Estrus Synchronization Using CIDR® in Heifers

Rodolfo B. VARGAS, Yutaka FUKUI, Akio MIYAMOTO and Yoshinori TERAWAKI
Laboratory of Animal Genetics and Reproduction, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

Abstract. The efficacy of the use of controlled internal drug releasing (CIDR®) device containing 1.9g progesterone in inducing estrus synchronization of heifers in Japan was first attested. Eighty-eight Holstein heifers were randomly designated for the 7, 12, and 14 days of CIDR® insertion periods. The effects of CIDR® with and without estradiol-benzoate (E2) and prostaglandin F2α (PGF2α) at 7, 12 and 14 days of insertion periods were evaluated and compared in terms of progesterone concentrations during the 1st and 2nd day of insertion periods, at CIDR® removal and at the AI time; retention rates, estrus incidence, and pregnancy rates.

An overall CIDR® device retention rate of 85.2% (75/88), estrus incidence of 90.7% (68/75) and pregnancy rate of 63.3% (38/60) were recorded. Specifically, estrus incidence and pregnancy rates in both 7 days (86.8 and 60.7%) and 12 days (93.3 and 61.5%) insertion groups showed encouraging results. No significant difference was found between treatment groups. However, the 14 days insertion period without the use of additional hormones showed the highest pregnancy rate of 83.3% (5/6). An E2 capsule implanted at insertion and PGF2α injected at CIDR® removal showed no effect on pregnancy rate, but the incidence of ‘standing’ estrus increased significantly (p<0.05) with PGF2α treatment.

Key words: CIDR®, Heifers, Synchronization, E2 and PGF2α.

The use of controlled internal drug releasing (CIDR®) device for synchronization and superovulation treatments in cattle was first introduced 10 years ago by Ruakura researchers in New Zealand [1]. A CIDR® device containing 1.9g progesterone has been effective in synchronization of the estrous cycle in dairy cattle [1–7], and has been successfully used in dairy heifers for minimized gestation intervals [1]. Also, CIDR® treatment without supplementary hormones has been reported capable of inducing acceptable synchrony and fertility in naturally-mated suckling beef cows and yearling heifers [1–6]. In Japan, to date, there are no available data on the use and effectiveness of CIDR® device in inducing synchronization of estrus in dairy heifers. Therefore, the present study was conducted to evaluate the efficacy of CIDR® device either used alone or with supplementary hormones such as estradiol-benzoate (E2) and prostaglandin F2α(PGF2α), as an aid to reproductive management of Holstein heifers in Japan.

Materials and Methods

Eighty-eight Holstein heifers aged 17.1±0.3 months old (mean±SEM) were used. The study was conducted from January 2nd to 22nd 1992 at Tawa Field Station, Hokkaido, Japan.

A CIDR® device (Eazi-breed, type B: The Carter...
Holt Harvey Plastic Products Ltd., New Zealand) containing 1.9g progesterone was inserted into the vagina of the heifers at unknown stages of the estrous cycle. In Fig. 1, the schedule of the different treatment groups are summarized. Groups I-IV (39 heads), groups V-VIII (39 heads) and group IX (10 heads) had 7, 12 and 14 days insertion periods, respectively. No additional hormones were used in groups I, V and IX. In groups II and VI, a capsule containing 10 mg E₂ was clipped on the CIDR(R) device at the time of insertion, and in groups III and VII, an intramuscular injection of 250 µg PGF₂α (Estrumate, Sumitomo Pharmaceutical Co., Japan) was administered at CIDR(R) removal. For groups IV and VIII, both E₂ capsule and PGF₂α were administered. A factorial design of 2×2×2+1 was employed in the study.

Blood was collected from the caudal vein of five selected heifers per group using 10 ml heparinized vacutainers. Blood was obtained four times; at the time of CIDR(R) insertion (Day 0), two days after the insertion, upon withdrawal of the CIDR(R) device and on the day of artificial insemination (AI). Immediately after blood collection, plasma was separated by centrifugation at 1600 g for 10 minutes and stored at −20°C until used. Progesterone (P₄) level in plasma was assayed after ether extraction (recovery rate: 85%) using a second antibody following the enzyme-immunoassay (EIA) method [12]. The intra and inter assay coefficient of variation was below 15%.

After the removal of the CIDR(R) device, estrus was observed at 0700, 1200, and 1600 h, one h each time for 4 days. Subsequently, AI was performed immediately after thawing 0.5 ml of frozen semen straws by the routine schedule (rectal palpation prior to AI) at the station. Pregnancy was determined by rectal palpation 60–90 days after insemination. Heifers that failed to conceive following the AI at the synchronized estrus, were given the 2nd or 3rd insemination. Data from the different insertion periods and of the additional hormones (E₂ and PGF₂α) in relation to estrus incidence including ‘standing’ behaviour and pregnancy rate were analyzed by general linear models and the chi-square method (p<0.05). Plasma of heifers lost CIDR(R) and did not show estrus were not included in the hormonal analysis.

