Comparison of Methods for the Synchronization of Estrous Cycles in Dairy Cows. 1. Effects on Plasma Progesterone and Manifestation of Estrus

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ABSTRACT

Dairy cows (n = 571) were treated with 1) one or two injections of prostaglandin F2α given 11 or 14 d apart, 2) two injections given 14 d apart and a progesterone coil inserted 8 d after the first injection for a 7-d period; or 3) a coil inserted for 7 d and prostaglandin injected 1 d before its removal.

Cows given two prostaglandin injections that conceived had higher progesterone concentrations during the luteal phase preceding AI than did cows that did not conceive. Cows with progesterone coils that conceived did not have higher progesterone concentrations than did cows that did not conceive. Older cows had lower progesterone concentrations than younger ones, and they appeared in estrus earlier after treatment.

In four daily observations, 75% of cows were seen in estrus within 7 d after treatment. Cows mounting two or more times had a conception rate (62%) similar to that of cows that stood to mount. Fewer cows (56%) treated with prostaglandin that had low concentrations of progesterone appeared in estrus than did cows with high progesterone (84%). Cows treated with prostaglandin differ from cows treated with progesterone coils in respect to manifestation of estrus and to the relationship between plasma progesterone and conception.

(Key words: estrous cycles, synchronization, progesterone)

INTRODUCTION

Many estrous periods in dairy cows are brief (8, 17), and at least three observation periods per day are required to detect most cows that manifest estrus. A schedule of three observations per day is inconvenient, time consuming, and therefore not often implemented. As a result, managing reproduction in dairy herds has been a long-standing problem. Approximately 50% of the estrous periods are not detected by the farmer (21, 26, 29), thus delaying conception and causing economic loss.

We have suggested (9) a system of reproductive management in which cows were observed for signs of estrus and inseminated during 6 d of each 3 wk. The synchronization method was inserting a progesterone-releasing intravaginal device (PRID) for 7 d and administering a prostaglandin F2α (PG) analogue 1 d before PRID removal. Cows inserted with PRID during d 1 to 10 of the estrous cycle had a conception rate of 69 rather than 42% in cows inserted with PRID during d 11 to 20 of the cycle. This result prompted the development of a new method for synchronization of estrus, which consisted of two PG injections given 14 d apart and a PRID inserted 8 d after the first injection for a period of 7 d.

The objective of the present study was to evaluate the potential of the new method for synchronization of estrus, which ensured that the PRID would be inserted in most cows during the first half of the estrous cycle. Another objective was to compare the reproductive performance of cows synchronized by the new method with that of cows synchronized by two PG injections given 14 d apart or by a PRID that was inserted at a random stage of the estrous cycle followed by a PG injection 1 d before PRID removal. In a preliminary experiment, the performance of cows synchronized by two PG injections given 14 d apart was com-
pared with that of cows treated with two PG injections at an 11-d interval. This paper presents the effects of these methods of synchronization on concentrations of plasma progesterone and manifestation of estrus, and a companion paper presents the effects of the three synchronization treatments on conception (10).

MATERIALS AND METHODS

Animals

Three experiments were performed on Israeli Holstein dairy cows. The cows were part of a 300-cow herd with an annual milk yield of approximately 9000 kg. Cows were kept in open sheds, bedded with straw or cotton gin trash.

During the dry period, cows were fed hay and silage to supply the maintenance and pregnancy requirements. During the last 2 wk of the dry period, cows also were fed approximately 3 kg of DM of the ration fed to lactating cows. After calving, cows were fed ad libitum a total mixed diet containing 70% concentrates and 30% hay and silage. Vitamins and minerals were added to the mixed diets in quantities recommended by the US NRC (20). The only modification was that 150,000 to 200,000 IU of vitamin A were fed per cow per day. Cows were allotted to experimental treatments according to parity, body condition score determined 6 to 7 wk after calving, and interval from calving. To avoid heat stress, experiments were conducted from November to June.

