on Day 17 (40%, 4/10) than cows with a high estra-

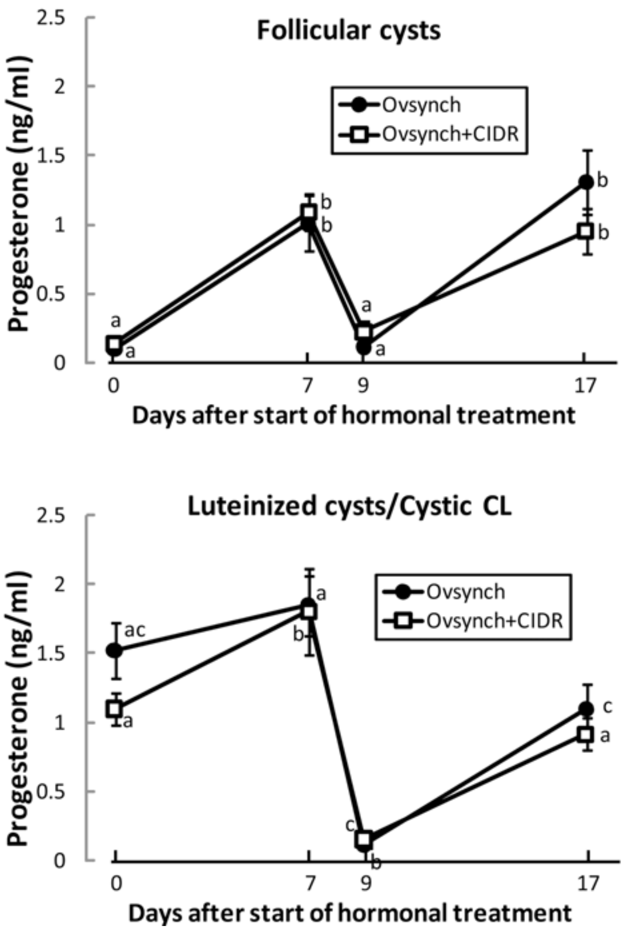

Fig. 2. Changes in plasma progesterone concentrations in dairy cows with cystic ovarian diseases in the Ovsynch and Ovsynch+CIDR groups. The progesterone concentrations are separately shown for follicular cysts (upper panel, Ovsynch; n=7, Ovsynch+CIDR; n=11) and luteinized cysts/cystic CLs (lower panel, Ovsynch; n=8, Ovsynch+CIDR; n=12). Data are expressed as mean ± SEM. Values with different superscripts differ between days within a group (P<0.05).

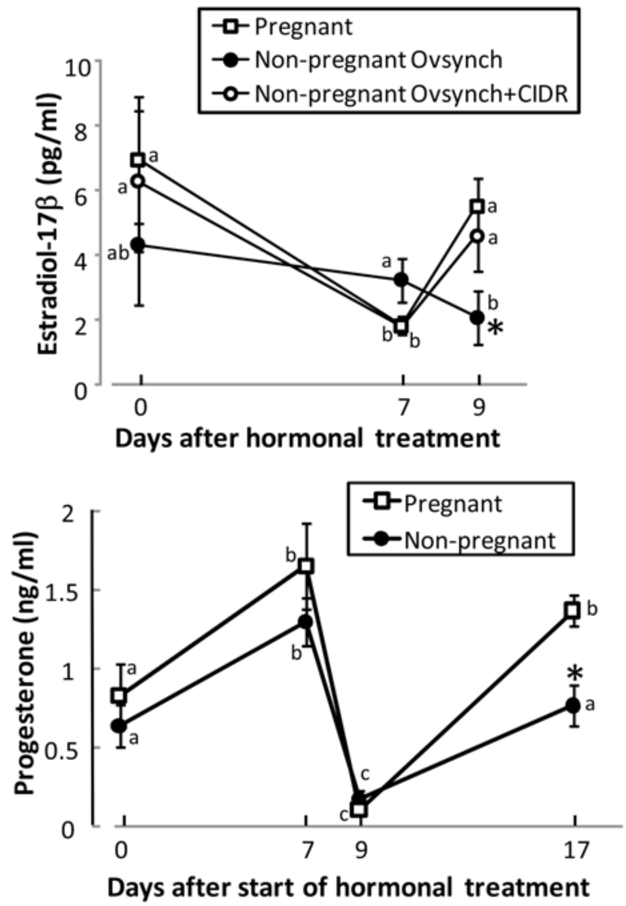

Fig. 3. Changes in plasma estradiol-17β and progesterone concentrations in dairy cows with cystic ovarian diseases treated with timed AI protocols in pregnant and non-pregnant animals. The estradiol-17β (upper panel, pregnant; n=17, non-pregnant Ovsynch; n=8, non-pregnant Ovsynch+CIDR; n=13) and progesterone (lower panel, pregnant; n=17, non-pregnant; n=21) are shown. Data for both types of cystic ovarian diseases are combined. Data are expressed as mean ± SEM. Asterisk indicates a difference compared with pregnant cows (P<0.05). Values with different superscripts differ between days within a group (P<0.05).

diol concentration (14.3%, 4/28). Cows with a low estradiol concentration on Day 9 (10%, 1/10) again showed lower conception rates (P<0.05) than cows with a high estradiol concentration (57.1%, 16/28). None of the cows (0%, 0/8) with a low progesterone concentration on Day 17 were pregnant and the conception rates were lower (P<0.01) than for the cows with a high progesterone concentration (56.7%, 17/30).

