GnRH Treatment at CIDR Insertion Influences Ovarian Follicular Dynamics in Japanese Black Cows

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ABSTRACT. Ovarian follicular dynamics and estrous synchronization after Gonadotropin-releasing hormone (GnRH) treatment at Controlled Internal Drug Releasing device (CIDR) insertion were investigated in Japanese Black cows. CIDR was inserted for eight cows at 7 days after estrus. Cows were allocated to either Group A: 8-day CIDR insertion with GnRH treatment on d 0 (n=4, d 0=CIDR insertion) or Group B: 8-day CIDR insertion (n=4). Both groups were injected with prostaglandin F₂α (PGF₂α) on d 7. Ultrasoundography and blood sampling were performed twice daily. Intensive sampling was performed every 15 min for 8 hr to determine the pulsatile release of LH on d -1, d 5 and d 10. Three of four cows showed intermediate ovulation within 2 days after GnRH treatment during CIDR insertion in Group A, whereas no ovulation was found in Group B. Three of four cows in Group A and all four cows in Group B ovulated after CIDR removal. Plasma progesterone concentrations from d 3 to d 7 in three intermediate ovulatory cows in Group A (8.4 ± 1.6 ng/ml) was significantly higher than those in Group B (4.1 ± 1.2 ng/ml; 4 cows) during CIDR insertion (P<0.01). Interval to estrus and ovulation after CIDR removal was observed at 60.0 ± 12.0 hr and 76.0 ± 6.9 hr in three cows in Group A, and 75.0 ± 15.1 hr and 93.0 ± 20.5 hr in Group B, respectively. There was a significant increase in LH pulse frequency on d 10 compared on d -1 or d 5 in both groups (P<0.05), in addition those on d 10 in Group A tended to be higher than in Group B. As a result, GnRH treatment at CIDR insertion at 7 days after estrus induced intermediate ovulation with formation of corpus luteum (CL) and rather synchronized emergence of ovulatory follicle during CIDR insertion. These induced CL increased plasma progesterone concentrations and contributed to precise synchronization.

KEY WORDS: CIDR, GnRH, LH pulse, progesterone, synchronization.

FULL PAPER

Theriogenology

The treatment aiming estrous synchronization or ovulation allows the effective management of timed artificial insemination (AI) in dairy cows without the need for estrous detection. Most estrous synchronization protocols require prostaglandin F₂α (PGF₂α) treatment for luteolysis to aid the synchrony of estrus [4]. Yet, they do not enhance the conception rate nor ineffective in anestrous cows where mature corpus luteum (CL) be absent. Progesterone-based treatment for estrous synchronization is to be considered as the most appropriate one for non-cyclic or anestrous cows. Controlled Internal Drug Releasing device (CIDR) is an intravaginal progesterone releasing device, which has been widely used in estrous synchronization and for the treatment of reproductive disorders [6, 12, 13, 20, 24]. Estrous synchronization is an effective method for estrous detection and improves pregnancy rates without being labor intensive. Furthermore, it establishes reproductive management for super ovulation programs [2, 3, 9].

Various methods of estrous synchronization have been developed, such as single or double injections of PGF₂α [4], Ovsynch program (GnRH–PGF₂α–GnRH) [17, 18], and intravaginal progesterone with or without a combination of other hormones [2, 6, 12, 13, 20, 25]. Ovsynch regimen increases pregnancy rate with timed AI [17]. However, if ovulation was not induced with first GnRH treatment, luteolytic effect with PGF₂α injection has been reduced. PGF₂α treatment requires functional CL in the ovaries for estrous induction [11], that is, PGF₂α alone is not effective on inactive ovaries nor follicular cysts. CIDR treatment has the advantage in this matter, where it improves estrous synchronization and ovulation with an acceptable percentage in anestrous cows [20].

