Interrelationships of Prostaglandin $F_{2\alpha}$ and Gonadotropins in the Immature Female Rat[1] (35298)

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Effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on functional luteal activity have been published in which the results *in vivo* conflict with those found *in vitro*. Pharriss and Wyngarden (1) originally reported that $PGF_{2\alpha}$ could terminate luteal steroidogenesis in pseudopregnant rats. However, in later studies *in vitro* with luteinized rat ovaries, Pharriss *et al.* (2) indicated that $PGF_{2\alpha}$ increased progesterone synthesis in a manner similar to that caused by luteinizing hormone. As these reports appeared contradictory, additional information was needed to resolve this problem. Studies were designed to measure $PGF_{2\alpha}$ imitation of, or antagonism to, gonadotropin activity in the immature female rat. Pregnant mare serum and human chorionic gonadotropin served as model gonadotropins against which $PGF_{2\alpha}$ was tested. Subsequently $PGF_{2\alpha}$ was tested for estrogen-like and estrogen-potentiating activity because of unusual results found in initial portions of this study.

Materials and Methods. Immature female pathogen-free rats (Upjohn-Sprague-Dawley) were fed Purina Laboratory Chow and water *ad libitum*. The animals were housed at 76°F with a light cycle of 14 hr light/10 hr darkness. Six animals were used for each experimental variable except where noted in tables.

Equinex serum gonadotropin (PMS; Ayerst) was reconstituted with the accompanying sterile diluent and the desired dosage administered in 0.5 ml. Human chorionic gonadotropin (HCG; Upjohn) was reconstituted with the accompanying diluent; and the desired dosage was administered by tail vein injection (0.25 ml). Prostaglandin $F_{2\alpha}$ (10 mg) was dissolved in 1 ml of 95% ethanol and 1.8 ml of $NaHCO_3$ (1 mg/ml), then was brought to the desired concentration with saline. Control vehicle was identical to the prostaglandin preparation except for the absence of $PGF_{2\alpha}$. A 20 mg/ml solution of sodium phenobarbital was prepared in sterile water and 10 mg were used/injection. Estradiol 17β (Upjohn) was dissolved in benzyl alcohol and diluted to the desired concentration with cottonseed oil. All solutions were injected subcutaneously except where noted.

Experimental design. Experiment 1 was designed to determine if $PGF_{2\alpha}$ imitated HCG activity by inducing ovulation. Five groups of 31-day-old rats received 20 IU of PMS at 8:00 a.m. on day 31. Ten mg of sodium phenobarbital was given to each animal at noon on day 33 and vehicle, 25 IU of HCG or $PGF_{2\alpha}$ (200 or 500 μg) was administered 1 hr later by tail vein injection. The rats were sacrificed at 8:00 a.m. on day 34. The ovaries were weighed and the oviducts were examined for ova by pressing the oviducts between two slides and viewing the ampulla under a dissecting microscope.

Experiment 2 was designed to reveal any similarity between the effect of $PGF_{2\alpha}$ and FSH-like activity. Five groups of animals were injected with vehicle, PMS (10 or 20 IU/rat) or $PGF_{2\alpha}$ (100 or 500 μg/rat) at 9:00 a.m. and 4:00 p.m. on days 31 and 32. On day 33 each animal received 10 mg of sodium phenobarbital at 1:00 p.m. and 25 IU of HCG 1 hr later. The rats were sacrificed at 9:00 a.m. on day 34, their ovaries were weighed and the oviducts were inspected for

[1] The research upon which this publication is based was performed pursuant to Contract No. NIH-69-2208 with the National Institutes of Health, Department of Health, Education and Welfare.

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the presence of ova.

Experiment 3 measured PGF_{2a} effect on PMS activity. At age 21 days, three of seven groups of rats were primed with 100 μg of PGF_{2a}/rat. On days 22, 23, and 24 PMS (1, 5, or 10 IU/rat) was administered b.i.d. to all animals except those of the control group. The PGF_{2a}-primed rats also received 100 μg of prostaglandin/rat b.i.d. on these days. The rats were sacrificed on day 25 and their ovarian and uterine weights were recorded. This experiment was repeated at a later date using a 500-μg dose of PGF_{2a}.

