The Effect of Prostaglandin $F_{2\alpha}$ on Ovarian and Plasma Progesterone Levels in the Pregnant Hamster[1] (35449)

G. D. GUTKNECHT, L. J. WYNGARDEN AND B. B. PHARRISS[2]
(Introduced by G. W. Duncan)
Fertility Research, The Upjohn Company, Kalamazoo, Michigan 49001

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment terminates pregnancy in the rat (1), rabbit (1), monkey (2), and human (3, 4). $PGF_{2\alpha}$ appears to have a luteolytic action since it causes : (a) a shortened metestrous condition in the pseudopregnant rat (5), (b) a visible regression of the corpora lutea in the pseudopregnant rabbit (5, 6), and (c) lowered plasma progestin levels in the pregnant monkey (2). In the rat $PGF_{2\alpha}$ acts ostensibly by terminating luteal steroidogenesis and lowering plasma progesterone levels since administration of a progestin will antagonize the action of $PGF_{2\alpha}$ and allow normal pregnancy to be maintained (1).

In the present study progesterone levels of plasma and ovarian tissue were compared during pregnancy in $PGF_{2\alpha}$- and placebo-treated hamsters. Peripheral plasma progesterone levels were also determined in untreated cycling female hamsters. Corpora lutea from placebo and $PGF_{2\alpha}$-treated hamsters were examined for histological differences. Progesterone was administered to pregnant hamsters treated with an antifertility dose of $PGF_{2\alpha}$ to determine if exogenous progestin would prevent pregnancy disruption.

Materials and Methods. Mature virgin female (~100 g) and proven fertile male Syrian Golden hamsters, *Mesocricetus auratus*, were maintained in light from 6:00 a.m. to 8:00 p.m. and darkness from 8:00 p.m. to 6:00 a.m. Sperm in the vaginal lavage designated day 1 of pregnancy.

Ovarian weight, condition of pregnancy at autopsy in mated animals, and plasma and ovarian tissue progesterone concentrations were determined in each of 3 groups of females.

Mated females received 0.5 ml of 0.9% saline or 0.1 mg of $PGF_{2\alpha}$ in 0.5 ml of 0.9% saline daily, given subcutaneously at 8:00 a.m. and 4:00 p.m. in two equal portions on each of days 5, 6, and 7 of pregnancy. These females were autopsied preceding the p.m. treatment on selected days of 1 through 15 of pregnancy and following parturition.

Nonmated, nontreated female hamsters were autopsied on each day of a normal 4-day estrous cycle. Following the procedure of Orsini (7), the appearance of the postestrous discharge was designated as day 1 of the estrous cycle, *i.e.*, the morning following the night in which the female was in heat.

All of the above animals were weighed and then anesthetized with Metofane. Blood drawn via inferior vena cava puncture with a heparinized 3-ml syringe and a No. 22 needle was centrifuged and the plasma was frozen. Ovaries were quickly removed, trimmed, weighed, and then frozen in 2.5% NaOH for progesterone determinations or stored in Bouin's solution for histological preparations. Plasma and ovarian progesterone were measured by the competitive binding technique described by Neill *et al.* (8).

Ovaries were homogenized in 5.0 ml of 2.5% NaOH and then extracted four times with equal volumes of diethyl ether. Ether extracts were washed twice with 0.1 vol of water, then dried, and dissolved in 2.0 ml of absolute ethanol. Aliquots of alcohol solutions were analyzed for progesterone before and after silica gel thin-layer separation in 2:5 diethyl ether:methylene chloride. "Progesterone" values corrected for percentage

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[2] Present address: Alza Corporation, Palo Alto, California.

TABLE I. Reversal of Antifertility Effects of Prostaglandin $F_{2\alpha}$ in Hamsters with Progesterone.

