Effects of prostaglandin F₂α administration on the time of parturition and post-partum ovulation in rats

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Summary. The time relationship between delivery and post-partum ovulation in rats treated with PGF₂α was investigated.

PGF₂α, 1.0 mg/rat, was s.c. injected at 21:00 on Day 19 (PG 19 group) or 09:00 on Day 20 (PG 20 group) of pregnancy.

In the PGF₂α-treated groups, first delivery occurred about 30 h after the treatment. About 90% of the rats delivered within 48 h after the treatment. Some rats which delivered from 11:00 to 19:00 on Day 21 of pregnancy in the PG 19 group had ovulated within 12 h after the delivery. In rats which delivered from 20:00 on Day 21 to 10:00 on Day 22 of pregnancy in the PG 20 group, ovulation was not detected until 23:00 on Day 22 of pregnancy.

Serum gonadotrophin levels (LH and FSH) increased within 2 h after the delivery in the PG 19 group. In the PG 20 group, however, gonadotrophin levels did not change until 12:00 on Day 22 of pregnancy.

These results suggest that the timing of the "critical period" for the release of gonadotrophins for post-partum ovulation in the PGF₂α-treated rats is between 11:00 and 20:00. The "critical period" is similar to normally delivering rats and broader than that in cyclic rats.

Introduction

Although there are several reports concerning effects of prostaglandins (PG) on the induction of premature parturition in rats,1-4) time of the post-partum ovulation5-6) in the PG-treated animals is obscure.

In the present study, the time intervals from delivery to ovulation and gonadotrophin release after premature delivery with PGF₂α treatment were investigated to compare with those of intact delivering rats.

Materials and Methods

Animals: Wistar strain rats inbred in our laboratory were used. They were kept in an artificially illuminated (12 h light, 12 h dark; light on at 06:00) room with controlled temperature (25-26°C). Only rats which had shown at least 3 consecutive regular 4-day cycles before initiation of the experiment were used. Females were caged with fertile males during proestrus and mating was checked by examination for spermatozoa in vaginal smear on the following morning (Day 1 of pregnancy).

Treatment and sampling: One mg of PGF₂α (Ono Pharmaceutical Co. Ltd, Japan) was dissolved in 1 ml of ethanol-saline. One mg PGF₂α/rat was sc injected at 21:00 on Day 19 (PG 19 group) or 09:00 on Day 20 (PG 20 group) of pregnancy, based on the preliminary observations, in which this dose of PGF₂α, induced premature delivery effectively during a period from 30 to 48 h after the
Delivery was checked hourly from 30 h after the treatment. The time of birth of the last neonate was designated as the time of delivery (0 h). Data were presented every 3 h after delivery which began at 11:00 on Day 22 in intact rats (11:00 to 13:00, 14:00 to 16:00, 17:00 to 19:00, 20:00 to 22:00, 23:00 to 01:00 (next day), 02:00 to 04:00, 05:00 to 07:00 and 08:00 to 10:00).

The animals in each group were killed at various intervals according to the experimental design (Tables 1, 2, 3 and 4), and oviducts were removed. Ovulation was checked by counting tubal ova. Blood samples were collected each time by decapitation and centrifuged. Sera were stored below -20°C in a deep-freezer until assayed.

Assay of gonadotrophins: Gonadotrophin levels in serum were estimated by double antibody radioimmunoassay using the RIA kits provided from the National Institute of Health. The limit of sensitivity for each assay was 20 ng. NIAMD Rat LH-1-3 and FSH-1-3 were used for radioiodination. The LH and FSH values were expressed as ng NIAMD Rat LH-RP-1 and FSH-RP-1/ml serum.

Results

Effects of PGF2α on the time of delivery and ovulation: The distribution in the time of delivery was shown in Fig. 1. All intact pregnant rats (n=59) delivered on Day 22 to 23 of pregnancy. About 80% of the rats delivered between 11:00 and 19:00 on both days and the remainder delivered between 20:00 (Day 22) and 10:00 (Day 23).

In both PGF2α-treated groups, however, first delivery occurred about 30 h after the treatment; and in the PG 19 group, 112/125 rats delivered from 11:00 to 19:00 on Day 21 of pregnancy. In the PG 20 group, 45/59 rats delivered from 20:00 on Day 21 to 10:00 on Day 22 of pregnancy.