Management of Reproduction

The herd was under veterinary health care throughout the experiment. Approximately 30 d after calving, the reproductive tracts of all cows were examined, and the cows were treated for any disorders. To maximize milk and calf production (28), primiparous cows were bred 80 to 100 d after calving and multiparous cows 60 to 80 d after calving. The management of reproduction was based on a scheme of estrous synchronization (9). All cows were given an estrous synchronization treatment prior to the first postpartum insemination. Healthy cows that calved within a 3-wk period were treated simultaneously. Thus, a cluster of cows was synchronized once every 3 wk. The synchronization of cows that were under veterinary observation or treatment was postponed to subsequent clusters. Visual observations for signs of estrus and AI following synchronization treatment were conducted during 6 d out of each 3 wk. These 6 d are designated as the EDI (estrus detection and insemination) week.

Visual observation of cows for signs of estrus during the EDI week were carried out approximately 6 h; each observation lasted more than 30 min. Observations of animals were not carried out during feeding or milking. The number of mountings (head or tail), standings to mount, and escapes were recorded in each observation. Cows manifesting standing estrus and cows mounting other cows two or more times during a single observation session or once or more during two consecutive observation sessions were considered to be estrual. Cows that mounted only once during a single observation session were regarded as not being in estrus. All cows that were observed in estrus during the EDI week were inseminated 2 to 20 h after detection of estrus. In addition, some cows in Experiment 1 and all cows in Experiment 2 received two fixed-time inseminations 72 and 96 h following the last PG injection. The timing of AI in each experiment is described in the companion paper (10).

Experimental Design

Experiment 1. Primiparous cows (n = 87) were allotted to two groups. Cows in the first group were given two PG injections (25 mg per injection of Lutalyse, Upjohn, Belgium) 11 d apart; cows in the second group were given two PG injections 14 d apart.

Experiment 2. Cows (n = 368) were allotted into the following three groups: A) two PG injections given 14 d apart (designated as PG1/d/PG); B) two PG injections given 14 d apart and a PRID without the estradiol capsule (CEVA Laboratories, France; 1.55 g of progesterone) inserted 8 d after the first PG and removed 1 d after the second PG injection (designated as PG8-PRID2/PG6); C) a PRID inserted for 7 d and PG injected 1 d before PRID removal (designated as PRID7/PG6).

Experiment 3. Multiparous cows (n = 116) were allotted to two groups. Cows of the first group received a single PG injection; cows of
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The experiments were performed during 3 consecutive yr. In the first half of the 1st yr, Experiment 1 was carried out with primiparous cows. In the same year, the multiparous cows were allotted equally between the three treatment groups of Experiment 2. In the 2nd yr and the first half of the 3rd yr of the experiment, primiparous as well as multiparous cows were assigned equally to the three treatment groups of Experiment 2. The analysis of some of the results of primiparous cows given the PG analogue treatment included cows of both Experiments 1 and 2. Consequently, more primiparous cows were in the PG analogue group than in the other two treatment groups. Experiment 3 was conducted on multiparous cows during the second half of the 3rd yr.

Blood Collection and Progesterone Assay. Blood was collected between 0600 and 0700 h from the jugular vein into heparinized vacutainers. The vacutainers were placed on ice and centrifuged, and the plasma was stored at −18°C. Determination of progesterone concentration in plasma was carried out by radioimmunoassay as previously described (24).

In Experiment 1, blood was collected on d 0, 3, 7, 9, 10, 11, 14, and 17 after the first PG injection. In Experiments 2 and 3 blood was collected on d 0 and 4 to 5 after the first PG injection and on 4 d during the week preceding AI. As synchronization treatments were terminated on the same day, the time of blood collection in relation to the time of PG injection differed by 1 d between the PG analogue group and the PG+PRID groups. To compare treatments, plasma progesterone concentrations 8, 10, and 12 d after the first PG injection were calculated for each cow of the PG analogue group as an average of the values measured on the 2 adjoining days.