Treatment[a]	Total (dose/day)	Days of treatment during pregnancy	No. pregnant/ no. bred	Av no. of implants at day 10
Saline	0.25 ml	5–10	11/12	14.3
$PGF_{2\alpha}$	0.1 mg	5	4/12	16.5
	0.2 mg	5	0/8	—
	0.1 mg[b]	5–7	0/9	—
Progesterone	12 mg[b]	5–10	13/16	12.5
$PGF_{2\alpha}$	0.1 mg	5		
+ progesterone	12 mg[b]	5–10	9/10	11.1
$PGF_{2\alpha}$	0.2 mg	5		
+ progesterone	12 mg[b]	5–10	7/9	14.6
$PGF_{2\alpha}$	0.1 mg[b]	5–7		
+ progesterone	12 mg[b]	5–10	14/15	12.7

[a] All treatments given subcutaneously in saline.
[b] Administered b.i.d.

recovery before and after thin-layer chromatography (TLC) were very similar, indicating that there were no large quantities of other progestins (17α-hydroxyprogesterone or 20α-hydroxy-4-pregnene-3-one) present to interfere in the protein-binding procedure. Plasma samples, extracted with petroleum ether, were not subjected to TLC separation and these values were not corrected for percentage recovery. Therefore, reported values for plasma progesterone refer to total progestin levels, although progestins other than progesterone are presumed to be negligible.

Standard hematoxylin and eosin preparations were made for ovarian tissue. Photomicrographs were taken of luteal tissue from placebo and $PGF_{2\alpha}$-treated females. An attempt was made to quantitate the degree of luteolysis by microscopically examining luteal tissue at 1500× and counting normal luteal cells per given area (100 μ^2).

Additional pregnant hamsters were assigned to 8 groups and treated according to the $PGF_{2\alpha}$–progesterone regimens in Table I to determine if administration of a progestin would maintain pregnancy during $PGF_{2\alpha}$ treatment. All of these animals were autopsied on day 10 of pregnancy.

Results. Pregnancy was terminated prior to day 7 in all 47 animals treated with 0.1 mg of $PGF_{2\alpha}$ on days 5 through 7 post-coitus, whereas 58 of 63 control animals were pregnant at autopsy. These data include only females autopsied on day 7 of pregnancy or later as visible uterine implants could not be identified in either treated or control animals before this time. Control animals found nonpregnant after day 7 were excluded from all subsequent analyses.

Treatment with $PGF_{2\alpha}$ on days 5, 6, and 7 of pregnancy significantly reduced plasma progesterone concentrations on days 5 ($p<0.001$), 6 ($p<0.01$), and 10 ($p<0.05$), ovarian progesterone concentrations on days 6 ($p<0.001$), 8, 9 ($p<0.01$), and 10 ($p<0.02$) (Figs. 1 and 2). $PGF_{2\alpha}$ treatment decreased plasma progesterone 1 day before a significant decrease in ovarian progesterone concentration occurred. Plasma progesterone concentration rebounded on days 7 through 9, but the level declined again shortly after the second decrease in ovarian values. Ovarian progesterone concentration returned to a nearly normal value on day 7 only and then fell sharply.

In control pregnant animals plasma progesterone levels tended to follow ovarian progesterone levels. Two peaks were evident for both, on around days 9 and 14; a sharp rise around day 4 was seen only in plasma levels. Following parturition, progesterone levels fell rapidly.

Ovarian weights in control pregnant animals increased beginning on day 4 (Fig. 3).

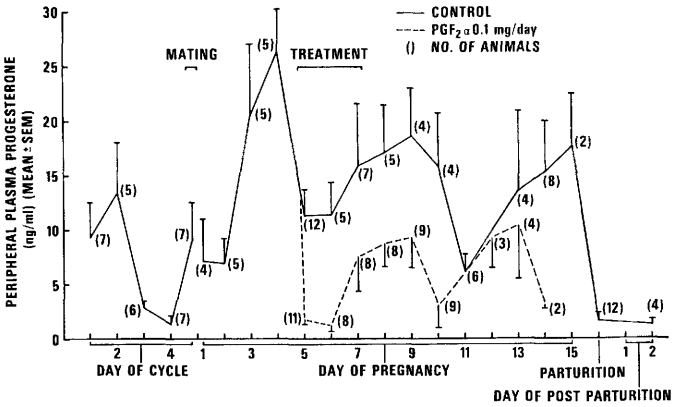

FIG. 1. Effect of prostaglandin $F_{2\alpha}$ on peripheral plasma progesterone levels in pregnant hamsters.