Results

Results of the nine treatments using CIDR(R) device are shown in Table 1. A total of 13 CIDR(R) device was lost, showing a retention rate of 85.2% (75/88). The 14 days insertion period had the highest percentage of loss (30%: 3/10) followed by the 12 days insertion period (23.1%: 9/39), and both are significantly (p<0.05) different from that of the 7 days insertion period (2.6%: 1/39).

Sixty eight out of 75 heads (90.7%) showed estrus within 4 days after the removal of CIDR(R). The maximum rate of estrus incidence (100%: 7/7) was obtained at 14 days insertion duration, followed by the 12 days insertion period (93.3%: 28/30) and the 7 days (86.8%: 33/38) of insertion period. There was no significant difference in the rates of estrus incidence among the three insertion periods. The mean (±SEM) interval times between the removal of CIDR(R) and estrus was 63.2% (43/68). The 14 days insertion group had a
significantly (p<0.05) low rate of 'standing' estrus (49.2%; 3/7) as compared with the 12 days (60.7%; 17/28) and 7 days (69.7%; 23/33) of insertion periods. The mean proportion of 'standing' estrus for CIDR® + E2 (groups II and VI; 47.9%) was significantly (p<0.05) less compared to the treatments with CIDR® only (groups I, V and IX; 52.4%), CIDR® + PGF2α (groups III and VII; 76.4%), and the CIDR® + E2 + PGF2α (groups VI and VIII; 74.6%) (Table 2).

P4 concentrations measured in plasma obtained at the times of insertion and removal of CIDR® (Table 3) showed no significant difference between treatment groups (I-IX) and between the days of insertion periods (7, 12 and 14). However, two days after CIDR® insertion, P4 concentrations significantly varied among groups. Also, at the time of AI, P4 concentration of group II (7 days) was significantly (P<0.05) higher as compared to all other groups. In groups I and IX, one heifer each had high P4 concentrations (4.2 and 4.3 ng/ml, respectively) at CIDR® removal but they were confirmed to be pregnant. Whereas, one heifer each in groups I, IV, VII and VIII, with low P4 concentra-

---

**Table 1.** Results of estrus synchronization using CIDR® with (+) or without (-) E2 and PGF2α in Holstein heifers

<table>
<thead>
<tr>
<th>CIDR® insertion periods/groups</th>
<th>Additional hormones</th>
<th>No. heifers treated</th>
<th>CIDR® retention No. (%)</th>
<th>estrus incidence (Interval times)</th>
<th>'standing' estrus</th>
<th>inseminated</th>
<th>pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days I</td>
<td>- -</td>
<td>10</td>
<td>10</td>
<td>7 (48.0±5.0)</td>
<td>4</td>
<td>5</td>
<td>2 (40.0)</td>
</tr>
<tr>
<td>II</td>
<td>+ -</td>
<td>10</td>
<td>9</td>
<td>8 (52.0±3.6)</td>
<td>5</td>
<td>8</td>
<td>4 (50.0)</td>
</tr>
<tr>
<td>III</td>
<td>- +</td>
<td>9</td>
<td>9</td>
<td>9 (54.3±4.3)</td>
<td>7</td>
<td>7</td>
<td>5 (71.4)</td>
</tr>
<tr>
<td>IV</td>
<td>+ +</td>
<td>10</td>
<td>10</td>
<td>9 (48.9±4.2)</td>
<td>7</td>
<td>8</td>
<td>6 (75.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39</td>
<td>38</td>
<td>33 (50.8±5.0)</td>
<td>23</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(97.4)a</td>
<td>(69.7)a</td>
<td>(60.7)a</td>
<td></td>
</tr>
<tr>
<td>12 days V</td>
<td>- -</td>
<td>9</td>
<td>7</td>
<td>7 (47.0±4.9)</td>
<td>4</td>
<td>7</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>VI</td>
<td>+ -</td>
<td>10</td>
<td>7</td>
<td>6 (51.8±7.0)</td>
<td>2</td>
<td>6</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td>VII</td>
<td>- +</td>
<td>10</td>
<td>8</td>
<td>8 (46.3±7.2)</td>
<td>6</td>
<td>7</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>10</td>
<td>8</td>
<td>7 (48.5±5.7)</td>
<td>5</td>
<td>6</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39</td>
<td>30</td>
<td>28 (48.5±6.2)</td>
<td>17</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(76.9)b</td>
<td>(93.3)</td>
<td>(60.7)b</td>
<td></td>
</tr>
<tr>
<td>14 days IX</td>
<td>- -</td>
<td>10</td>
<td>7</td>
<td>7 (51.0±3.1)</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(70)b</td>
<td>(100)</td>
<td>(42.9)b</td>
<td>(83.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>75</td>
<td>68</td>
<td>43</td>
<td>60</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(85.2)</td>
<td>(90.7)</td>
<td>(63.2)</td>
<td>(63.3)</td>
</tr>
</tbody>
</table>

* Average number of hours (mean±SEM) between the removal of CIDR® and the onset of estrus (p>0.05). a, b: The same superscripts in each column show no significant different (p>0.05).