Experiment 4 was designed to determine if PGF_{2a} would inhibit ovulation or ovarian weight gain induced by HCG. Four groups of animals received the following treatments on days 30 through 33: Group I, vehicle b.i.d. for 4 days; Group II, 500 μg of PGF_{2a} b.i.d. for 4 days; Group III, 20 IU of PMS day 31 only; and Group IV, 500 μg of PGF_{2a} b.i.d. for 4 days and 20 IU of PMS on day 31. All animals received 25 IU of HCG at 1:00 p.m. on day 33 and were sacrificed on day 34, their ovaries were weighed and the oviducts were examined for ova.

Experiment 5 was designed to determine if PGF_{2a} has estrogen-like activity. Female rats (22 days old) were ovariectomized and divided into five groups. The animals received vehicle, 0.02 or 0.05 μg of estradiol or 100 or 500 μg of PGF_{2a} on days 24 through 28. After sacrificing the rats on day 29, the uteri were removed, blotted dry, and weighed.

Experiment 6 was designed to establish any estrogen-potentiating activity of PGF_{2a}. Rats ovariectomized on day 22 were divided into five groups. On days 24 through 28, the animals received vehicle, 0.05 μg of estradiol or 0.05 μg of estradiol plus 50, 100, or 500 μg of PGF_{2a}. On day 29 the rats were sacrificed, the uteri were removed, blotted dry, and weighed.

The Students' t test of significance was used to compare two means and Tukey's test to measure difference between several means. Values of $p<0.05$ were considered significant.

Results. Expt. 1. Compared to the control group, the groups dosed with HCG showed an increase in ovarian weight and number of ova present in the oviducts (Table I).

TABLE I. Response of PMS (20 IU)-Primed Rats to Vehicle, HCG, and PGF_{2a}.

Treatment	Ovarian wt (mg)	Ova/rat
Vehicle	63.1 ± 5.0	0.0
5 IU HCG (5 rats)	90.8 ± 3.3[a]	14.8 ± 4.9[a]
25 IU HCG	80.9 ± 5.9[a]	20.2 ± 5.1[a]
200 μg PGF_{2a}	72.8 ± 7.2	1.2 ± 1.3
500 μg PGF_{2a}	79.8 ± 13.4	0.0

[a] $p<0.02$ when compared to vehicle.

Prostaglandin F_{2a} did not significantly stimulate ovarian weight gain or ovulation. Only one rat ovulated among the six animals receiving 200 μg of PGF_{2a}.

Expt. 2. Table II indicated that ovarian growth and ovulation were significantly increased in the PMS-treated groups compared to the control and PGF_{2a}-primed groups. Prostaglandin failed to affect the weight gain response of the ovaries but it inhibited the effect of HCG as measured by the number of ova found in the ampullae. The lower number of ova seen in the 20 IU PMS group compared to the 10 IU PMS group is probably due to a premature ovulation with the high dose of gonadotropin.

Expt. 3. Table III shows that the 100-μg dose of PGF_{2a} had no marked effect on ovarian weight gain at all levels of PMS treatment. The animals receiving PMS with 500 μg of PGF_{2a} had significantly increased uterine weights over animals treated with the corresponding doses of PMS alone. Rats receiving 10 IU of PMS/dose had lower uterine weight gains than those receiving injections of 1 or 5 IU of PMS, either with or without addition of PGF_{2a}.

TABLE II. Response of Vehicle, PMS, and PGF_{2a} Primed Rats to 25 IU of HCG.

Priming[a]	Ovarian wt (mg)	Ova/rat
Vehicle	40.5 ± 4.9	6.3 ± 2.2
10 IU PMS	150.5 ± 14.5[b]	26.7 ± 3.4
20 IU PMS	219.7 ± 14.7[b]	9.2 ± 3.9
100 μg PGF_{2a}	33.7 ± 4.5	0.7 ± 0.7[c]
500 μg PGF_{2a}	37.8 ± 5.1	1.0 ± 1.1

[a] b.i.d. for 2 days.