Ovarian weights of $PGF_{2\alpha}$-treated hamsters were significantly less than those of control animals on days 10 ($p<0.001$) through parturition. While ovarian weights of control pregnant females increased significantly ($p<0.05$) after day 9, ovarian weights of the females previously treated with $PGF_{2\alpha}$ decreased significantly ($p<0.05$) between days 8 and 10 post-coitus and remained significantly ($p<0.001$) below the control weights (Fig. 3). The ovarian weight increases largely reflected changes in luteal growth (9).

Plasma progesterone concentrations of cycling females are presented in Fig. 1. The concentration of progesterone on day 4 of the estrous cycle was significantly lower ($p<0.05$) than levels found on days 1 and 2. Day 3 concentration was significantly different ($p<0.05$) compared to that of day 2 of the cycle.

Comparison of plasma progesterone concentrations in cycling untreated females to those of $PGF_{2\alpha}$-treated females on days 7 through 14 after mating suggest that the treated animals had resumed a "normal" cycling pattern following pregnancy termination. Aligning the low values of treated hamsters on days 6, 10, and 14 with the day 4 value of the cycle, there were no significant differences in progesterone concentration between these two groups on the days examined.

Variations in ovarian weights of cycling females correlated with plasma progesterone levels. Days 1 and 2 of the estrous cycle showed significantly heavier ovarian weights than days 3 and 4 of the cycle (Fig. 3). Ovarian weights of the treated females beginning 8 days after mating were similar to those of females during the 4-day estrous cycle

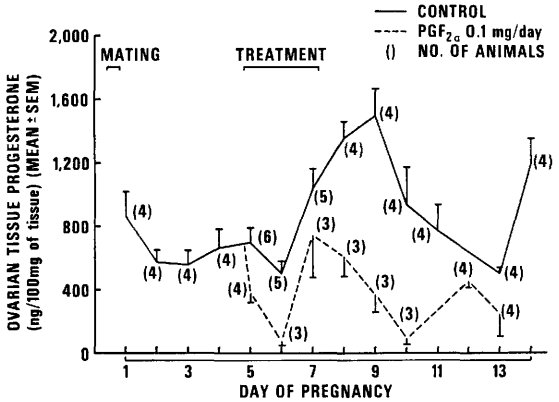

FIG. 2. Effect of prostaglandin $F_{2\alpha}$ on ovarian tissue progesterone levels in pregnant hamsters.

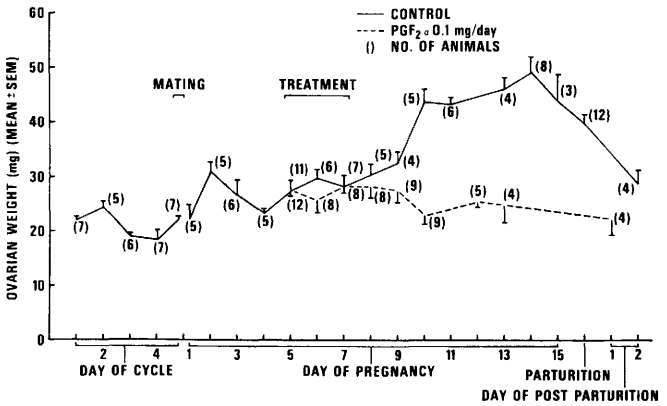

FIG. 3. Effect of prostaglandin $F_{2\alpha}$ on ovarian weight in pregnant hamsters.

(Fig. 3).