Statistical Analysis. Plasma progesterone concentrations were analyzed by one-way or factorial ANOVA in conjunction with Duncan’s multiple range test using general linear models procedure of SAS (25). The model included treatment (n = 3); parity (primiparous vs. multiparous); conception to first AI (n = 2), and the two- and three-way interactions. Differences in frequency distributions were evaluated by chi-square analysis. Procedure CATMOD of SAS (25) was used to analyze the fraction of cows manifesting estrus and the fraction of cows with different progesterone concentrations (n = 2 for each analysis). The model included treatment (n = 2), parity (n = 2), and the two-way interactions. The effects of treatment (n = 2), parity (n = 2), and progesterone concentration (≤3.0 ng/ml vs. ≥3.1 ng/ml) on the fraction of cows that manifested estrus within 78 h of PG injection were also analyzed by the CATMOD procedure. Pearson’s coefficients of correlation were determined wherever appropriate.

RESULTS

Plasma Progesterone Concentration

Cows given two PG injections 14 d apart had average plasma progesterone of ≥5 ng/ml for a longer period than cows given two PG injections 11 d apart (Table 1). On the day of the second injection, 80% of cows given two injections 14 d apart had plasma progesterone concentrations of >5.1 ng/ml compared with 53% of the cows given two PG injections 11 d apart (P <.001). Cows that subsequently conceived had higher (P <.03) plasma progesterone concentrations during d 9 to 11 following the first PG injection than did cows that did not conceive.

In Experiment 2, plasma progesterone concentrations on the day of PRID insertion were 2.1, 2.8, and 3.5 ng/ml in primiparous cows of the PG analogue group and the PG+PRID groups. To compare treatments, plasma progesterone concentrations 8, 10, and 12 d after the first PG injection were calculated for each cow of the PG analogue group as an average of the values measured on the 2 adjoining days.

In Experiment 2, plasma progesterone concentrations on the day of PRID insertion were 2.1, 2.8, and 3.5 ng/ml in primiparous cows of the PG analogue, PG analogue/PG analogue, and PG+PRID groups, respectively (P <.05). In multiparous cows, progesterone on those same days was 1.9, 2.3, and 3.3 ng/ml for the three treatments, respectively. During the 2 d after PRID insertion, plasma progesterone increased in primiparous and multiparous cows of the PG analogue/PG analogue treatment by 1.6 and 1.2 ng/ml, respectively. There was no significant difference between parities. In the PG analogue/PG analogue treatment, however, the increase in progesterone concentrations in primiparous cows was 3.0 ng/ml and was greater (P <.02) than in multiparous cows of the same treatment (Table 2). The increase in plasma progesterone did not differ between treatments in cows that conceived but was higher in primiparous (P <.05) and multiparous (P <.01) cows of the PG analogue/PG analogue treatment that did not conceive compared with the PG analogue/PG analogue group, which failed to conceive (Table 2).
TABLE 1. Plasma progesterone concentration (ng/ml) of primiparous cows following the first of two prostaglandin F2α (PG) injections given 11 or 14 d apart in Experiment 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cows</th>
<th>Days after the first PG injection</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hours</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PO1</td>
<td>24</td>
<td>4.3</td>
<td>1.9*</td>
<td>3.8</td>
<td>4.6</td>
<td>5.6</td>
<td>6.6</td>
<td>4**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PO14</td>
<td>20</td>
<td>4.7</td>
<td>.6</td>
<td>2.0</td>
<td>4.7</td>
<td>6.2</td>
<td>7.3</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conception</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conceived from first AI</td>
<td>29</td>
<td>4.8</td>
<td>1.5</td>
<td>3.6</td>
<td>5.4*</td>
<td>6.7**</td>
<td>7.8*</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Did not conceive from first AI</td>
<td>15</td>
<td>3.8</td>
<td>.9</td>
<td>2.0</td>
<td>3.3</td>
<td>4.3</td>
<td>5.2</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SEM</td>
<td>1.3</td>
<td>.5</td>
<td>.9</td>
<td>.9</td>
<td>.8</td>
<td>1.0</td>
<td>.6</td>
<td>.1</td>
</tr>
</tbody>
</table>

*Higher than the other group (P<.03).
**Different from the other group (P<.01).