Ovulation rates within 12 h after delivery in the PG 19 group were significantly different from those of the intact delivered group (χ² test), but the mean number of eggs/ovulating...
Table 1. Comparison of the time interval from delivery to post-partum ovulation between intact and premature birth in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of delivery (hours in day)</th>
<th>11:00-19:00</th>
<th>20:00-10:00</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intact</td>
<td>PG 19</td>
<td>intact*</td>
</tr>
<tr>
<td>Time after delivery (h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4/5b (7.3±5.5)c</td>
<td>2/18* (8.5±2.2)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5/5 (12.2±1.8)</td>
<td>11/23 (12.9±5.3)</td>
<td>0/1</td>
</tr>
<tr>
<td>16</td>
<td>5/5 (15.8±1.3)</td>
<td>11/17 (14.5±3.0)</td>
<td>0/1</td>
</tr>
<tr>
<td>18</td>
<td>5/8 (14.6±2.3)</td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td>0/1</td>
</tr>
</tbody>
</table>

* This group consists of the rats which delivered from 20:00 to 22:00.

b Number of ovulating rats/number of examined rats.

c The mean (±sp) number of eggs ovulated/ovulating rat.

* P<0.05, Significantly different from those in intact.

PG 19 group; PGF2α was injected at 21:00 on Day 19 of pregnancy.
PG 20 group; PGF2α was injected at 09:00 on Day 20 of pregnancy.

Table 2. Serum LH and FSH level in late pregnancy and post-partum in intact rats

<table>
<thead>
<tr>
<th>Time of sampling</th>
<th>No. of rats</th>
<th>LH level (Mean±SEM), ng/ml</th>
<th>FSH level (Mean±SEM), ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 (09:00)</td>
<td>6</td>
<td>46.6±23.3**</td>
<td>450.0±76.5</td>
</tr>
<tr>
<td>18 (09:00)</td>
<td>6</td>
<td>44.1±27.9**</td>
<td>490.0±44.5</td>
</tr>
<tr>
<td>20 (09:00)</td>
<td>6</td>
<td>94.1±32.4**</td>
<td>392.0±62.7</td>
</tr>
<tr>
<td>22 (09:00)</td>
<td>7</td>
<td>218.5±19.3</td>
<td>345.7±12.4</td>
</tr>
<tr>
<td>Time after delivery (h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>310.0±77.0</td>
<td>505.0±90.8</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1907.5±369.0**</td>
<td>979.1±114.5**</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>2630.0±268.9**</td>
<td>1300.0±74.7**</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>1135.7±293.4**</td>
<td>841.4±90.7**</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>488.3±88.7*</td>
<td>909.1±72.4**</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>251.0±32.2</td>
<td>521.0±144.1</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>176.0±15.4</td>
<td>655.0±146.6</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>159.0±16.6*</td>
<td>795.0±79.9**</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>139.0±14.0*</td>
<td>488.0±75.1</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, Significantly different from those in Day 22 of pregnancy (Student's t test).

Only post-partum rats which delivered from 11:00 to 19:00 were used for hormones assay.

rat did not differ significantly.

In the PG 20 group the intervals from delivery to ovulation varied with delivering time in rats which delivered after 19:00. They were 22, 20, 18 and 16 h for the rats which delivered 23:00 to 01:00, 02:00 to 04:00, 05:00 to 07:00 and 08:00 to 10:00, respectively. When these data were arranged
according to the time of day, rats which ovulated at 23:00, 24:00 (Day 22), 01:00, 02:00 and 03:00 (Day 23) in the PG 20 group were 3/10, 6/10, 9/10, 8/8 and 2/2, respectively. The mean number of eggs (± sd)/ovulating rat were 5.7±4.0, 8.5±5.7, 14.9±2.4, 12.5±3.9 and 11.0±4.2, respectively.

Changes in serum LH and FSH levels: Results were shown in Tables 2, 3 and 4. In the intact delivering group, LH in serum remained at a low level throughout the period from Day 16 to 22 of pregnancy. The level increased significantly within 2 h to a peak 4 h after delivery (P<0.01), then declined to 16 h.

In the PG 19 group, LH level began to in-
crease within 2 h and rose to a peak at 6 h after delivery. The level returned to the basal level within 14 h. In the PG 20 group, no significant increase in serum LH level following delivery occurred until 12:00 (Day 22). The level recovered to the basal level by 02:00 (Day 23).

The FSH level in serum during late pregnancy in the intact delivery group was not markedly changed, but remained at relatively higher level than LH. FSH value started to increase within 2 h to a peak 4 h after delivery \((P<0.01)\). The level declined to 0 h level at 16 h.

In the PG 19 group, the FSH level reached maximum at 6 h after delivery, then decreased slightly to 14 h. In the PG 20 group, the level increased sharply at 14:00 \((P<0.05)\) to a peak at 16:00 on Day 22 of pregnancy \((P<0.01)\). It fell slightly at 18:00 and remained constant thereafter.