Photomicrographs of ovaries taken from $PGF_{2\alpha}$- and placebo-treated animals on days 5 and 7 of pregnancy demonstrate luteal disorganization, irregularly shaped luteal cells and pyknotic nuclei associated with $PGF_{2\alpha}$ treatment (Fig. 4). After prostaglandin treatment, luteal cells were also less numerous than in normal corpora lutea. This is shown quantitatively in Fig. 5. On days 6 and 7 post-coitus, luteal tissue from $PGF_{2\alpha}$-treated hamsters contained significantly ($p<0.05$ and $p<0.001$, respectively) fewer normal luteal cells than did luteal tissue from placebo-treated females. When the dose of $PGF_{2\alpha}$ was increased 10-fold this difference was magnified and a significant ($p<0.01$) reduction in normal luteal cell numbers was observed within 5 hr after the initial treatment. By day 8, luteal tissue from treated hamsters no longer showed evidence of disorganization, and on day 9 luteal cell numbers were significantly ($p<0.001$) higher than luteal cell numbers from placebo-treated pregnant hamsters.

Exogenous progesterone maintained pregnancy in hamsters that had received either single or multiple $PGF_{2\alpha}$ treatments (Table I).

Conclusions. The immediate decreases in plasma and ovarian progesterone concentrations in the pregnant hamster after treatment with $PGF_{2\alpha}$ argues strongly for a luteolytic effect. Eliminating the source of progesterone in the hamster by ovariectomy before parturition terminates pregnancy (10). The maintenance of pregnancy in $PGF_{2\alpha}$-treated hamsters with exogenous progesterone supports the concept that $PGF_{2\alpha}$ acts directly upon the ovary and suggests that the constant source of progesterone needed during gestation is interrupted by treatment with this prostaglandin.

$PGF_{2\alpha}$, however, had only a transient effect on progesterone levels. While plasma levels of progesterone decreased after one treatment of 0.05 mg of $PGF_{2\alpha}$ and ovarian levels dropped 24 hr later, a rebound occurred on day 7, the last day of treatment. Considering subsequent changes in progesterone levels, an apparent explanation is that cyclic ovarian activity had been reinitiated.

Histological examination of luteal tissue from treated females provides an explanation for the reduced steroid levels and also supports the concept of renewed ovarian cyclicity. Luteal cell breakdown (irregularly shaped luteal cells, pyknotic nuclei; etc.) was observed on days 6 and 7 post-coitus. Increasing the dose of $PGF_{2\alpha}$ 10-fold caused a more rapid and magnified response. On days 8 and 9 post-coitus luteal cell breakdown was not observed, and luteal tissue appeared similar to that of cycling hamsters on days 2 and 3 of their cycles. It has been shown that hamsters treated with $PGF_{2\alpha}$ on day 5 of pregnancy returned to estrus on day 7 (11). Thus, it is interesting to note the apparent similarity of plasma progesterone levels and luteal cell numbers from hamsters after treatment with $PGF_{2\alpha}$ and from untreated cycling hamsters. This similarity may indicate that after the original corpora lutea of pregnancy

have been inhibited by $PGF_{2\alpha}$ treatment and pregnancy has been terminated, the animals then begin to recycle with new generations of corpora lutea becoming active.

A second possibility is that the increases in progesterone levels and in number of normal luteal cells resulted from corpora lutea which had "recovered" from the effect of $PGF_{2\alpha}$. Thus, the second significant drop in progesterone levels and the decline in number