The data in Figure 1 indicate that within 2 d after PRID insertion, plasma progesterone concentrations in the PG8-PRID7/PG6 and the PRID7/PG6 treatment groups plateaued at concentrations that were higher (P<.0001) than those of the PG14/PG group. During the following 4 d, until the second PG injection, plasma progesterone changed very little in cows of the PG8-PRID7/PG6 group and decreased slightly in cows of the PRID7/PG6 group. Unlike cows treated with PRID, plasma progesterone of cows given two PG injections did not reach a plateau and their plasma progesterone concentration continued to increase up to the day of the second PG injection (Figure 1). On the day of the last PG injection, the proportion of cows with plasma progesterone of ≥3.1 ng/ml was not higher in the groups treated with PRID than in cows synchronized with PG alone (Table 3).

Cows of the PG14/PG treatment that conceived following the first AI had higher (P<.01 and P<.05) progesterone concentrations 10 and

TABLE 2. Average increase in plasma progesterone concentration (ng/ml) during 2 d after the insertion of a progesterone-releasing intravaginal device (PRID) and in cows given two injections of prostaglandin F2α (PG) during the two corresponding days (Experiment 2).

<table>
<thead>
<tr>
<th>No. of cows</th>
<th>PG1/PG1</th>
<th>PG8-PRID7/PG6</th>
<th>PRID7/PG6</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conceived from first AI</td>
<td>1.8</td>
<td>2.9</td>
<td>2.5</td>
<td>.45</td>
<td>NS</td>
</tr>
<tr>
<td>Did not conceive</td>
<td>1.4</td>
<td>3.0</td>
<td>2.2</td>
<td>.44</td>
<td>.05</td>
</tr>
<tr>
<td>All cows</td>
<td>1.6</td>
<td>3.0</td>
<td>2.3</td>
<td>.31</td>
<td>.01</td>
</tr>
<tr>
<td>Multiparous cows</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conceived from first AI</td>
<td>1.5</td>
<td>1.8</td>
<td>1.6</td>
<td>.26</td>
<td>NS</td>
</tr>
<tr>
<td>Did not conceive</td>
<td>1.1</td>
<td>2.3</td>
<td>1.4</td>
<td>.26</td>
<td>.01</td>
</tr>
<tr>
<td>All cows</td>
<td>1.2</td>
<td>1.9</td>
<td>1.5</td>
<td>.18</td>
<td>.03</td>
</tr>
</tbody>
</table>

*a,bMeans within a row with different superscripts differ (P≤.05).

*Increase in plasma progesterone concentration in primiparous cows is higher (P≤.02) than that in multiparous cows.

1Two injections of prostaglandin F2α (PG) 14 d apart.
2Two injections of PG 14 d apart and a PRID inserted 8 d after the first PG for 7 d.
3PRID was inserted for 7 d and PG injected 1 d prior to PRID removal.
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12 d, respectively, following the first PG injection than cows that did not conceive. In the two other treatments, cows that subsequently conceived did not have higher progesterone (Figure 1). During the luteal phase primiparous cows had higher (P<.05) plasma progesterone than did multiparous cows in the PG14/PG and PG8-PRID7/PG6 treatments but not in the PRID7/PG6 treatment (Figure 1).

Parity affected the proportion of cows that had plasma progesterone concentrations of 3.1 ng/ml or more on the day of the last PG injection (Table 3). Only 45% of cows in their fourth and later lactations that were treated with a PRID had progesterone concentrations above 3.0 ng/ml compared with 84% of the primiparous cows (P<.001).

Manifestation of Estrus

Data in Table 3 indicate that in multiparous cows synchronized by two PG injections the manifestation of estrus was related to plasma progesterone concentrations on the day of the last PG injection. Only 56% of the cows synchronized with two PG injections, which had a plasma progesterone concentration of ≤3 ng/ml, manifested estrus following synchronization, compared with 84% among cows of the same treatment with plasma progesterone concentrations of ≥3.1 ng/ml (P<.03) and compared with 85% among multiparous cows of the PRID groups with plasma progesterone concentrations of ≤3.0 ng/ml (P<.02). In primiparous cows of the PG14/PG group and in the PRID-treated groups, plasma progesterone concentrations had no effect on the manifestation of estrus.