Ovarian follicles in cattle develop in a wave-like pattern [8]. Shortly after follicular wave emergence, one follicle becomes dominant, and suppresses continuous growth of other subordinate follicles [1, 17]. Normally, first wave DF grows until 6 to 7 days after estrus, maintains its diameter from 6 to 10 days, and regresses gradually soon after [22]. CIDR treatment increases estrous behavior, but in this method pregnancy rate fluctuates depending on the oocyte competence at CIDR removal. Recently, various studies have revealed about a combination of other hormones at CIDR treatment [3, 15, 16]. GnRH treatment at CIDR insertion induces intermediate ovulation, or turnover of the dominant follicles [22]. If GnRH was injected at the growth phase of DF, ovulation occurs from 24 to 48 hr after GnRH injection. This causes the emergence of a new follicular wave, synchronizing estrus and ovulation [16]. Induction of CL with GnRH treatment increases serum progesterone (P₄)
concentrations during CIDR treatment [25]. Exogenous progesterone treatment suppresses pituitary luteinizing hormone (LH) secretion, and prevents early estrus or premature ovulation [15]. After CIDR removal, a surge-like release of estradiol-17β (E2) leads to LH surge and ovulation. When comparing the estrous synchronization with CIDR insertion alone and those with CIDR and GnRH combination in dairy cows, several authors have favored for CIDR–GnRH combinations in conception rates [22, 25]. There is scope for further research with CIDR and GnRH protocols on the mechanisms of follicular dynamics in beef cows. In the present study, we hypothesis that first wave DF at 7 days after estrus attains near the maximum diameter and to be sufficient for the ovulatory response to GnRH injection.

The objective of this study was to monitor ovarian follicular dynamics and estrous synchronization after GnRH treatment at CIDR insertion in Japanese Black cows.

MATERIALS AND METHODS

Animals: Eight Japanese Black cows with normal estrous cycles were employed during the experiment in October 2001 to June 2002. They were housed in a stanchion barn under natural light and temperate conditions at the Animal Hospital in Kagoshima University, Japan. These cows were 10.4 ± 3.0 years old (mean ± sd), had calved 6.9 ± 3.6 times, and were 211.3 ± 171.3 days postpartum. Their estrous cycle was previously synchronized by 25 mg of PGF2α (Pronalgon F, Pharmacia, Tokyo, Japan), and animals were observed for the standing estrus twice daily. CIDR (CIDR-B, InterAg, Hamilton, NZ) was inserted intravaginally at seven days after induced estrus, and cows were allocated to either Group A: 8-day CIDR insertion with 100 µg of GnRH (Suporunen, Denka Pharmaceutical, Tokyo, Japan) treatment at the same time of insertion (d0=CIDR insertion, n=4), and Group B: CIDR insertion alone (n=4). Both groups were injected with 25 mg of PGF2α on d 7 a.m. to induce immediate regression of CL after CIDR removal. All animals in this study were cared for according to the standards set by the Kagoshima University on Animal Care (1995).

Ultrasonography: The ovarian structures were examined daily with an ultrasound scanner (EUB-405, Hitachi Medical Co., Tokyo, Japan) equipped with 7.5 MHz transrectal linear-array transducer (EUP-033J, Hitachi Medical Co., Tokyo, Japan), from the day of preceding PGF2α for estrus inducement until CIDR insertion. Then intensive examination was performed twice daily at 12 hr intervals from CIDR insertion to ovulation after CIDR removal. After ovulation, observation using ultrasonography was performed once daily for 7 days to confirm the CL formation. The size together with the location of follicles (> 5 mm in diameter) and CL in the ovaries was recorded, and the future ovulatory follicle (OF) was retrospectively identified. Time of ovulation was defined as the mean between the two consecutive scanning before and after the disappearance of the dominant follicle (DF).

Hormonal analysis: After each ultrasonography, blood was sampled from the jugular vein with a heparinized syringe. Plasma was separated immediately and frozen at −20°C until analysis. The double antibody radioimmunoassay (RIA) method was used to determine the concentrations of plasma P4, E2 [23] and LH [7]. Blood was collected every 15 min for 8 hr one day before CIDR insertion (d -1), 5 days after CIDR insertion (d 5) and 2 days after CIDR removal (d 10) to measure LH pulses. The intra- and inter-assay coefficients of variation were 4.2% and 8.0% for P4, 6.7% and 8.2% for E2, 8.8% and 13.0% for LH, respectively.

