Induction of Estrus and Promotion of Fertility by Prostaglandin F$_2$α Administration in Mares

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To reduce the intervals from parturition to conception in normal multiparous mares and to promote the fertility in mares with reproductive failures, 13 mares, a total of 17 cases, were intramuscularly injected with prostaglandin F$_2$α (PG) in the luteal phase. Ten mares came into estrus after the first injection of 5 mg of PG, whereas 3 mares required two or three PG treatments to induce estrus. Estrus was induced 5–8 days after PG treatment in 10 out of 14 cases that showed signs of estrus. The duration of estrus was 3.9 days on the average and 11 out of 17 cases ovulated 8–12 days after PG treatment. The number of matings was 1–5 times during the induced estrus and 11 mares conceived. A “star-like layering of the folds” in the uterine cavity appeared 2–6 days after PG treatment, and then disappeared 1–2 days before ovulation on ultrasonic monitoring. The plasma levels of progesterone (P) rapidly decreased following PG treatment, whereas the plasma levels of inhibin (INH) and estradiol-17β (E$_2$) increased after PG treatment. The plasma concentrations of LH gradually increased following PG treatment and typical prolonged LH surges were observed during the periovulatory period. In contrast, the plasma concentrations of FSH decreased after PG treatment, followed by an increase after ovulation. There was an inverse relationship between the plasma concentrations of FSH and INH. The results of the present study clearly demonstrated that treatment with PG is a useful method for reducing the intervals from parturition to conception in multiparous mares, and for improving reproductive efficiency in mares with reproductive failure.

Key words: induction of estrus, luteal phase, mare, PG, promotion of fertility

Farmers in the equine industry who do not have a teaser stallion have few chances to detect the estrus in mares, because almost all mares show the estrous signs only in sexual behavior with a stallion (the teaser) [4]. Although the beginnings of the first postpartum estrus (foal heat) are generally given as 7 to 9 days after parturition [4], some mares are not mated because of quiet ovulation or feeble estrus [15]. Even if mated at this estrus, many reports agree that pregnancy rates are lower for mares mated during the first postpartum estrus as compared to mating in estrus during the normal estrous cycle [7, 14].

We therefore designed a treatment with prostaglandin F$_2$α (PG) for efficient production in mares, namely early conception after parturition in normal multiparous mares, and the induction of estrus and promotion of fertility in mares with reproductive failure. We also determined changes in circulating progesterone (P), estradiol-17β (E$_2$), inhibin (INH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) in relation to ovarian activity.

Materials and Methods

Thirteen mares were used in this study with a single injection of PG (Pronalgon F, 1 ml; 5 mg of
Table 1. Summary of mares studies

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mare</th>
<th>Number of mares and history</th>
<th>Number of PG treatments</th>
<th>Number of experiment cases (Number of mares used)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1–5</td>
<td>6 multiparous mares with foal heat</td>
<td>1</td>
<td>5 (5 mares)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>2</td>
<td>2 (1 mare)</td>
</tr>
<tr>
<td></td>
<td>7–8</td>
<td>4 multiparous mares with anestrus</td>
<td>1</td>
<td>2 (2 mares)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>2</td>
<td>2 (1 mare)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>after parturition</td>
<td>3</td>
<td>3 (1 mare)</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>1 multiparous mare with anestrus after abortion</td>
<td>1</td>
<td>1 (1 mare)</td>
</tr>
<tr>
<td></td>
<td>12, 13</td>
<td>2 maiden mares with feeble estrus</td>
<td>1</td>
<td>2 (2 mares)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

Dinoprost: Takeda Co. Ltd. Osaka, Japan) from March to May, 1992. Six multiparous mares (Group A) (average age, 9.8 years) were given PG intramuscularly in the side of the neck at 7–8 days after the first ovulation. Of the remaining 7 mares (Group B), 5 multiparous mares (average age, 7.5 years) with anestrus after parturition or abortion, and 2 maiden mares (2–3 years old) with feeble estrus were also given PG in the luteal phase which was determined by transrectal ultrasound echography of ovaries and by the peripheral levels of P (higher than 5.2 ng/ml) with an Ovuchek Rapid Well Kit (Denka Co. Ltd., Kawasaki, Japan). For the 2 mares which did not show estrus by the first or second PG treatment and resulted in quiet ovulation, an additional PG treatment in the subsequent luteal phase was carried out. Altogether, 17 cases (13 mares) were investigated in this study (Table 1). The multiparous mares were progressing favorably after parturition, and there were no abnormal findings in any of the mares on examining the vagina and uteri at the time of PG treatment.

In Group A, morphological changes in ovaries and uteri were monitored every day by transrectal ultrasound echography. The estrous signs in all mares were detected by the sexual behavior with a teaser stallion. All mares were mated with a fertile stallion when estrous signs appeared.

