Inhibition of Prostaglandin $F_{2\alpha}$ or LH Induced Luteolysis in the Pseudopregnant Rabbit by 17β-Estradiol[1] (36154)

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In vivo studies show luteolysis occurs after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment in rats (1), hamsters (2, 3), guinea pigs (4), sheep (5), and rhesus monkeys (6, 7). Administration of $PGF_{2\alpha}$ also interrupts pregnancy in laboratory animals (8), rhesus monkeys (6, 9) and humans (10, 11) either indirectly by reducing ovarian and plasma progesterone levels as in hamsters (3) and rats (1, 12) or directly by stimulating uterine smooth muscle contractions as in monkeys (9) and humans (10, 11).

$PGF_{2\alpha}$ is also luteolytic in the rabbit when administered either subcutaneously or intravenously on 4 consecutive days of pseudopregnancy (13). In this species, a single intravenous administration of luteinizing hormone (LH) on day 9 of pseudopregnancy causes luteal regression within 72 hr (14). This lytic effect can be antagonized by concomitant subcutaneous injections of 17β-estradiol (15).

In contrast to *in vivo* observations, addition of either $PGF_{2\alpha}$ or LH *in vitro* to incubation media containing rat ovaries (16) or bovine luteal slices (17) stimulates progesterone synthesis. It is suggested that both of these substances play a role in the activation of 3′,5′-cyclic adenosine monophosphate (18, 19). Others, investigating mouse ovaries, postulated that a prostaglandin receptor functions as a necessary intermediate in the action of LH since a prostaglandin antagonist competitively blocked the effects of both prostaglandins and LH on ^{14}C-cyclic AMP accumulation. However, additive effects were observed (18).

By monitoring corpora lutea weights and peripheral plasma progestin concentrations, the present study was designed to determine if exogenous estrogen is antagonistic to the luteolytic effect of $PGF_{2\alpha}$ in the pseudopregnant rabbit as it is to LH-induced luteolysis. The data gained would be used to further assess the interaction of LH and $PGF_{2\alpha}$ on luteal function and to gain information on the mode of action of $PGF_{2\alpha}$.

Materials and Methods. Forty-eight pseudopregnant Dutch Belted female rabbits (av body wt = 2.2 kg), mated with vasectomized males, were distributed among six groups. The day of mating was designated as day 0 of pseudopregnancy. Pseudopregnancy was confirmed by progestin determinations on day 7. Treatment regimes were:

Group A. 0.5 ml of 0.9% saline subcutaneously b.i.d. on days 9 through 14 of pseudopregnancy.

Group B. 50 μg of LH (NIH-LH-S14) in 0.25 ml of 0.9% saline via the maginal ear vein on day 9 of pseudopregnancy.

Group C. 50 μg of LH (same as Group B) plus 20 μg of 17β-estradiol in 0.1 ml of cottonseed oil–5% benzyl alcohol subcuteously on days 9 through 14 of pseudopregnancy.

Group D. 500 μg of $PGF_{2\alpha}$ in 0.5 ml of 0.9% saline subcutaneously b.i.d. on days 9 through 12 of psueduopregnancy.

Group E. 500 μg of $PGF_{2\alpha}$ (same as Group D) plus 20 μg of 17β-estradiol (same as Group C).

Group F. 2,500 μg of $PGF_{2\alpha}$ (same as Group D) plus 20 μg of 17β-estradiol (same as Group C).

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TABLE I. Effect of Exogenous 17β-Estradiol on Maintenance of Original Corpora Lutea in Pseudopregnant Rabbits Treated with a Luteolytic Dose of LH or Prostaglandin $F_{2\alpha}$.

Group	Treatment	Av corpus luteum wt (mg) [a]	
		Per animal	Per group ± SEM
A	Saline (0:5 ml sc b.i.d. on days 9–14 of pseudopregnancy)	9.0, 10.6, 13.6, 15.0, 16.0, 16.8	13.5 ± 1.3 [b]
B	LH (50 μg iv) on day 9 of pseudopregnancy)	3.3, 4.6, 5.7, 6.6	5.1 ± 0.7 [d]
C	LH (same as B) + 17β-estradiol (20 μg sc on days 9–14 of pseudopregnancy)	10.1, 11.9, 13.2, 17.7	13.2 ± 1.6 [bc]
D	$PGF_{2\alpha}$ (500 μg sc b.i.d. on days 9–12 of pseudopregnancy)	5.2, 5.6, 6.0, 6.5, 7.2, 7.6	6.4 ± 0.4 [d]
E	$PGF_{2\alpha}$ (same as D) + 17β-estradiol (same as C)	6.2, 6.4, 6.7, 8.0, 8.8, 11.4, 11.6, 13.2, 14.8	9.7 ± 1.1 [cd]
F	$PGF_{2\alpha}$ (2500 μg sc b.i.d. on days 9–12 of pseudopregnancy) + 17β-estradiol (same as C)	3.7, 4.5, 7.9, 9.9	6.5 ± 1.5 [dc]

[a] Day 14 of pseudopregnancy.