Data in Table 4 indicate that parity and treatment affected the interval to estrus and that older cows appeared in estrus earlier than younger ones (P<.02). The proportion of cows that manifested estrus within 78 h of the second PG injection was reduced (P<.001) in cows synchronized with PRIDs, which had plasma progesterone concentrations of ≥3.1 ng/ml, on the day of the last PG (P<.001). The correlation coefficients between plasma progesterone on the day of the second PG injection and the interval to estrus were .45, .48, and .46 for cows of the PG14/PG, PG8-PRID7/PG6, and PRID7/PG6 groups, respectively (P<.0001 in all cases).
TABLE 3. Effect of synchronization treatment and parity on plasma progesterone concentration on the day of the last prostaglandin F2α (PG) and manifestation of estrus in Experiments 1 and 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parity</th>
<th>% of Cows with Progesterone ≥3.1 ng/ml(a)</th>
<th>Manifested estrus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG1/PG1</td>
<td>1</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2+3</td>
<td>72</td>
<td>55*</td>
</tr>
<tr>
<td></td>
<td>≥4</td>
<td>67</td>
<td>60*</td>
</tr>
<tr>
<td>PG8-PRID7/PG52</td>
<td>1</td>
<td>84</td>
<td>80</td>
</tr>
<tr>
<td>and PRID7/PG63</td>
<td>2+3</td>
<td>67</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>≥4</td>
<td>45</td>
<td>88</td>
</tr>
<tr>
<td>*Different from cows of the same treatment-parity subclass with plasma progesterone ≥3.1 ng/ml (P&lt;.03) and from cows with the same progesterone concentrations in the PRID group (P&lt;.02).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1Two injections of prostaglandin F2α (PG) 14 d apart.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2Two injections of PG 14 d apart and a progesterone-releasing intravaginal device (PRID) inserted 8 d after the first PG for 7 d.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3PRID was inserted for 7 d and PG injected 1 d prior to PRID removal.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4ANOVA by the CATMOD procedure of SAS (25) revealed a parity effect (P&lt;.06) and a parity by treatment interaction (P&lt;.03).</td>
<td></td>
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</tr>
</tbody>
</table>

The effect of the different synchronization treatments on the time interval from the last PG injection to the onset of estrus is presented in Table 5. In Experiment 1, 75% of the cows given two PG injections 11 d apart and 79% of the cows given two injections 14 d apart were detected in estrus within 7 d following the second PG injection. In Experiment 3, 69% of the cows given the PG8-PRID7/PG6 treatment and 58% of the cows given a single PG injection were detected in estrus within 7 d following the PG injection.

TABLE 4. Effect of treatment and plasma progesterone concentration on the day of the last prostaglandin F2α (PG) injection on the percentage of cows manifesting estrus within 78 h of the last PG injection in Experiments 1 and 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parity</th>
<th>Progesterone ≤3.0 ng/ml 1</th>
<th>Progesterone &gt;3.1 ng/ml 1</th>
<th>Total 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of cows</td>
<td>Interval ≤78 h (%)</td>
<td>No. of cows</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG1/PG3</td>
<td>1</td>
<td>6</td>
<td>67 (%)</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>2+3</td>
<td>6</td>
<td>67 (%)</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>≥4</td>
<td>3</td>
<td>100 (%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>15</td>
<td>73 (%)</td>
<td>73</td>
</tr>
<tr>
<td>PG8-PRID7/PG64</td>
<td>1</td>
<td>8</td>
<td>50 (%)</td>
<td>42</td>
</tr>
<tr>
<td>and PRID7/PG65</td>
<td>2+3</td>
<td>24</td>
<td>75 (%)</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>≥4</td>
<td>15</td>
<td>80 (%)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>47</td>
<td>72 (%)</td>
<td>98</td>
</tr>
</tbody>
</table>