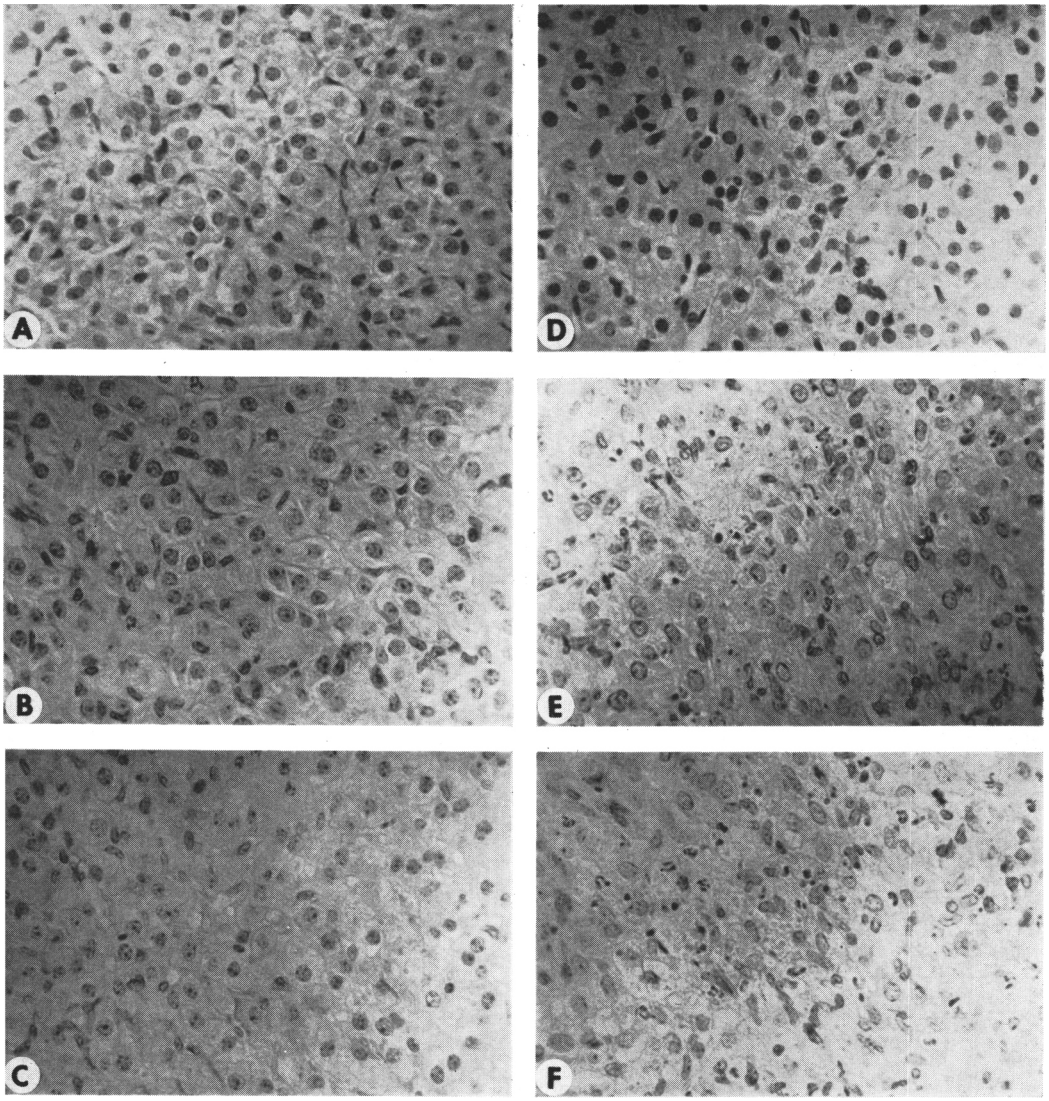

FIG. 4. Hematoxylin and eosin stained luteal tissue from pregnant hamsters; ×630: (a) placebo-treated hamster, day 5 of pregnancy (0.5 ml of 0.9% saline 5 hr preceding autopsy); (b) $PGF_{2\alpha}$-treated hamster, day 5 of pregnancy (0.05 mg 5 hr preceding autopsy). (c) $PGF_{2\alpha}$-treated hamster, day 5 of pregnancy (0.5 mg 5 hr preceding autopsy). Note beginning of luteal disorganization. (d) Placebo-treated hamster, day 7 of pregnancy (0.5 ml of 0.9% saline b.i.d. on days 5 through 7 of pregnancy). (e) $PGF_{2\alpha}$-treated hamster, day 7 of pregnancy (0.05 mg b.i.d. on days 5 through 7 of pregnancy). Note luteal disorganization, irregularly shaped luteal cells, and numerous pyknotic nuclei. (f) $PGF_{2\alpha}$-treated hamster, day 7 of pregnancy (0.5 mg b.i.d. on days 5 through 7 of pregnancy). Note high degree of luteal disorganization and numerous irregularly shaped luteal cells and pyknotic nuclei.