Statistical analysis: Emergence of follicles was defined by the day when the follicle diameter exceeded 5 mm. The emergence of OF after CIDR insertion, maximum diameter of OF and interval to ovulation after CIDR removal were compared between the groups. In Group A, two cows ovulated both during CIDR insertion and after CIDR removal, one cow (#2234) ovulated after CIDR removal without intermediate ovulation and the other cow (#2159) ovulated immediately but not after CIDR removal. Therefore, cows in Group A were sub-grouped according to the ovulatory stage during CIDR insertion and after CIDR removal. All data was reported as the mean ± sd, and the respective mean value was analyzed by student’s t-test. Mean plasma concentrations of P4, E2 and LH during CIDR insertion were divided into two stages, Stage 1 ranged from d 0 p.m. to d 2 p.m. and Stage 2 from d 3 a.m. to d 7 a.m. The interval to peak concentrations of E2 and LH after CIDR removal was compared between groups, and analyzed by student’s t-test. Fluctuations in plasma LH was determined to be pulses if: (1) the values were at least 40% above the LH concentration immediately preceding the rise and (2) they were followed by at least two successive values that were declining or represented basal levels [19]. Pulse amplitude was determined by subtracting basal values from the highest value associated with the pulse. Basal LH concentrations were calculated from values not associated with either the ascending or descending portions of a pulse. LH pulse frequency was characterized by the number of pulses in 8 hr. Results were subjected to analysis of variance for repeated measures. For LH pulse frequency during the intensive bleeding on d -1, d 5 and d 10, data was first normalized by square root transformation in order to achieve homogeneity of variance. One factor ANOVA was performed on the transformed data followed by Dunn’s procedure in a multiple comparison procedure. A value of P<0.05 was considered to be significant.

RESULTS

Characteristics of OF and the interval to ovulation after CIDR removal were tabulated in Table 1. All cows showed standing estrus in 60.0 ± 12.0 hr and 75.0 ± 15.1 hr after CIDR removal in Group A and B, respectively. Two of four cows in Group A ovulated both after GnRH injection during CIDR insertion and after CIDR removal (Fig. 1.a). Both of cows showed ovulation of the first wave DF in 42 hr after GnRH injection, and replaceable DF emerged on d 3 or d 4,
became OF after CIDR removal. Intermediate ovulation was not induced in a cow (#2234) after GnRH injection in Group A, where DF with 5.7 mm in diameter on d 0 developed to be 21.1 mm in diameter on d 5 p.m. after GnRH treatment, but soon regressed thereafter (Fig. 1. b). Then another DF emerged at 144 hr after CIDR insertion and ovulated at 90 hr after CIDR removal with a maximum diameter of 13.3 mm. Another cow (#2159) showed intermediate ovulation at 18 hr after GnRH injection (Fig. 1. c). This cow showed estrus at 60 hr after CIDR removal, but did not ovulate. Data are characterized with mean ± sd.

OF: ovulatory follicle after CIDR removal.

Table 1. Characteristics of ovulatory follicle and interval to ovulation after CIDR removal determined by the treatment with GnRH injection (Group A) or without (Group B) at CIDR insertion. In Group A, cows were sub-grouped according to the ovulatory state during CIDR insertion and after CIDR removal

<table>
<thead>
<tr>
<th>Characteristics of OF</th>
<th>Group A-a (n=2)</th>
<th>Group A-b (n=1)</th>
<th>Group A-c (n=1)</th>
<th>Group B (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval to estrus after CIDR removal (hr)</td>
<td>54.0 ± 8.5</td>
<td>72</td>
<td>60</td>
<td>75.0 ± 15.1</td>
</tr>
<tr>
<td>Maximum diameter of OF (mm)</td>
<td>14.5 ± 1.3</td>
<td>13.3</td>
<td>–</td>
<td>13.4 ± 0.6</td>
</tr>
<tr>
<td>Interval to OF emergence after CIDR insertion (hr)</td>
<td>96.0 ± 0.0</td>
<td>144</td>
<td>–</td>
<td>84.0 ± 82.0</td>
</tr>
<tr>
<td>Interval to ovulation after CIDR removal (hr)</td>
<td>72.0 ± 0.0</td>
<td>84</td>
<td>–</td>
<td>93.0 ± 20.5</td>
</tr>
<tr>
<td>Interval to ovulation after OF emergence (hr)</td>
<td>168.0±0.0</td>
<td>132</td>
<td>–</td>
<td>201.0 ± 76.8</td>
</tr>
</tbody>
</table>