**Table 2.** Results of estrus synchronization by CIDR® combined with additional hormones (Pooled data)

<table>
<thead>
<tr>
<th>Treatment groups*</th>
<th>Number of heifers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>treated</td>
</tr>
<tr>
<td></td>
<td>retained CIDR®</td>
</tr>
<tr>
<td></td>
<td>estrus incidence</td>
</tr>
<tr>
<td></td>
<td>'standing' estrus</td>
</tr>
<tr>
<td></td>
<td>pregnant</td>
</tr>
<tr>
<td>A</td>
<td>29</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>19</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
</tr>
</tbody>
</table>

* A: only CIDR® groups I, V, and IX; B: Estradiol-benzoate (E2) groups II and VI; C: Prostaglandin F2α (PGF2α) groups III and VII; D: E2 and PGF2α groups IV and VIII. #Mean (±SEM) between the removal of CIDR® and the onset of estrus. *: b: The same superscripts in each column are not significantly different (p>0.05).
tions (0.1–1.1 ng/ml) was inseminated at detected estrus but not conceived. Likewise, 3 heifers each in groups III and V with low P4 concentrations (0.2–1.1 ng/ml) were inseminated at detected estrus but not conceived.

Overall, 38 out of 60 inseminated heifers (63.3%) conceived by the first insemination at the synchronized estrus. The mean pregnancy rates in all the treatments and individual groups were not significantly different, although pregnancy rate was highest in the 14 days insertion period (83.3%: 5/6).

Discussion

Previous reports have demonstrated a 92–99.5% of CIDR® retention rates in Holstein cattle [1–7] with an overall average of 98% [3]. In the present study, the 7 days insertion period showed the highest retention rate of 97.4% (38/39). This contradicts the high retention rates occurring in more than 12 days of insertion periods in parous cows as reported by Macmillan et al. [3–5]. We surmise that the difference in our findings could have been influenced by the small vagina in heifers as reported by Macmillan [3].

Tjondronegoro et al. [7] specifically demonstrated that when a progesterone releasing intravaginal device (PRID) was inserted at random during the estrous cycle, the concentration of P4 varies at the time of removal of the device causing variation in the timing of estrus and ovulation. Our findings collaborate to their conclusion that the stage of estrous cycle at the time of insertion is an important factor influencing the interval from withdrawal of progesterone treatment to ovulation. As shown in Table 3, at CIDR® removal, P4 concentration values were not significantly different between all groups. At the time of AI, the plasma P4 concentration levels had declined to less than 1 ng/ml except groups I, II, and IX. One heifer each with high P4 level in groups I and IX, and 2 heifers in group II likely affected to the increase in P4 concentrations. As the administration of CIDR®s were performed at the unknown stage of the estrous cycle of heifers in the present study, exogenous progesterone were a reflection of cyclical variation rather than a treatment effect [5] which may affect P4 to ascend. On the other hand, low levels of (less than 1.1 ng/ml) P4 have been recorded in one heifer each for groups I, IV, VII and VIII, and 3 heifers in groups III and V, at the time of AI, but they were not conceived. Munro and Moore [15] described factors which influenced calving rates must have exerted their effects on fertilization or during the early stages of pregnancy.

Pregnancy rates in all groups were not significantly different. The 14 days of CIDR® insertion had a significantly low percentage of ‘standing’ estrus as compared to those in 7 and 12 days of insertion periods. Without the use of PGF₂α, we found a significantly (P<0.05) lower rate of ‘standing’ estrus as compared to that with PGF₂α.

Table 3. Plasma progesterone levels in heifers treated with CIDR® with (+) or without (-) E2 and PGF₂α

<table>
<thead>
<tr>
<th>CIDR® insertion periods/groups</th>
<th>Number of animals examined</th>
<th>Additional hormones</th>
<th>Plasma progesterone levels (ng/ml: mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E₂</td>
<td>1st*</td>
</tr>
<tr>
<td>7 days I</td>
<td>3</td>
<td>–</td>
<td>1.62±0.73*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>2.52±1.16*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>2.88±0.47*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>2.44±0.62*</td>
</tr>
<tr>
<td>12 days V</td>
<td>4</td>
<td>–</td>
<td>3.90±2.04*</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>2.16±0.85*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>4.93±0.73*</td>
</tr>
<tr>
<td>14 days IX</td>
<td>3</td>
<td>–</td>
<td>4.06±1.42*</td>
</tr>
</tbody>
</table>