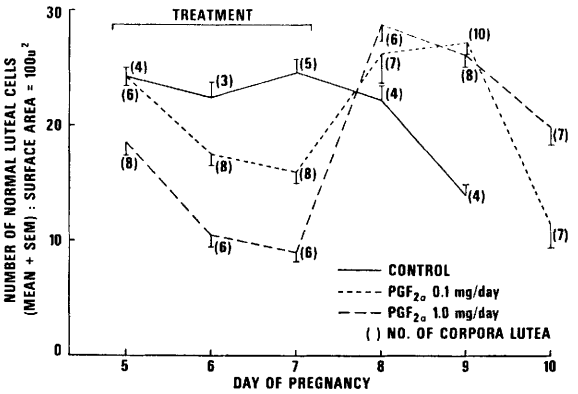

FIG. 5. Effect of prostaglandin $F_{2\alpha}$ on luteal cell numbers in pregnant hamsters.

of normal luteal cells on day 10 post-coitus would have resulted from naturally occurring luteolysis, as would happen in late stage pseudopregnancy. Implants were resorbed by day 7, eliminating the source of a fetal or placental feedback signal or of luteotropin (prolactin) to the ovary. To support this, it was noted that ovarian weights of treated animals were not significantly lower than those of control pregnant animals until day 10, indicating that luteal development had not been completely arrested until then. This would partially explain why, except for an immediate but transient steroidal depression on days 5 and 6, the corpora lutea did not regress until day 10. It also would appear that there was a steroidal and morphological recovery and that when present, morphological luteal degeneration appeared to lag behind physiological and biochemical luteolysis.

Although previous studies demonstrating the luteolytic effect of $PGF_{2\alpha}$ in pseudopregnant rats (5, 12) and rabbits (5, 6), and in hysterectomized guinea pigs (13) would argue against a uterine related effect, $PGF_{2\alpha}$ does stimulate myometrial activity and cause uterine contractions leading to early term pregnancy disruption in monkeys (14) and humans (3, 4). Isolated uteri of rats are caused to contract by $PGF_{2\alpha}$ (15), as are human myometrial strips (16). Since evidence for complete luteolysis caused by $PGF_{2\alpha}$ treatment was not obtained in this study, it may be suggested that $PGF_{2\alpha}$ was operating at a second site, the myometrium. Pregnancy may have been terminated in this way between days 5 and 7, conceivably resulting in lowered progesterone levels and corpora lutea regression around day 10. The exogenous progesterone, which maintained pregnancy in the $PGF_{2\alpha}$-treated hamster, may have protected the uterus and myometrium and instituted a quiescent state. It has been shown that progesterone added *in vitro* depresses the sensitivity of both guinea pig and rat uteri to $PGF_{2\alpha}$ (17).

The decline in plasma progesterone level before that of ovarian tissue may indicate that $PGF_{2\alpha}$ causes a constricting effect on the utero-ovarian vein which prevents ovarian progesterone from entering the systemic vascular system. As hypothesized (5), this constricting effect could lead to corpus luteum degeneration through insufficient blood perfusion. Studies in rats and rabbits on the effect of $PGF_{2\alpha}$ on ovarian blood flow demonstrated a reduced utero-ovarian blood flow (6, 18), and in rabbits, simultaneous luteolysis (6).

Summary. The effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on peripheral plasma and ovarian progesterone concentrations has been studied in pregnant hamsters. $PGF_{2\alpha}$ administered subcutaneously at a dose of 0.1 mg/day on days 5 through 7 post-coitus lowered both plasma and ovarian progesterone levels and terminated pregnancy in all animals. Ovaries from $PGF_{2\alpha}$-treated hamsters showed histological evidence of luteal disorganization on days 6 and 7 post-coitus. A 10-fold increase in the dose of $PGF_{2\alpha}$ (1 mg/day) caused an indication of luteal breakdown within 5 hr of the initial treatment. Ovarian weights, plasma progesterone levels, and luteal morphology

of females after treatment and pregnancy termination were similar to those of untreated cycling females. Exogenous progesterone maintained pregnancy in $PGF_{2\alpha}$-treated females.

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