LUTEOLYTIC EFFECTS OF PROSTAGLANDINS
IN RAT PREGNANCY,
AND REVERSAL BY LUTEINIZING HORMONE

By
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ABSTRACT

The influence of prostaglandin infusions on plasma progesterone (P) was determined in pregnant rats at various stages of gestation. Serial blood samples were collected from the right atrium through an indwelling catheter before, during and after prostaglandin (PG) administration. PGE_2 and PGF_2α was given as intravenous infusions of 6 hours duration through the same catheter at rates varying from 0.33 to 5.5 µg/min (total dose: 125 to 2000 µg/rat). Control rats received an equal amount of 0.9% saline solution.

Prostaglandins suppressed plasma P levels at all stages of gestation, PGF_2α being much more effective than PGE_2. The effect of PGF_2α was not dose-dependent above a threshold level of about 125 µg/rat. The decrease in plasma P was evident within 3 hours of the start of the infusion, with further decrease in the subsequent 3 hours of infusion. The degree of luteal suppression by PGF_2α varied in the course of gestation, being greatest on days 10 and 18 of pregnancy and smallest on days 4 and 16.

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On days 10 and 18, plasma P was reduced by 75%, on the average, during the infusion of PGF<sub>2α</sub> with further decrease to 15% of initial values within 24 hours. Pregnancy was terminated in these rats, but was maintained in most rats treated with PGF<sub>2α</sub> at other stages of gestation. Plasma P decreased, on the average, by 60% during the infusion of PGF<sub>2α</sub> in all rats that remained pregnant, and progesterone concentrations remained at a level of 30% of initial values for at least 2 days. Plasma P levels as low as 20% of initial values were compatible with undisturbed gestation.

Concomitant treatment with LH prevented the decrease in plasma P induced by PGF<sub>2α</sub> on days 4, 7 and 10, but had little effect on day 18. Prolactin, given alone or with oestradiol, was completely ineffective on day 10 but on day 4, prolactin had a marginal inhibitory effect on the PGF<sub>2α</sub>-induced fall in plasma progesterone.

Prostaglandins are considered to be luteolytic when administered in vivo, in contrast to their steroidogenic effects in in vitro preparations. This luteolytic effect has been particularly well documented in the ewe (McCracken et al. 1970; Aldridge et al. 1970; Thorburn & Nicol 1971; Chamley et al. 1972) and in pregnant hamsters (Gutknecht et al. 1971) in which prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) administration results in a significant drop in plasma progesterone levels. On the other hand, the evidence for a luteolytic effect in the monkey appears equivocal as judged from plasma progesterone determinations (Kirton et al. 1970; Caldwell et al. 1972), and in non-pregnant women no such luteolytic effect has been detected (Jewelewics et al. 1972; Lemaire & Shapiro 1972) and PGF<sub>2α</sub>-induced abortion often occurs without any preceding fall in plasma progesterone levels (Symonds et al. 1972; Speroff et al. 1972). Administration of PGF<sub>2α</sub> to pregnant mice, rats and rabbits has been shown to interfere with gestation (Gutknecht et al. 1969; Labhsetwar 1970; Chang & Hunt 1972; Barthe et al. 1972) but the effective doses were quite large, in the range of 3-4 mg/kg, and had to be given over several days. The observed antifertility effects in these species may, therefore, be expressions of the great pharmacological potency of prostaglandins, and may not have any specific physiologic significance for the regression of the corpus luteum of these animals under normal conditions.

A detailed investigation of the effects of prostaglandins in pregnant rats showed that PGF<sub>2α</sub> infused intravenously over 6-7 h is effective in terminating pregnancy only during two limited periods of gestation; namely, between days 9 and 12, and from day 18 of gestation onward. On these days, 20 µg/h/rat (0.4-0.5 mg/kg) was 100 per cent effective in all instances, whereas even much higher doses failed to interrupt pregnancy at other stages (Fuchs & Mok 1973).

To gain more information on the effect of PGF<sub>2α</sub> on luteal function in the
rat, the present experiments were carried out to assess critically the effect of PGF₂α infusion on plasma progesterone levels at different stages of gestation.

The ability of various luteotrophic hormones to counter or block the prostaglandin-induced changes in plasma progesterone levels was also investigated.