[b] <0.001 when compared to vehicle; [c] $p<0.05$.

TABLE III. Response in the Rat to PMS and PMS with $PGF_{2\alpha}$.

Treatment[a]	Ovarian wt (mg)	Uterine wt (mg)
100 μg of $PGF_{2\alpha}$/dose		
Vehicle	17.8 ± 0.5	33.0 ± 2.0
1 IU PMS	19.0 ± 1.0	165.1 ± 10.6
5 IU PMS	69.0 ± 7.5	161.4 ± 5.1
10 IU PMS	161.9 ± 9.8	129.5 ± 2.9
1 IU PMS + $PGF_{2\alpha}$	21.4 ± 1.6	160.0 ± 14.0
5 IU PMS + $PGF_{2\alpha}$	60.0 ± 9.7	152.5 ± 11.4
10 IU PMS + $PGF_{2\alpha}$	148.4 ±14.8	133.4 ± 8.9
500 μg of $PGF_{2\alpha}$/dose		
Vehicle	13.0 ± 1.8	28.0 ± 3.2
1 IU PMS	26.6 ± 2.4	139.4 ± 7.9
5 IU PMS	77.2 ± 8.1	139.8 ± 11.0
10 IU PMS	107.7 ± 9.8	108.8 ± 5.0
1 IU PMS + $PGF_{2\alpha}$	18.0 ± 1.7[b]	171.8 ± 7.8[b]
5 IU PMS + $PGF_{2\alpha}$	48.8 ± 4.5[b]	153.0 ± 7.9
10 IU PMS + $PGF_{2\alpha}$	79.7 ± 2.4[b]	121.6 ± 3.8

[a] b.i.d. for 3 days.

[b] Different ($p<0.02$) from corresponding dose of PMS without $PGF_{2\alpha}$.

Expt. 4. In both PMS-primed and unprimed animals, $PGF_{2\alpha}$ retarded ovarian growth (Table IV). The number of ova seen in PMS–HCG-treated rats was reduced over 50% by $PGF_{2\alpha}$ administration.

Expt. 5. Rats receiving the two doses of estradiol exhibited the expected dose response relationship (Table V). The uterine weights of the rats receiving $PGF_{2\alpha}$ were not different from those of the control animals.

Expt. 6. Compared to the control animals (Table VI) the rats dosed with estradiol exhibited a significant gain in the uterine weights. However, this estrogen induced weight gain was not changed when $PGF_{2\alpha}$ was administered at these three doses with the estrogen.

TABLE IV. Response of Vehicle, PMS, $PGF_{2\alpha}$, and PMS with $PGF_{2\alpha}$ Primed Rats to 25 IU of HCG.

Priming	Ovarian wt (mg)	Ova/rat
Vehicle[a]	33.8 ± 4.1	2.1 ± 1.8
500 μg $PGF_{2\alpha}$[a] (5 rats)	20.0 ± 2.0[b]	0.0
20 IU PMS	107.2 ± 12.1	34.5 ± 2.4
500 μg $PGF_{2\alpha}$[a] + 20 IU PMS	53.6 ± 7.6[b]	14.6 ± 2.4[b]

[a] b.i.d. for 4 days.

[b] Different ($p<0.02$) from similar group without $PGF_{2\alpha}$.

Discussion. Prostaglandin $F_{2\alpha}$ terminates functional luteal activity in various species (3–5). Data gathered *in vitro* suggests $PGF_{2\alpha}$ may have an LH-like effect on the ovary (2). In an attempt to determine if this effect *in vitro* plays a role in luteolysis, $PGF_{2\alpha}$ was investigated for both gonadotropin-like activity and gonadotropin antagonism. The results reported here indicate that the prostaglandin may inhibit ovulation and ovarian growth brought about by gonadotropins.