Discussion

In the present study, the effects of adding a CIDR to the Ovsynch protocol on changes of plasma steroid concentrations and conception were assessed for dairy cows with cystic ovarian diseases. As the results, the addition of a CIDR to Ovsynch did not improve the conception rates in both types of cystic ovarian diseases, i.e., follicular cysts and luteinized cysts/cystic CL. The conception rates by timed AI of both protocols for the cystic ovarian diseases were around 45% in this study. These conception rates are higher than those in previous reports in which conventional Ovsynch was used (18.3% [11] and 23.1% [12]) and are almost equivalent to those in the previous reports in which CIDR-based timed AI protocols were used (52.3% [18] and 36.7% [19]). Conception rates in dairy cows could be affected by various factors including postpartum interval, parity and milk yield [24, 25]. Treatment history for the ovarian diseases before the start of the ovulation synchronization may also influence conception. In this study, it was elucidated that postpartum interval, age and style of cowshed do not affect conception. However, detailed information on other factors such as milk yield and treatment history was lack-

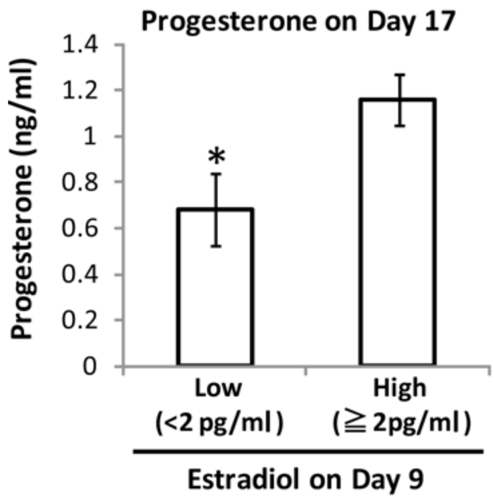

Fig. 4. Comparison of plasma progesterone concentrations on Day 17 between cows with low (<2 pg/ml, n=10) and high (≥2 pg/ml, n= 28) estradiol-17β concentrations on Day 9. Both types of cystic ovarian diseases and both protocols are combined. Data are expressed as mean ± SEM. The asterisk indicates a difference compared with pregnant cows (P<0.05).

ing, and the effects of those factors on conception were unclear in the present study. It has been previously suggested that higher milk-yield of dairy cows enhances metabolism of sex steroid hormones in the liver [26, 27], and thus milk yield may affect ovulation synchronization and subsequent conception rates.

In this study, the Ovsynch plus CIDR protocol did not increase the plasma progesterone concentrations on Day 7 compared with the Ovsynch protocol in the cows with follicular cysts and luteinized cysts/cystic CLs. In a recent report that used lactating dairy cows at the follicular phase [28], plasma progesterone concentrations increased rapidly after CIDR insertion and were maintained at the maximal level for the initial 2 days (1 ng/ml) and then decreased to a lower level (0.6 ng/ml) 7 days after insertion. On the other hand, GnRH treatment for dairy cows with ovarian cysts gradually increased the plasma progesterone concentrations over the course of 9 days [29]. Thus, in a future study, the progesterone concentrations for the initial few days after the start of the protocols should be compared between the Ovsynch and Ovsynch plus CIDR protocols in dairy cows with cystic ovarian diseases.

The addition of a CIDR to the Ovsynch protocol did not alter the plasma estradiol-17β concentrations on Day 7 and Day 9 in the cows with cystic ovarian diseases although an increase of the concentrations from Day 7 to Day 9 was clearly evident with addition of a CIDR to the Ovsynch protocol. The unclear effect of addition of the CIDR on the estradiol-17β concentrations is probably due to the unaltered progesterone concentrations on Day 7, as described above.

In this study, the estradiol-17β concentrations from Day 7 to Day 9 in the non-pregnant cows treated with Ovsynch decreased, while those of the non-pregnant cows treated with Ovsynch+CIDR and those of the pregnant cows increased. A similar decrease of the plasma estradiol concentrations from the time of the $PGF_{2\alpha}$ treatment to the time of the second GnRH treatment has been previously shown in Ovsynch-treated non-pregnant beef cows [14]. This may be partly due to earlier maturation and ovulation of follicles and addition of a CIDR to the Ovsynch protocol being able to prevent the premature ovulation [14, 16]. It is unclear in the present study whether such a premature ovulation occurred or not in the Ovsynch-treated cows with cystic ovarian diseases that were later found to non-pregnant.

In the present study, associations between plasma estradiol-17β and progesterone concentrations in the process of performing the protocols and between the steroids and conception by timed AI were also examined using the combined data of the two different protocols and both types of cystic ovary. As a result, cows with low estradiol concentrations (<2 pg/ml) on Day 9 showed reduced plasma progesterone concentrations on Day 17 and conception rates, suggesting that a low estradiol level on the day of the second GnRH treatment can be a predictor for impaired luteal function and lower likelihood of pregnancy after timed AI. If a rapid method of completing the estradiol assay for cows within a few hours is developed, unpromising treatments such as timed AI and/or the second GnRH would be avoidable for cystic cows with a low estradiol concentration on that day.

In conclusion, in dairy cows with cystic ovarian diseases, the addition of a CIDR to the conventional Ovsynch protocol had no remarkable effects on plasma estradiol-17β and progesterone concentrations during and after the treatments or on conception after timed AI. This study suggests that a low plasma estradiol concentration (<2 pg/ml) at the second GnRH treatment in timed AI protocols can be a predictor for impaired luteal formation and lower likelihood of pregnancy in dairy cows with ovarian cystic diseases.

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