[bcde] Weights with any part of the superscript the same are not significantly different from each other.

Treatments administered twice a day were given at 8:00 a.m. and 4:00 p.m. 17β-estradiol was administered prior to the a.m. treatment of $PGF_{2\alpha}$ and prior to the noon treatment of LH on day 9. Blood for plasma progestin determinations was drawn via cardiac puncture from all rabbits on days 7, 9, 11, and 14 of pseudopregnancy. The plasma was analyzed for total progestins following the procedure of Neill *et al.* (20). All rabbits were autopsied in the p.m. on day 14 of pseudopregnancy. The ovaries of 33 rabbits were removed and the original corpora lutea (as distinct from LH treatment-induced corpora lutea) were dissected out and weighed to the nearest one tenth milligram.

Results. LH significantly ($p < .001$) reduced the average weight of the original corpora lutea to 5.1 mg from 13.5 mg by day 14 of pseudopregnancy (Table I). This demise occurs concomitantly with luteinization of newly ovulated follicles due to the LH treatment and therefore is not apparent from assessment of peripheral plasma progestin concentrations on days 9, 11, or 14 (Fig. 1). Corpora lutea weights of rabbits treated with LH plus estradiol did not differ significantly from controls and were significantly ($p < .001$) heavier than corpora from rabbits treated with LH alone. This group of rabbits also had the greatest plasma progestin concentrations on day 14, reflecting the presence of two sets of active corpora lutea.

$PGF_{2\alpha}$ was also luteolytic as assessed by changes in corpora lutea weight consistently reducing the average weight to 6.4 mg from 13.5 mg (Table I). In contrast to LH treatment, no luteinization resulted from $PGF_{2\alpha}$ treatment, hence only original corpora lutea were present and plasma progestin concentrations were nondetectable within 48 hr of starting treatment (Fig. 1).

Estradiol concomitant with $PGF_{2\alpha}$ treatment resulted in two populations of responders. As a group, corpora lutea weights were significantly ($p < .05$) heavier than in rabbits treated with $PGF_{2\alpha}$ alone (Table I). Total plasma progestin levels were also significantly ($p < .05$) higher on days 11 and 14 in animals which received $PGF_{2\alpha}$ plus 17β-estradiol when compared to animals receiving only $PGF_{2\alpha}$ (Fig. 1). In 6 out of 9 rabbits, estradiol effectively antagonized the luteolytic effect of $PGF_{2\alpha}$—both corpora lutea weights and plasma progestin values were comparable to controls. In the remaining rabbits, estradiol afforded little or no protection; corpora lutea weights were equivalent to $PGF_{2\alpha}$-treated rabbits and plasma progestins were $\leq$ 1.0 ng/ml by day 11.