MATERIALS AND METHODS

One hundred rats purchased from Charles River Breeding Labs were used. They were kept in temperature- and light-controlled quarters (14 h of light and 10 h of darkness), and mating was verified by the finding of spermatozoa in the vaginal smear on the morning following pro-oestrus. This day was designated day one of gestation. Polyethylene catheters (i. d. 0.06 cm, o. d. 0.1 cm) were inserted into the vena cava superior or into the right heart via the right external jugular vein at an operation performed under pentobarbital anaesthesia (30 mg/kg) one day before the scheduled infusion. The catheters were filled with heparin-saline, sealed and exteriorized through the skin of the neck. They were concealed in a small plastic capsule attached to the skin when not in use. It was usually possible to obtain blood samples repeatedly through these catheters. The prostaglandins were infused through the same catheters in 10 ml of physiologic saline over six to seven hours between 9 a.m. and 4 p.m. Control rats received the same amount of saline. Blood samples, 0.5 ml each, were collected in the morning before the infusion, at noon during the infusion and immediately after the infusion was completed. Blood was also taken in the morning of the days following, until the rats were sacrificed to ascertain the condition of the uterus and the ovaries. From 3 to 8 blood samples were taken from each rat. Plasma was separated and frozen until assayed by means of radioimmunoassay by the method described by Thorneycroft & Stone (1972) following an ether extraction of a 1:100 dilution of plasma with 30 to 12 volumes of diethyl ether. The antibody used was prepared against progesterone 11-succinyl-bovine serum albumin (1 HT-R 1516-1). The recovery of progesterone from plasma samples was over 90% as determined by addition of known amounts of progesterone to plasma from castrated adrenalectomized rats. The validity of the assay was established by comparing the results with a competitive protein binding method already established in our laboratory (Henzl et al. 1971).

Various doses of prostaglandin F₂α (PGF₂α) ranging from 125 µg to 2000 µg/rat, and two doses of prostaglanding E₂ (PGE₂) (250 and 1000 µg/rat) were administered. Experiments were carried out on days 4, 7, 10, 14, 16 and 18 of gestation. Groups of 2 to 4 rats were used on each day and for each dose of prostaglandin. In other groups of animals, either prolactin (NIH-P-S-9 ovine) or luteinizing hormone (NIH-LH-S-7 ovine) was administered intramuscularly in 0.1–0.2 ml 5% gelatin together with the prostaglandin infusions and until the rats were sacrificed. The difference between experimental and control groups was evaluated by Student's t-test, and values with $P < 0.05$ were considered significant.

RESULTS

The initial plasma progesterone levels, determined at 8 a.m. on the day of
Peripheral plasma progesterone levels in pregnant rats determined at 8 a.m. on various days of gestation. Each value is mean of at least 6 rats. The bars represent SE.

Infusion, varied considerably according to the stage of gestation, and from animal to animal. Fig. 1 shows the progesterone concentrations in peripheral plasma of these rats before the infusion was started. The progesterone levels were already high on the morning of day 4, with further increase to day 7. On day 9, the plasma progesterone level was decreasing and on days 10 and 14, the values were significantly lower than on day 7 or day 16 when the progesterone levels appeared to reach maximal values. On day 18, the levels were decreasing, but were still high compared to non-pregnant values.

The experimental procedures, namely the infusion of 10 ml of physiologic saline and daily withdrawal of blood samples, had a certain influence on the plasma progesterone levels in themselves. In rats infused with saline on day 4, the plasma progesterone concentration on day 7 was lower than the concentration on the same day of pregnancy but determined in the initial sample from rats scheduled for infusion on day 7 (67 ± 10.7 ng/ml vs. 98 ± 8.2 ng/ml). This difference was significant at a level of \( P < 0.05 \). On the other hand, in rats infused with saline on day 7, the plasma progesterone levels on day 10 did not differ significantly from the initial values measured on day 10 (51 ± 3.8 ng/ml vs. 56 ± 8.1 ng/ml). The same applied to rats infused with saline on day 10. Plasma progesterone concentration on day 14 in these rats (47 ± 7.5 ng/ml) was not significantly different from the initial values found on day 14 (58 ± 9.5 ng/ml).
Infusion of 125 µg of PGF₂α per rat over 6 h on days 10 and 18 always terminated pregnancy. Resorption of the embryos was evident within 24 h after infusion on day 10, while rats infused on day 18 aborted living foetuses at an average interval of 48 h after the start of the infusion. Most of the rats infused on days 4, 7, 14 and 16 remained pregnant and the rats that were not killed after laparotomy 3–5 days later delivered living offspring at normal term.