Group A-a: Two cows showed ovulations both intermediately during CIDR insertion and after CIDR removal. Group A-b: One cow (#2234) ovulated after CIDR removal without intermediate ovulation. Group A-c: One cow (#2159) ovulated during CIDR insertion, but not ovulated after CIDR removal. Group B: All four cows ovulated after CIDR removal, but not intermediate ovulation. Data are characterized with mean ± sd.
As for the P₄ concentrations in Stage 1; from d 0 p.m. to d 2 p.m., levels were similar between the two groups (Table 2). However, P₄ concentrations in Stage 2 were tended to be higher in three intermediate ovulatory cows (2 cows in Group A-a and a cow in Group A-c, Table 2) than those in Group B (Group A: 156.0 ± 20.8 hr, Group B: 201.0 ± 76.8 hr).

As for the E₂ concentrations in Stage 1; from d 0 p.m. to d 2 p.m., levels were similar between the two groups (Table 2). However, P₄ concentrations in Stage 2 were tended to be higher in three intermediate ovulatory cows (2 cows in Group A-a and a cow in Group A-c, Table 2) than those in Group B (Group A: 8.4 ± 1.6 ng/ml, Group B: 4.1 ± 1.2 ng/ml). In addition within intermediate ovulatory cows in Group A, there was a significant difference in P₄ concentrations between stages (Stage 1: 5.6 ± 2.6 ng/ml, Stage 2: 8.4 ± 1.6 ng/ml, P<0.05). There was neither difference in E₂ concentrations nor LH concentrations between two groups. However, again within intermediate ovulatory cows in Group A, E₂ concentrations were significantly higher in Stage 1 than those in Stage 2 (5.1 ± 4.2 pg/ml, 2.8 ± 2.2 pg/ml, P<0.05).

There was no change in the interval from CIDR removal to E₂ peak between two groups (Table 3). The interval after CIDR removal to LH peak in three ovulatory cows in Group A (two cows in Group A-a, and one cow in Group A-b, Table 3) was shorter than those in Group B (Group A: 60.0 ± 12.0 hr, Group B: 72.0 ± 13.9 hr). There was neither difference in LH peak nor the interval from LH peak to ovula-

| Table 2. Plasma concentrations of progesterone (P₄), estradiol-17β (E₂) and LH characterized in two stages during CIDR insertion. In Group A, cows were sub-grouped according to the ovulatory state |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Group A-a | Group A-b | Group A-c | Group B          |
| P₄ concentrations in Stage 1 (ng/ml) | 6.0 ± 3.0  | 5.3 ± 0.8  | 4.5 ± 1.0  | 4.9 ± 1.2        |
| E₂ concentrations in Stage 1 (ng/ml) | 8.7 ± 1.6  | 4.5 ± 1.1  | 7.3 ± 1.0  | 4.1 ± 1.2        |
| LH concentrations in Stage 1 (ng/ml) | 4.8 ± 3.2  | 1.2 ± 0.8  | 6.1 ± 3.8  | 3.5 ± 2.5        |
| P₄ concentrations in Stage 2 (ng/ml) | 2.5 ± 1.7  | 1.6 ± 1.4  | 3.2 ± 2.4  | 2.5 ± 1.8        |
| E₂ concentrations in Stage 2 (ng/ml) | 1.6 ± 1.0  | 2.1 ± 0.8  | 1.7 ± 0.9  | 2.2 ± 0.5        |
| LH concentrations in Stage 2 (ng/ml) | 1.6 ± 1.5  | 2.0 ± 1.1  | 1.9 ± 0.8  | 2.3 ± 0.7        |