TABLE V. Response of Ovariectomized Rats to Vehicle, Estradiol, and $PGF_{2\alpha}$.

Treatment[a]	Uterine wt (mg)
Vehicle	23.1 ± 1.7
0.02 μg Estradiol	65.6 ± 3.4
0.05 μg Estradiol	118.6 ± 6.4
100 μg $PGF_{2\alpha}$	26.9 ± 1.8
500 μg $PGF_{2\alpha}$	20.0 ± 1.2

[a] Daily for 5 days.

TABLE VI. Response of Ovariectomized Rats to Vehicle, Estradiol, and Estradiol with $PGF_{2\alpha}$.

Treatment[a]	Uterine wt (mg)
Vehicle	25.4 ± 2.0
0.05 μg Estradiol	122.8 ± 5.3
+ 50 μg $PGF_{2\alpha}$	124.1 ± 6.4
+ 100 μg $PGF_{2\alpha}$	111.5 ± 7.3
+ 500 μg $PGF_{2\alpha}$	116.4 ± 5.5

[a] Daily for 5 days.

Interpretation of this data to resolve the conflict of our previous results *in vivo* and *in vitro* is difficult. While the higher doses of $PGF_{2\alpha}$ cause antagonism to gonadotropin activity (which could be the mechanism for luteolysis) these doses are much higher on an animal weight basis than those used to bring about the luteolytic effect in the mature animal (3). The mechanism of $PGF_{2\alpha}$ gonadotropin antagonism has not been established, but one effect of prostaglandin activity is reducing utero-ovarian venous blood

flow (6). This could represent a reduction in ovarian perfusion and either negate the usual hyperemic effect of HCG or just restrict exposure of the ovary to the gonadotropins.

Recently, the prostaglandins have been implicated in the actions of many peptide hormones and especially those originating from the anterior pituitary (7). Their ability to activate adenyl cyclase (8), enhance ovarian steroidogenesis (2, 9) and their presence in luteal tissue suggest a role in gonadotropin activity. However, in the present study their inability to stimulate growth of the ovary and ovulation suggest that if the above implication is true, then the steroidogenic effects of the gonadotropins might be mediated through a mechanism different from that of ovulation and ovarian growth. Such effects are not inconsistent with observations in similar studies involving cyclic AMP (10).

One interesting but confusing result was the enhancement of uterine weight gain when 500 μg of PGF_{2a} was administered with PMS. Should this difference be meaningful, three explanations are offered. PGF_{2a} either has or potentiates estrogenic activity, there is a reduction in circulating progesterone (luteolytic effect) which allows the endogenous estrogen to more fully express itself or PGF_{2a} actually stimulates estrogen production/secretion by the ovary. The first suggestion is ruled out by Expts. 5 and 6. Since uterine weight was unaffected by PGF_{2a}, either alone or in the presence of low levels of estrogen the stimulation must come about indirectly. Neither of the remaining possibilities can be selected based on present data. Progesterone can antagonize estrogen uterine weight gain but ratios of several thousand to one, progesterone to estradiol, are necessary (11). Whether or not the conditions of this experiment can allow selective depression of progesterone release without affecting concomitant estrogen output is unknown. The third reason for the uterine weight changes appears most promising and it is interesting to hypothesize that a possible shift in steroid synthesis from progesterone preference to estrogen synthesis might in some way be responsible not only for luteolysis but for other reproductive effects of the prostaglandins as well.

Summary. Prostaglandin F_{2a} was tested in immature female rats to measure its effect on gonadotropin activity. Neither ovarian weight gain nor ovulation were stimulated by PGF_{2a} administration. Prostaglandin F_{2a} did have an inhibiting effect on the results of PMS and HCG treatment, decreasing ovarian weight gain, and ovulation. PMS stimulation of uterine growth was enhanced by PGF_{2a}. This enhancement is probably due to increased secretion of estrogen by the ovary or by altering the ratio of estrogens to progestogens in the circulation.

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Received Sept. 30, 1970. P.S.E.B.M., 1971, Vol. 136.