Levels of P, E₂, INH, FSH and LH in peripheral blood collected once or twice a day were measured by specific radioimmunoassays (RIA) [25]. Plasma concentrations of FSH were determined with anti-human FSH serum (#66) and equine FSH (E265B) for radioiodination and the reference standard. LH were measured with anti-ovine LH serum (YM#18) and equine LH (E263B) for radioiodination and the reference standard. Plasma concentrations of inhibin were determined with antiovine inhibin serum (TDNH-1) and bovine 32 KDa inhibin for radioiodination and the reference standard [8]. Plasma levels of P and E₂ were determined as described previously [25] with antisera to P (GDN337) and E₂ (GDN 244).

Results

In this study, 10 mares came in estrus after the first injection of PG, but 3 mares required two or three PG treatments to induce estrus (Table 2).

In multiparous mares, 9 out of a total of 14 cases were given PG within 30 days after parturition. Four were treated with PG during the 31–50 days after parturition. The remaining one was first injected with PG 17 days after parturition, and diagnosed as fertile 18 days after mating, but this mare aborted by the 38th day and then she received a second PG treatment 68 days after parturition (Table 2).

As shown in Table 3, estrus was induced 5.3 days on average in 14 cases after PG treatment, but 3 cases did not show signs of estrus. The duration of estrus was 3.9 days on average in 14 cases. All mares ovulated following every PG treatment. Ovulation was induced 8.2 days on average in all
cases after PG treatment. All 13 mares were mated 1–5 times, 2.4 times on average, during the induced estrus. Thereafter, pregnancy was diagnosed by transrectal ultrasound echography in 11 mares. Of the remaining 2 mares, the fetal sac disappeared at 39 days after the final mating in one mare, and the other was sold before pregnancy was checked.

On transrectal ultrasound echography, a "star-like layering of the folds" appeared in the uterine cavity from 2–6 days after PG treatment, and then disappeared 1–2 days before ovulation (Fig. 1).

The relationship among the growth of dominant follicles, corpus luteum and five hormones in a multiparous mare is shown in Fig. 2 as a representative example. In this animal, one dominant follicle was identified during the period of study. The diameter of the dominant follicle was 17 mm on the day of PG treatment (7 days after the postpartum ovulation), and reached the maximum size (57 mm in diameter) on the day of ovulation. Ovulation was detected 11 days after PG treatment. The diameter of the corpus luteum was 34 mm on the day of PG treatment and there followed a marked decrease in size in the 4 days after the PG treatment.

Plasma concentrations of LH were maintained at the low basal levels during the luteal phase but increased from the day of PG treatment to the day of the onset of the LH surge. The LH surge started 8 days after the PG treatment (2 days before ovulation), peaked at 12 days after the PG treatment (3 days after ovulation), followed by a gradual decline to the 6th day after ovulation. The total duration of the LH surge was 8 days in this animal. On the other hand, the plasma concentrations of FSH decreased gradually from the day of PG treatment to the day of onset of the FSH surge. The FSH surge occurred at the same time and had the same pattern as the LH surge. Dramatic changes in the plasma concentrations of INH were observed during the period of this study. The plasma concentrations of INH increased rapidly after the day of PG treatment, and reached a peak on the 8th day after PG treatment followed by an abrupt decline when the LH surge started. There

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**Table 2. Appearance of estrus and fertility after each PG treatment**

<table>
<thead>
<tr>
<th>Number of PG treatments</th>
<th>Appearance of estrus and conception following each PG treatment</th>
<th>Number of mares and fertility condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>first</td>
<td>second</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Although this mare was diagnosed as fertile 18 days after final mating, the fetal sac had disappeared by the 38th day. –: quiet ovulation.

**Table 3. Induction of estrus and ovulation after PG treatment**

<table>
<thead>
<tr>
<th>Days to estrus from PG</th>
<th>Duration of estrus</th>
<th>Days to ovulation from PG</th>
<th>Number of matings per estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>no estrus (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4 days (4)</td>
<td>1–2 days (5)</td>
<td>1–4 days (3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>5–7 days (9)</td>
<td>3–5 days (7)</td>
<td>5–7 days (3)</td>
<td>2–3 (8)</td>
</tr>
<tr>
<td>8 days (1)</td>
<td>8–9 days (2)</td>
<td>8–12 days (11)</td>
<td>4–5 (3)</td>
</tr>
</tbody>
</table>

Mean: 5.3 days, 3.9 days, 8.2 days, 2.4

The number of mares in each group is shown in parenthesis.
An ultrasound echogram of the uterine cavity at the time of PG treatment in a mare. No “star-like layering of the fold” is seen.

An ultrasound echogram of the uterine cavity in the same mare more 6 days after PG treatment (4 days before ovulation). A clear “star-like layering of the fold” appears.

![Diagram](image)

<table>
<thead>
<tr>
<th>No. of mare</th>
<th>PG</th>
<th>Duration of appearance of a “star-like layering of the folds” in uterine cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

○ : Mating, ● : Duration of appearance of a “star-like layering of the folds” in uterine cavity.