Data are characterized with mean ± sd.
Stage 1: averages through d 0 p.m. to d 2 p.m.
Stage 2: averages through d 3 a.m. to d 7 a.m.
a) Significant differences in concentrations of P₄ and E₂ were noted between Stage 1 and Stage 2 within intermediate ovulatory cows in Group A (2 cows in Group A-a and a cow in Group A-c, P<0.05).

| Table 3. Peak concentrations of estradiol-17β (E₂) and LH, and the interval to ovulation in seven days after CIDR removal. In Group A, cows were sub-grouped according to the ovulatory state |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Group A-a | Group A-b | Group A-c | Group B          |
| E₂ peak concentrations (ng/ml) | 7.2 ± 1.9  | 14.7 ± 6.5  | 13.1 ± 2.4  | 9.9 ± 4.2        |
| Interval to E₂ peak after CIDR removal (hr) | 54.0 ± 8.5  | 60 ± 7.1  | 72 ± 10.6  | 54.0 ± 6.9        |
| LH peak concentrations (ng/ml) | 12.4 ± 6.7  | 7.1 ± 1.0  | 84 ± 15.1  | 15.1 ± 6.5        |
| Interval to LH peak after CIDR removal (hr) | 54.0 ± 8.5  | 72 ± 1.0  | 84 ± 15.1  | 15.1 ± 6.5        |
| Interval to ovulation after LH peak (hr) | 18.0 ± 8.5  | 12 ± 3.8  | - ± 7.3  | 27.0 ± 22.7        |

Data are characterized with mean ± sd.
–: One cow (#2159) in Group A-c did not ovulate after CIDR removal and therefore no data on ovulatory event.
tion between two groups.

During the intensive samplings, mean LH concentration in Group A (4 cows) was significantly higher on d 10 compared to those on d -1 or d 5 (d 10: 6.8 ± 2.4 ng/ml, d -1: 2.5 ± 0.5 ng/ml, d 5: 2.4 ± 0.4 ng/ml, P<0.01), whereas no difference was noted in Group B (d 10: 4.5 ± 1.7 ng/ml, d -1: 2.1 ± 0.5 ng/ml, d 5: 3.1 ± 1.7 ng/ml). There was an increase in LH pulse frequency on d 10 compared to those on d -1 or d 5 in both groups (Fig. 2). In addition, LH pulse frequency on d 10 in Group A tended to be higher than that in Group B.

**DISCUSSION**

In the present study, 8 cows were allocated into two groups; one with (Group A) and the other without (Group B) GnRH injection at CIDR insertion. In Group A, three of four cows showed ovulation at 34 hr after GnRH injection, while one cow (#2234) failed ovulation. When CIDR was inserted at seven days after estrus, DF in three ovulatory cows was at the growing phase; whereas DF of #2234 (unovulatory cow) was already at the regressing phase. It has been reported that cows treated with GnRH at CIDR insertion ovulated only 8 out of 16 cows in 1.5 ± 0.3 d, and succeeding follicular wave soon emerged [17]. Martinez et al. [16] reported that if GnRH was injected to the DF at the later growing stage or early static stage in the ovary, ovulation usually occurs. However, if GnRH was given to the DF at the regressing stage, ovulation is the least likely to occur. In the similar reasons, DF of #2234 was not responded to the LH surge induced by GnRH treatment.

In the present study, every cow showed estrus in two to four days after CIDR removal and ovulation was induced except one cow (#2159) in Group A. In this cow, an intermediate ovulation of DF (13 mm in diameter) at 18 hr after CIDR insertion was induced by GnRH treatment, while a coexisting larger sized follicle (18.0 mm in diameter) remained through the entire experiment. It has been reported that persisted follicular cyst suppresses both new follicular wave and ovulations [24]. In the present study, such large follicle may have acted as a cystic follicular pattern, and suppressed further ovulation of newly emerged follicle (10.6 mm in diameter) after CIDR removal.