In spite of the variable effect on gestation at different stages of pregnancy, PGF₂α infusions resulted in a significant decline of plasma progesterone levels in all rats as compared with saline-infused control rats. Fig. 2 shows the changes in plasma progesterone concentration induced by infusions of either PGF₂α or saline in the experimental rats. The initial impact of PGF₂α on plasma progesterone levels was of approximately equal magnitude at all stages as evidenced by the drop in the first 3 h of infusion, with the exception of rats infused on day 16, when the initial drop was insignificant. In the subsequent 3 h, the progesterone levels decreased significantly in all rats, but at a greater
rate on days 10 and 18 than on other days. In these rats, non-pregnant levels were reached within 24–48 h. Plasma progesterone remained significantly below control values for at least 3 days also in the other groups in spite of sustained pregnancy.

Table 1 shows the effect of PGF$_{2\alpha}$ on plasma progesterone levels expressed as a percentage of initial values at these different stages of gestation. During the “critical periods”, when PGF$_{2\alpha}$ infusion resulted in termination of pregnancy, the average progesterone concentration at the end of the infusion was 25.3% of the initial values. Outside these “critical periods”, the levels were, on the average, reduced to 40.0% at the end of the infusion. Day 7 seems to represent a transitional stage; the plasma progesterone decreased during the entire infusion at the same rate as on day 10, but did not decrease significantly thereafter. Pregnancy was interrupted in rats in which plasma progesterone dropped to 15%, but was maintained when plasma progesterone fell to 20% of its initial values.

*Pregnancy interrupted following the infusion; rats in other groups remained pregnant.*

Table 1.
The effect of PGF$_{2\alpha}$ infusions on plasma progesterone levels in pregnant rats at various stages of gestation. (All values are expressed as per cent of initial values, and are means from 4–12 rats).

<table>
<thead>
<tr>
<th>Day of gestation (n)</th>
<th>Time of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day of infusion</td>
</tr>
<tr>
<td></td>
<td>8 a.m.</td>
</tr>
<tr>
<td>4 (6)</td>
<td>100</td>
</tr>
<tr>
<td>7 (10)</td>
<td>100</td>
</tr>
<tr>
<td>10* (12)</td>
<td>100</td>
</tr>
<tr>
<td>14 (4)</td>
<td>100</td>
</tr>
<tr>
<td>14* (2)</td>
<td>100</td>
</tr>
<tr>
<td>16 (6)</td>
<td>100</td>
</tr>
<tr>
<td>18* (6)</td>
<td>100</td>
</tr>
<tr>
<td>All rats with interrupted pregnancy</td>
<td>100</td>
</tr>
<tr>
<td>All rats with maintained pregnancy</td>
<td>100</td>
</tr>
</tbody>
</table>

* *
Effect of various doses of PGF<sub>2α</sub> on plasma P levels in pregnant rats on day 7

The effect of various doses of PGF<sub>2α</sub> on plasma progesterone concentrations in rats infused on day 7 of pregnancy. Numbers in brackets indicate number of rats used for each dose. Shaded bar in this and following figures indicates time of infusion.

The effect of PGF<sub>2α</sub> was not dose-related above a threshold dose, as illustrated in Fig. 3. The threshold dose was about 125 µg/rat over 6 h, corresponding to an infusion rate of 20 µg/h/rat.

PGE<sub>2</sub> also lowered plasma progesterone levels, but was less effective than PGF<sub>2α</sub>. At the end of infusion, plasma progesterone had decreased by 50%, on the average, both on day 6 and day 10, and remained at a level of about 40% of initial values on the following days. Pregnancy was not interrupted in any of the rats infused with 250 µg of PGE<sub>2</sub>, but infusion of 1000 µg of PGE<sub>2</sub> on day 10 terminated pregnancy in 2 out of 4 rats.

The effect of LH and prolactin was investigated in rats treated with PGF<sub>2α</sub> on days 4, 7 and 10. As already mentioned, the administration of PGF<sub>2α</sub> alone on day 10 resulted in termination of pregnancy in all instances, but simultaneous treatment with 50 µg of LH twice daily prevented this effect of PGF<sub>2α</sub>. Fig. 4 shows the plasma progesterone levels in these rats. In rats infused with PGF<sub>2α</sub> and given no other treatment, the progesterone levels were significantly lower than in saline-treated controls, whereas no change in plasma progesterone was apparent in rats treated with LH and PGF<sub>2α</sub>. By contrast, prolactin treatment (doses varying from 0.2 mg to 2 mg twice daily were tried) alone or
Fig. 4.
The effect of concomitant administration of LH (50 μg b. i. d., im) or prolactin (2 mg b. i. d., im) on the PGF₂α-induced changes in plasma progesterone levels. All rats were 10 days pregnant and received 250 μg PGF₂α. Values are means ± se of 4 to 8 rats.

supplemented with oestradiol benzoate (0.1 μg twice daily) failed to prevent either the termination of gestation or the drop in progesterone levels induced by PGF₂α. In fact, the progesterone concentration decreased more rapidly in PGF₂α and prolactin-treated rats than in rats infused with PGF₂α alone. Three hours after the start of the infusion, plasma progesterone levels were 65% of initial values with PGF₂α alone, and 11.5% in rats treated concomitantly with prolactin. Because of the large individual variations in the 3 h values in PGF₂α-treated rats, the difference in plasma progesterone concentrations between these two treatment groups is only of borderline significance (P < 0.10).