* Represent blood collection on the 1st (Day 0) and 2nd day after CIDR® insertion, 3rd and 4th blood collection at the time of CIDR® removal and AI, respectively. a, b, c : The same superscripts in each column are not significantly different (p>0.05).
findings is consistent with the results of previous
studies [6] with the use of intravaginal device plus
PGF₂α in acyclic cows [13] and heifers [14] show-
ing a convincing estrus incidence. Fukui et al. [9]
compared the effect of regulating estrus and the
subsequent fertility of repeat-breeder and
anestrous Holstein heifers using PRID and found
that the use of PRID combined with PGF₂α was
more effective than the two injection regime of
PGF₂α, and the E₂ capsule may be unnecessary
when PGF₂α was administered one day before
PRID removal. Our findings have supported ear-
lier extensive works [1-11] showing that a satisfac-
tory synchrony and pregnancy rates can be ob-
tained by using intravaginal devices as a source of
exogenous progesterone for at least 14 days. Over-
all, the pregnancy rate of 63.3% (38/60) obtained
in the present study is similar to other reports rang-
ing from 50 to 82% using CIDR® in large-scale field
trials [1-7]. Our study did not show significant
difference in pregnancy rates between treatments
inspite of the lowest rate of ‘standing’ estrus inci-
dence in the 14 days of insertion period. Treat-
ment between 12 to 21 days of insertion period
without additional hormones has been suggested
by reports [1-5] to be financially beneficial to cattle
farmers. Results of our study reveal that an accept-
able rate of pregnancy ranging from 60.7% to 83.3%
(Table 1) can be achieved with or without hor-
monal treatment such as E₂ and PGF₂α at insertion
periods of 7 to 14 days. Nevertheless, an extensive
experimentation using a larger number of animals
is needed before the application of CIDR® device
either used alone or with hormones for estrus syn-
chronization in cattle.

Acknowledgments

The authors wish to express their thanks to the
Manager and Staff of Tawa Field Station, Sibecha-
cho for their assistance and the use of animals and
facilities. To Carter Holt Harvey Plastic Products
Ltd., of New Zealand and Mr. Y. Miyawaki, Surge
Miyawaki Co. Ltd., we thank for the supply of
CIDR® devices.

References

1. McMillan WH, Macmillan KL. CIDR®-B for man-
age reproduction in beef cows and heifers. Proc N

2. Macmillan KL, Taufa VK, Barnes DR, Day AM.
Oestrous detection in synchronized heifers. In:
Proc 11th Int Cong Anim Reprod AI; 1988; Abstract
4: 443.

3. Macmillan KL, Taufa VK, Day AM, Baraggins TJ.
Onset of oestrus and fertility in heifers synchro-
ised with progesterone from a CIDR®

4. Macmillan KL, Taufa VK, Barnes DR, Henry R.
Development of CIDR dispensers for oestrous con-
trol in dairy cattle. New Zealand Ministry of Agri-
culture and Fisheries Agricultural Research Divi-

5. Macmillan KL, Taufa VK, Barnes DR, Day AM,
Henry R. Detecting estrus in synchronized heifers
using tailpaint and aerosol raddle. Theriogenology

6. Broadbent PJ, Tregaskes LD, Dolman DF,
Franklin MF, Jones RL. Synchronization of estrus
in embryo transfer recipients after using a combi-
nation of PRID or CIDR®-B plus PGF₂α.

7. Tjondronegoro S, Williamson P, Sawyer GJ,
Atkinson S. Effects of progesterone intravaginal
devices on synchronization of estrus in postpar-

8. Aoyagi Y, Iwazumi Y, Wachi H, Fukui Y, Ono H,
Horie T. Application of Progesterone Releasing
Device (PRID) in Holstein cows. Jpn J Vet Assoc

9. Fukui Y, Kobayashi M, Tsubaki M, Kikuchi N,
Ono H. Regulating estrus and therapy of repeat-
breeder and anestrous Holstein heifers using pro-
gesterone releasing intravaginal devices (PRIDs).

10. Fukui Y, Mutoh K, Tsubaki M, Odagiri I, Masuto
Y, Ono H, Ygura H. The use of a progesterone re-
leasing intravaginal device (PRID) on synchroni-
zation of oestrus in Japanese Black Cattle. Jpn J

Evaluation of three oestrus synchronization regi-
mens for use in extensively managed Bos indicus
and Bos indicus / taurus heifers in Northern Aus-

12. Miyamoto A, Okuda K, Schweigerr FJ, Schams
D. The effect of basic fibroblast growth factor,
transforming growth factor- and nerve growth fac-
tor on the secretory function of the bovine corpus