Fig. 1. Appearance of a “star-like layering of the folds” from No. 1 and mating schedule during estrus induced by PG treatment.
was therefore a sharp temporary increase in the plasma concentration of INH in accordance with the time of ovulation and followed by a gradual decline thereafter. There was a significant inverse relationship between the plasma levels of FSH and INH throughout the period of this study ($r = -0.508$, $n=30$, $p<0.01$). The plasma concentrations of $E_2$ increased gradually from 2 days after the PG treatment to the day of the LH surge, followed by an abrupt decline accompanying the onset of the LH surge. The plasma concentration of P was 8.0 ng/ml on the day of the PG treatment, followed by an abrupt decline after the PG treatment. After ovulation, there was a progressive increase in the plasma concentration of P throughout the period of this study. Although the other mares were also studied concerning the relationship among the growth of dominant follicles, corpus luteum and five hormones, there were no apparent differences among the cases.

**Discussion**

To induce estrus by PG treatment in mares, the following 4 points should be considered, that is, 1) determining the correct timing of treatment, 2) uniformity of the number of days from treatment to the appearance of estrus, 3) induction of estrus to permit mating, and 4) facilitation of high fertility.

In the present study, PG administration resulted in a higher rate of estrus induction and fertility in normal multiparous mares after parturition and in mares with reproductive failure. In particular, the therapeutic treatment with PG of mares with anestrus or feeble estrus was very effective for the theriogenological controls. However, there remain questions concerning the promotion of fertility after the induction of estrus. Miyake et al. [16] stated in their paper that some mares in which estrus was induced by PG showed signs of insufficient hyperemia of the vaginal mucous membrane and loose portio vaginals uteri. It is necessary to continue clinical and endocrinological studies on successful induction of estrus.

The present results on the induction of estrus by PG treatment in mares were in general in accordance with those of previous works [2, 11, 13, 16–18, 27]. However, there was a slight difference from mares during the normal estrous cycle in the number of days from treatment to the appearance of estrus. Ginther [4] reported that there are 1–2 follicular waves including a dominant follicle during the estrous cycle resulting in secondary ovulation during diestrus in some mares. It is therefore, predictable that variations in the num-
ber of days from treatment to estrus may depend on the correlation between the time of PG treatment and the stage of the follicular waves. If PG was administrated at the presence of a large ovulatory dominant follicle, the time to estrus may be shortened. Conversely in the absence of a large dominant follicle, the time to estrus may be lengthened.

In one mare, ovulation occurred immediately after PG treatment. It has been indicated that PG or PG analogues may be able to induce estrus or ovulation in mares without a corpus luteum or with very low P concentrations [4, 26]. Furthermore, it is also reported that the administration of PG or PG analogues caused an increase in circulating LH and FSH within 2 hours [3, 11, 17] in mares, and these results were in accordance with those in rats [20, 22, 24]. Another mechanism is also postulated: PG acts directly on the ovary to induce ovulation [21, 23]. From these results, it is surmised that the increase in LH from the pituitary gland after PG administration may induce ovulation, or the action of PG in hastening ovulation may occur at the follicle rather than at the pituitary in the case of ovulation immediately after PG treatment.

Because the ovarian structure in mares is different from that of cattle, it is difficult to determine the activity of ovaries by rectal palpation alone. On that point, it is possible to confirm the changes in ovarian activity under direct vision by ultrasound echography [1, 5, 6, 19]. It is reported that with the advance of estrus, the prominent endometrial folds, a "star-like layering of the folds", in the uterine cavity is seen in the ultrasonic images [4, 10]. These researchers stated that the echogenic areas are attributable to the reflections of tissue-dense central portions of the folds, and the nonechogenic areas are attributable to edematous portions of the folds. In this study, it was shown that these changes in the uterus were in accordance with the increase in peripheral E2 levels and the period of permissive action of mares with a stallion.

In this study, hormonal changes after PG treatment in mares were characterized in relation to the findings of regression of the corpus luteum and development of the ovulatory follicle. It was clearly shown that these changes are similar to the changes in normal mares before and after estrus. The administration of PG is therefore very effec-

tive for efficient production in mares, namely early conception after parturition in normal multiparous mares, and the induction of estrus and promotion of fertility in mares with reproductive failure. The INH levels increased prior to the increase in E2 levels after PG treatment, and these changes were similar to the results in rats [28], pigs [9] and cattle [12]. It was shown that after a rapid decrease in INH and E2 levels in accordance with the onset of the LH surge, INH levels increased temporarily at ovulation. This temporary increase in INH levels is not shown in other animals. It is still not known why INH levels increase at the time of ovulation in mares. Further studies are needed to determine the mechanisms responsible for these findings. The significant inverse correlation between the changes in INH and FSH levels in plasma may indicate that INH plays an important role in the regulation of FSH secretion in mares similar to that in other animals.

The present study clearly demonstrated that treatment with PG is one of the useful methods for reducing the interval from parturition to conception in multiparous mares, and for improving reproductive efficiency in mares with reproductive failure.

In this study, we could not fully investigate the peculiar clinical findings and changes in hormonal levels including the slow increase in E2 in cases with prolongation of the interval from parturition to the first postpartum estrus (foaling heat) and the rapid decrease in P in cases with embryonic loss. In the near future, we will continue to study the remaining problems causing reproductive failure in mares.

Acknowledgments

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Wakabayashi (Gunma University, Japan) for anti-rabbit IgG and Teikoku Zoki Co. Ltd. for progesterone and estradiol-17β.

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