In rats infused with PGF₂α on day 4 or day 7, LH treatment also prevented the decrease in plasma progesterone following PGF₂α infusions. As can be seen in Figs. 5 and 6, the progesterone levels in LH-treated rats did not differ from those found in saline-treated rats, while in rats that were treated with PGF₂α alone, plasma progesterone was significantly below control levels for at least 3 days.

Contrary to the findings on day 10, prolactin had also an effect on the plasma progesterone levels of rats treated with PGF₂α on day 4, although this
Effect of LH and prolactin on plasma progesterone in pregnant rats infused with PGF$_2\alpha$ on day 4

![Graph showing plasma progesterone levels with different treatments]

**Fig. 5.**
The effect of LH (50 µg b. i. d., im) and prolactin (2 ng b. i. d., im) administration on rats infused with 250 µg PGF$_2\alpha$ on day 4 of pregnancy. Values are means ± se of 4 to 8 rats.

effect was much less than that of LH. With concomitant prolactin treatment, plasma progesterone decreased less during the infusion than with PGF$_2\alpha$ alone. However, the progesterone levels in prolactin- and PGF$_2\alpha$-treated rats were

**Table 2.**
Influence of LH on plasma progesterone levels in pregnant rats treated with 250 µg PGF$_2\alpha$ on day 18. (Dose of LH: 50 µg b. i. d., im).

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>Time of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 18</td>
</tr>
<tr>
<td></td>
<td>8 a.m.</td>
</tr>
<tr>
<td>PGF$_2\alpha$ (4)</td>
<td>96 ± 22</td>
</tr>
<tr>
<td>PGF$_2\alpha$ + LH (3)</td>
<td>93 ± 4.8</td>
</tr>
<tr>
<td>PGF$_2\alpha$ + LH (1)</td>
<td>76</td>
</tr>
</tbody>
</table>

* Values are in ng P/ml ± se.
Plasma Progesterone Levels in PGF$_{2\alpha}$ Infused Rat on Day 7

![Graph showing plasma progesterone levels in PGF$_{2\alpha}$ infused rat on Day 7.]

**Fig. 6.**
The effect of LH treatment on plasma progesterone levels in rats infused with 250 µg of PGF$_{2\alpha}$ on day 7.

 considerebly lower than in LH- and PGF$_{2\alpha}$-treated rats throughout the period studied.

The effect of prolactin on day 7 was not investigated.

LH treatment had very little effect in rats treated with PGF$_{2\alpha}$ on day 18, as seen in Table 2. In 4 rats given 50 µg LH b.i.d., the initial decrease in plasma progesterone was considerably reduced, but in spite of continuous treatment with LH, the progesterone levels declined on the following days and premature delivery occurred in all 4 rats. However, the delivery occurred, on the average, 15 h later than in rats treated with PGF$_{2\alpha}$ alone. In one of the LH-treated rats, shown separately in Table 2, a sudden increase in plasma P occurred on the third day; presumably as a result of new ovulation.

**DISCUSSION**

There have been several reports dealing with the luteolytic action of prostaglandins in pregnant rats, mostly utilizing the termination of pregnancy as an indication of luteolysis (Nutting & Cammarata 1969; Gutknecht et al. 1969; Labhsetwar 1970, 1972; Behrman et al. 1971c; Deis 1971).
In the present study, the prostaglandin-induced changes in plasma progesterone levels were used as an index of changes in luteal function. The corpus luteum is the main source of progesterone in pregnant rats, and since it has been shown that PGF$_2\alpha$ reduces ovarian progesterone output (Behrman et al. 1971b; Pharriss 1972), a luteal site of action is indicated. This is supported by the results of histochemical studies of PGF$_2\alpha$-treated rat ovaries (Fuchs & Mok 1974).

The data presented demonstrate that PGF$_2\alpha$ has a rapid and sustained inhibitory effect on luteal function at all stages of gestation. They are in agreement with the report of Behrman et al. (1971c) who found that on day 8, a single subcutaneous injection of PGF$_2\alpha$ reduced ovarian progesterone output by 50 to 80% within 12 h. The degree of this inhibition varies significantly in the course of gestation, being smallest on days 4 and 16. After day 6 the prostaglandin effect becomes successively more pronounced, and on day 10 plasma progesterone levels are reduced to non-pregnant values within 24 h. In the second half of gestation, the corpus luteum becomes more resistant to the luteolytic action of PGF$_2\alpha$ until day 18 when PGF$_2\alpha$ again is highly effective.