1ANOVA by the CATMOD procedure of SAS (25) revealed a progesterone concentration effect (P<.02). |
| 2ANOVA by the CATMOD procedure of SAS revealed a treatment and parity effect on the percentage of cows that appeared in estrus within ≤78 h (P<.02 for both factors). |
| 3Two injections of prostaglandin F2α (PG) 14 d apart. |
| 4Two injections of PG 14 d apart and a progesterone-releasing intravaginal device (PRID) inserted 8 d after the first PG for 7 d. |
| 5PRID was inserted for 7 d and PG injected 1 d prior to PRID removal. |

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In Experiment 1, cows given the PG11/PG treatment appeared in estrus earlier after the second PG injection than cows given the PG14/PG treatment. In Experiment 2, cows treated with PG only appeared in estrus earlier than cows treated with PRID and PG (Tables 4 and 5). However, in multiparous cows treated with PRID, the delay in appearance of estrus following PG administration was less than 24 h and shorter than that in primiparous cows (P<.003). Of 196 cows in estrus following one or two PG injections, 70.9% appeared in estrus within 48 h and of 232 cows in estrus following treatment with PRID and PG, 70.3% appeared in estrus during the same period.

In Experiment 2, during 7 d following the first postpartum synchronization treatment, 72.3% of the cows manifested standing estrus and 10.9% were detected displaying only mounting activity (Table 6). Three types of mounting activity could be discerned: cows that mounted twice or more during a single observation session, once or more during two consecutive sessions, or only once during a single session. Cows that displayed the first two types of mounting had a conception rate of 61.9%; cows that displayed only a single mounting had a conception rate of 26.3% (P<.025). Thus, cows with multiple mountings had a conception rate similar to that of cows that manifested standing estrus, whereas cows with a single mounting had a low conception rate. Therefore, cows with multiple mountings were concluded to have been in estrus, whereas cows with a single mounting were not regarded to have been in estrus.

### Table 6. Frequency distribution (%) of type of estrous behavior following synchronization and conception rate in Experiment 2.

<table>
<thead>
<tr>
<th>Type of estrous manifestation</th>
<th>No. of cows</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing estrus</td>
<td>266</td>
<td>72.3</td>
</tr>
<tr>
<td>Mounting twice or more</td>
<td>21</td>
<td>5.7</td>
</tr>
<tr>
<td>Mounting once</td>
<td>19</td>
<td>5.2</td>
</tr>
<tr>
<td>No estrous behavior</td>
<td>62</td>
<td>16.8</td>
</tr>
</tbody>
</table>

1. Mounting twice or more during a single observation session or once or more during two consecutive observation sessions.


**DISCUSSION**

Most of the cows in the PG₈-PRID₇/PG₆ treatment group received the PRID on d 4 to 6 of their estrous cycle, because the first PG injection had been administered 8 d before PRID insertion. As the device was applied without the estradiol capsule, no luteolysis was induced following PRID insertion. In contrast to the PG₈-PRID₇/PG₆ group, most of the cows in the PRID₇/PG₆ treatment group did not undergo luteolysis before PRID insertion and were at various stages of the luteal phase. Some of the cows in this treatment group received the PRID at the late luteal phase and underwent luteolysis during PRID treatment. The differences in the stage of the estrous cycle at the time of PRID insertion account for the higher plasma progesterone concentrations on that day, in cows of the PRID₇/PG₆ group compared with the two other treatments (Figure 1). The lower average plasma progesterone during the PRID treatment period in the PRID₇/PG₆ group, compared with the PG₄-PRID₇/PG₆ group, reflects the demise of the corpus luteum, which had occurred in some cows of the former group.