As for the characteristics of OF in Group A and B, neither difference was found in the maximum diameter before ovulation nor the interval to OF emergence. However, the interval to ovulation after OF emergence tended to be shorter in three ovulatory cows in Group A compared to Group B (156.0 hr vs. 201.0 hr). A study by Xu et al. [25] using post-partum dairy cows revealed similar results where the interval from OF emergence to ovulation was shorter in cows with GnRH treatment at CIDR insertion compared to those without (9.0 d vs. 10.3 d). They also found that the diameter of OF was slightly enlarged (19.3 mm vs. 18.0 mm). Rivera et al. [20] using 8 days of CIDR insertion in postpartum beef cows reported that ovulation was confirmed at 8.8 ± 0.4 d after the emergence of OF, which is similarly to the present result in Group B; CIDR insertion alone (201.0 hr).

Plasma P₄ concentrations in Stage 2 from d 3 a.m. to d 7 a.m. during CIDR insertion in three intermediate ovulatory cows in Group A were higher than those in Group B. A similar increase in P₄ concentrations was reported four days after GnRH treatment at CIDR insertion compared to those without GnRH treatment in dairy cows [25]. These data suggested that higher P₄ concentration during Stage 2 in Group A was attributed to the induced CL by GnRH treatment. Plasma E₂ concentrations in Stage 1 from d 0 p.m. to d 2 p.m. were significantly higher than those in Stage 2 in cows with intermediate ovulation in Group A, whereas no difference was found in cows without intermediate ovulation in Group B. Cow # 2234 without intermediate ovulation in Group A showed lower mean concentrations of E₂ during Stage 1 and Stage 2 (1.2 ± 0.8 pg/ml, 1.6 ± 1.4 pg/ml, respectively). If DF grows more than 7 mm in diameter, estradiol production from DF increases according to the size of follicle [14]. We have supposed that elevated E₂ in Stage 1 was secreted from pre ovulatory DF, and then lower E₂ in Stage 2 was indicated as disappearance of DF in Group A.

There was no difference in peak concentrations of E₂ and LH between the two groups. However, the interval to peak LH concentrations after CIDR removal tended to be shortened in three ovulatory cows in Group A compared to those in Group B (60.0 hr vs. 72.0 hr). Cavalieri et al. [5] reported that E₂ peak occurred 1.5 ± 5.8 hr after LH surge, and the interval from LH surge to ovulation was 24.4 ± 1.0 hr in cows after 10 days of ear implantation (3 mg of progesterone) with PGF₂α injection at removal. In another study [10], the interval from LH surge to ovulation was 29.9 ± 3.9 hr after 12 days CIDR insertion with CIDIROL (capsule containing 10 mg EB, InterAg, NZ). GnRH injection induced rather synchronized DF emergence during CIDR insertion, and these may have contributed to the shortened intervals to LH peak from CIDR removal and also interval to the following ovulation in Group A. However, LH surge or exact concentrations of E₂ peak was not specified with 12 hr interval of blood sampling in the present study, therefore more frequent samplings would be necessary to determine the exact time in peak concentrations of E₂ and LH.

Mean concentrations of LH during intensive blood sampling for 8 hr significantly increased on d 10 compared to those on d -1 or d 5 in Group A. Significant increase in the number of LH pulses for 8 hr was noted on d 10 when compared with those on d -1 or d 5 in both groups. In addition, LH pulses on d 10 in Group A tended to be higher, compared to those in Group B. These suggest that the higher frequency of LH pulses on d 10 in Group A with GnRH treatment at CIDR insertion contributed to the synchronized short intervals to LH surge after CIDR removal. Thus, the synchronized LH surge favored the narrow range of ovulation time compared to those in the treatment of CIDR insertion alone.

In conclusion, GnRH treatment at CIDR insertion at 7 days after estrus induced intermediate ovulation followed formation of CL during CIDR insertion, and these increased plasma progesterone concentrations and synchronized
emergence of new follicular wave. Furthermore, cows treated with GnRH injection at CIDR insertion showed the narrow range of ovulation time compared to those in CIDR insertion alone.

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