According to our results, the normally circulating plasma progesterone levels in pregnant rats exceed the minimal requirements for maintenance of gestation with a wide margin. Plasma levels as low as 20% of initial values were compatible with undisturbed gestation, whereas a drop to 15% was associated with termination of gestation.

The critical period for PGF$_2\alpha$ action at mid-gestation coincides with the period when LH is of supreme importance for the maintenance of luteal function (Alloiteau 1966), and when neutralization of endogenous LH with antiserum produces consequences similar to PGF$_2\alpha$ (Loewit et al. 1969; Madhwa Raj & Moudgal 1970). During this period, LH completely prevented the effects of PGF$_2\alpha$ on plasma progesterone levels, as well as on the histochemically demonstrable changes in luteal steroid dehydrogenases (Fuchs & Mok 1974), suggesting that PGF$_2\alpha$ exerts its luteal action by interfering with LH action. The failure of Pharriss (1972) to demonstrate an antagonistic effect of LH and PGF$_2\alpha$ in pregnant rats is probably due to the low dose of LH employed by this investigator. Our previous findings suggest that the inhibition of PGF$_2\alpha$ action by LH is competitive in nature (Fuchs & Mok 1973).

The possibility of a pituitary effect of PGF$_2\alpha$ through inhibition of LH release cannot be ruled out, but the observation that ovulation was not inhibited in rats treated with PGF$_2\alpha$ at pro-oestrus, while the response to exogenous gonadotrophin was reduced in PGF$_2\alpha$-treated immature rats speaks against such a possibility (Labhsetwar 1970; Pharriss & Hunter 1971).

LH might counteract the luteolytic effect of PGF$_2\alpha$ also by stimulating progesterone secretion from extraluteal compartments of the ovary. Cortes et al.
(1971) have demonstrated that an ovulatory dose of LH produces a rise in progesterone secretion in androgen-sterilized rats which lack corpora lutea, and, in these rats, the progestins released just before ovulation are, therefore, derived from non-luteal tissue.

However, histologic examination of the ovaries of PGF$_{2\alpha}$- and LH-treated rats (Fuchs & Mok 1974) did not show evidence of luteinization of follicles nor of formation of a new set of corpora lutea. Moreover, histochemical signs of luteolysis were not observed in the corpora lutea of pregnancy of the PGF$_{2\alpha}$- and LH-treated rats, in contrast to the rats treated with PGF$_{2\alpha}$ alone (Fuchs & Mok 1974), all of which speaks against the possibility that LH may have stimulated progesterone from extraluteal ovarian tissues. Moreover, the experiments of MacDonald et al. (1966) and of Anderson et al. (1973) suggest that LH does not normally stimulate ovarian progesterone production in the absence of corpora lutea.

Prolactin and oestrogen also have luteotrophic properties in the rat. The lack of effect of these compounds on plasma progesterone levels in PGF$_{2\alpha}$-treated rats indicates that the primary effect of PGF$_{2\alpha}$ is not on prolactin-mediated cellular processes, as suggested by Behrman et al. (1971a) and by Pharriss (1972).

PGF$_{2\alpha}$ may have additional effects on the rat ovary, beside those that are reversed by LH. This is indicated by the fact that PGF$_{2\alpha}$ induces premature delivery when infused on day 18, at which stage luteal function is independent of hypophyseal support. We have shown that the effect on day 18 is also independent of the myometrial effects of PGF$_{2\alpha}$ (Fuchs & Mok 1973), and its action at this stage needs further clarification. Chronic treatment with PGF$_{2\alpha}$ was associated with changes in ovarian cholesterol turnover which were not overcome with either LH or prolactin (Behrman et al. 1971a). Such effects on cholesterol turnover may play a part in the prostaglandin-induced luteolysis at the end of gestation.

It is not possible to draw any conclusions on the physiological significance of these findings. There is no information on the production rates of PGF$_{2\alpha}$ in rats, but the effective infusion rate, 20 $\mu$g/h, exceeds the calculated rate of synthesis in human females (Samuelsson 1973). It is of interest that the luteolytic efficacy of PGF$_{2\alpha}$ is greatest in those periods when luteal regression normally occurs. This findings is compatible with the hypothesis that endogenous prostaglandins play a part in normal luteolysis in the rat, although direct support for such a view is still lacking.

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