The difference in plasma progesterone preceding AI in the PG₁₄/PG and PG₁₄/PG groups, between cows that conceived and those that did not conceive (Table 1; Figure 1) is in agreement with previous studies carried out with untreated animals where progesterone was measured in plasma (5, 11, 12, 15, 24). Of three studies in which progesterone was measured in whole milk (3, 7, 19), a positive relationship between milk progesterone and conception was found in only one (19). Richards et al. (23) reported that a disparity between plasma and milk progesterone concentrations may exist and that progesterone concentrations in whole milk may depend on the content of milk fat in the sample, which in turn may depend on the timing and method of sampling (2, 22). The correlation between plasma progesterone concentrations and conception may be higher in PG-synchronized cows than in untreated cows because their luteal phase is shorter than that of normally cycling cows. If progesterone secretion during the cycle preceding AI has a cumulative effect on conception, then the length of the interval between the two PG injections might affect the conception rate of the synchronized cows. In addition, increasing the interval between the two PG injections from 11 to 14 d prolonged the luteal phase of the latter group and increased the proportion of cows that attained higher progesterone concentrations prior to the second PG injection. The possible reasons why cows of the PRID groups did not show a positive relationship between progesterone and conception are discussed in the companion paper (10).

In the PG₁₄/PG treatment group, endogenous plasma progesterone concentrations 8 and 10 d following the first PG injection did not differ significantly between primiparous and multiparous cows (Figure 1), indicating that progesterone concentrations on those days were not affected by parity. Following PRID insertion, the increase in plasma progesterone was higher in cows of the PG₈-PRID₇/PG₆ group than in cows of the PG₁₄/PG group. Therefore, a significant proportion of the additional progesterone was probably released by PRID. The greater increase in plasma progesterone in cows of the PG₈-PRID₇/PG₆ treatment that did not conceive than in cows that conceived (Table 2) may be related to the negative correlation between plasma progesterone and conception in this treatment (10).

The significantly greater increase in plasma progesterone in primiparous cows in comparison with multiparous cows during the 2 d following PRID insertion (Table 2) suggests that in primiparous cows the rate of clearance of progesterone is reduced compared with multiparous cows. The lower milk production of primiparous cows, and thus the lower clearance of progesterone in milk, can explain only a small part of this difference (14). In PRID-treated multiparous cows, the corpus luteum may secrete less progesterone than in primiparous cows due to the shorter interval from parturition to PG injection in multiparous cows or as a result of a feedback mechanism (1, 13, 16, 18). However, since primiparous and multiparous cows both received the first PG injection later than 46 d after parturition, the postpartum interval probably did not affect progesterone concentrations. Earlier work shows no difference in progesterone concentrations between consecutive estrous cycles starting later than 35 d postpartum (3, 6). Also, no evidence suggests that the sensitivity of the hypothalamic-hypophyseal axis to the negative
feedback of progesterone is affected by the interval from parturition or by age of the cows.

Carrick and Shelton (4) reported that the expression of estrus was more normal when ovariectomized cows were primed with progesterone before being challenged with estrogen. Another study showed that the efficiency of estrous expression was improved in cows treated with PRID+PG compared with cows treated with PG only (27). The present work indicates that only in the PG14/PG treatment group, the percentage of cows manifesting estrus was reduced in cows with low plasma progesterone concentrations (Table 3). However, in PRID-treated groups, estrous manifestation was high even in cows with low plasma progesterone concentrations. These results indicate again that in cows treated with PRID, conception rates are less related to plasma progesterone than in cows treated with PG only.

The higher percentage of cows of the PG14/PG treatment that appeared in estrus within 78 h following the PG injection (Table 4) is probably the result of the additional day that a source of progesterone was available in cows of the PRID treatments but not in cows of the PG14/PG group. The effect of parity on the interval from PG injection to estrus probably reflects the fact that a higher proportion of older cows had lower progesterone concentrations than younger cows (Table 3).

The distribution of the interval from the termination of synchronization treatment to the onset of estrus varied considerably between the three experiments (Table 5). The data in Table 5 show that if the cows had been inseminated only at one or two fixed times, the timing of AI would not have been optimal for about 30% of the cows that manifested estrus. On the other hand, the results in Table 6 indicate that 14.5% of the cows that were not detected in estrus, under a 4 times per day observation schedule, conceived following a fixed time insemination. Thus, fixed time AI may result in the conception of an additional 2.4% (.168 × .145) of all treated cows.

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