**Discussion**

In the present study, we have shown that PGF$_{2\alpha}$ effectively induced premature delivery within 48 h after the treatment, as reported by other researchers\(^5\)\(^-\)\(^4\) and the time interval from delivery to ovulation and the release of gonadotrophins following premature delivery was similar to that of rats which delivered normally.\(^7\)\(^-\)\(^9\)

These findings will be interesting in respect that the hour of delivery seems to be determined by biological rhythm which depends on photoperiodical conditions during gestation.\(^10\)\(^-\)\(^11\) The delivering hour may be sifted by PGF$_{2\alpha}$ treatment, but the time relationship between delivery and ovulation is similar to that of intact delivered rats. Photoperiod may effect on the onset of endocrine changes for delivery, especially by increasing of PGF$_{2\alpha}$ synthesis.\(^12\)

Until now, the time interval from delivery to the release of gonadotrophins for rats which delivered during dark period has not been clarified. In this study, rats which delivered from 23:00 to 01:00 following PGF$_{2\alpha}$ treatment did not ovulate for at least 21 h after the delivery and the release of gonadotrophins began 12:00 in the following morning. Some rats which delivered before 20:00 with PGF$_{2\alpha}$ administration ovulated within 12 h after delivery and the release of gonadotrophins began within 2 h after delivery. These results are similar to that of normally delivered rats.\(^7\)

The number of ovulated rats, which delivered from 11:00 to 19:00 by the PGF$_{2\alpha}$ treatment on Day 19, was smaller than that of intact delivered rats but the rate of ovulation in the PG 19 group is not significantly different from that of previous data.\(^7\) Hoffmann and Schwartz\(^6\) reported that about two-thirds of rats which delivered during daytime ovulated on the following day, and remainders delayed.

It appears that ovulation and the release of gonadotrophins following premature delivery were not caused by direct action of PGF$_{2\alpha}$. If gonadotrophins secretion is directly stimulated by the PGF$_{2\alpha}$ treatment, the interval from the treatment to gonadotrophins secretion should have remained constant regardless of the time of delivery. In fact, the timing of release of gonadotrophins did not depend on the time of PGF$_{2\alpha}$ administration but on the delivery time. Keyes and Houk\(^13\) reported that gonadotrophins secretion prior to post-partum ovulation is stimulated by steroids secreted from non-luteal tissues in the ovary.

In the present study, some rats in the PG 19 group which had delivered from 11:00 to
20:00, ovulated during 12-16 h after the delivery (2/18, 11/23 and 11/17, respectively) irrespective of the time of delivery and earliest ovulation was detected at 23:00 in the PG 20 group (3/10). In the strain of rats we used, the neurogenic stimulus for the pre-ovulatory surge of LH occurred during 13:00 to 14:00 on proestrus and most rats had ovulated at 02:00 on the morning of estrus. From these data it is possible to estimate that the "critical period" for post-partum ovulation is between 11:00 and 20:00 and broader than that of cyclic rats, assuming that the time interval between the "critical period" and ovulation is about 12 h. This estimation is consistent with the results of other reports in normal post-partum rats. The reason why the period of gonadotrophins secretion is bordered on 20:00 in post-partum rats is not clear.

The delivered time may be an important factor to estimate the timing of release of gonadotrophins for post-partum ovulation. The period of release of gonadotrophins for post-partum ovulation may depend on biological rhythm similar to the "critical period" in the cyclic rats. However, the time interval from delivery to the release of gonadotrophins in each rat is closely related to delivery hour in day.

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References

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ラットの分娩および後分娩排卵に及ぼす
プロスタグランジン F2α の影響

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竹内三郎・正木淳二

(PG 19 群) または 妊娠 20 日の 09:00 (PG 20 群) に皮下投与した。
PG2α 投与により早期に分娩を誘起したラットにおける分娩終了時刻とその後の排卵について検討した。
PG 19 群では、妊娠 21 日目の 11:00～19:00 の間に分娩を終了した動物の 2 匹が分娩終了後 12 時間目で排卵した。一方、PG 20 群の場合、妊娠 21 日目の 20:00～22 日目の 10:00 に分娩を終了した動物では妊娠 22 日目の 23:00 まで排卵が始まらなかった。

血中性腺刺激ホルモン (LH & FSH) 含量（以下 GTH と略）をラジオイムノアッセイにより測定した結果、PG 19 群では分娩終了後 2 時間目以上時間が始め、6 時間目で最高値を示した。一方、PG 20 群では、いずれの個体も妊娠 22 日目の 12:00 まで変化を示さなかった。

これらの結果は、PG2α により妊娠期に分娩を誘起した場合、分娩後の排卵機構は正常分娩したラットの後分娩排卵の場合と極めてよく似ており、GTH 放出は 11:00～20:00 の間にあることを示唆している。また、この GTH 放出の時間帯は正常性周期を示すラットの発情期間での放出時間帯よりも幅が広いことを示している。