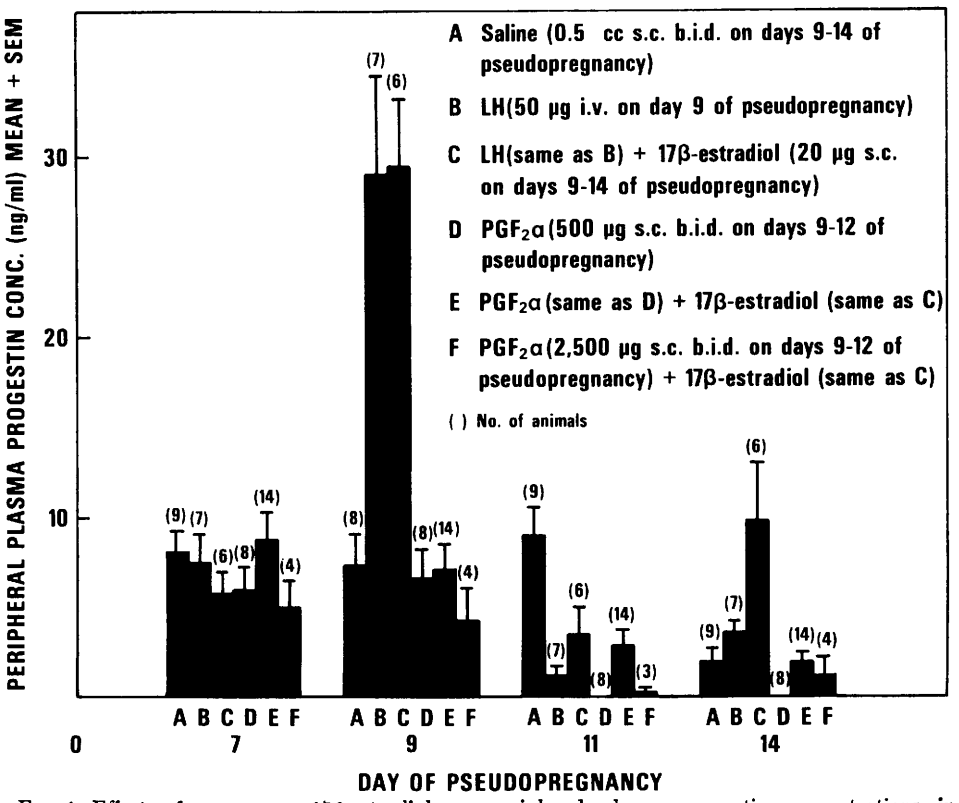

FIG. 1. Effect of exogenous 17β-estradiol on peripheral plasma progestin concentrations in pseudopregnant rabbits treated with a luteolytic dose of LH or prostaglandin F$_{2\alpha}$.

When the dose of PGF$_{2\alpha}$ was increased 5-fold in four rabbits, administration of exogenous estradiol still provided a partial luteotropic effect in two of the rabbits as determined by corpora lutea weights and in three of the rabbits as evidenced by detectable plasma progestin levels on day 14. There were no differences in the average number of original corpora lutea in any of the groups studied.

Conclusion. PGF$_{2\alpha}$ and LH are uniformly luteolytic in the rabbit. Concomitant administration of estradiol protects corpora lutea from the luteolytic action of these agents. Based upon our limited experience with LH plus previously published studies, estradiol appears consistently effective in antagonizing the luteolytic effect of LH. Whereas estradiol can completely antagonize the luteolytic effect of PGF$_{2\alpha}$, the corpora lutea of some rabbits (for yet unestablished reasons) are not protected at all.

It has been postulated that LH has a direct effect on rabbit ovarian interstitial tissue (21), and that the luteolytic effect of exogenous LH may be due to an alteration of estrogen production and/or release from the follicles below that necessary for maintenance of the original corpora lutea (22). Evidence has been presented showing that estrogen is not only essential for structural maintenance of the corpora lutea, but that it is also steroidogenic (23). It was concluded that estrogen acts directly on the corpus luteum in the rabbit and that 3 µg of exogenous 17β-estradiol/day effectively maintains the weight of corpora in rabbits where the endogenous estrogen source has been destroyed by X-irradiation (23). This conclusion is supported by recent evidence which shows the presence of an estrogen receptor in pseudopregnant rabbit corpora lutea which decreases in concentration as the corpora lutea regress (24). Thus, a substance such as LH, which induces

luteolysis by interference with the secretion of estrogen needed for the morphological maintenance of the luteal cell, and presumably not by a process such as luteal cell destruction, and is capable of being antagonized by exogenous estrogen, should then be antagonized by sufficient estrogen regardless of the dose of LH. Sufficient exogenous estrogen was shown to consistently antagonize the luteolytic properties of LH.

$PGF_{2\alpha}$, however, was not fully antagonized in all the animals at either of the two dosing regimes studied, even though sufficient exogenous estrogen was available. This would indicate that, in certain cases, the luteotropic properties of estradiol are overcome by the seemingly direct action of $PGF_{2\alpha}$ on the corpus luteum and/or the interstitial tissue. LH would seem to have multiple ovarian receptor sites as this substance is able to induce ovulation and luteolysis in the same ovary. If LH has a single receptor site and luteolysis is actually a consequence of ovulation, then it is likely that $PGF_{2\alpha}$ and LH have different receptor sites. In contrast to LH, $PGF_{2\alpha}$ did not induce ovulation with the formation of new corpora lutea.

Examining the results from all treatment groups, it seems important to observe that luteal cells are better able to utilize the luteotropic properties of exogenous estradiol after treatment with LH than they are after treatment with $PGF_{2\alpha}$.

Nevertheless, the luteolytic action of either $PGF_{2\alpha}$ or LH in the rabbit may be very similar in nature and closely connected at the molecular and biochemical level governing steroidogenesis.

It may be that luteal tissue in some cases has been physically altered by $PGF_{2\alpha}$, rendering it incapable of utilizing available estrogen. Studies in pregnant hamsters have shown that luteal cell breakdown begins 5 to 24 hr after initiation of $PGF_{2\alpha}$ treatment, accompanied by a decrease in peripheral plasma and ovarian tissue progesterone levels and finally pregnancy termination (3). However, implants were maintained in hamsters receiving $PGF_{2\alpha}$ and a luteotropic complex (FSH + prolactin). Since this "tropic" support could be overcome by increasing the dose of $PGF_{2\alpha}$, a competitive interaction of $PGF_{2\alpha}$ with luteotropic requirements was suggested (25). In addition, exogenous progesterone will maintain pregnancy in $PGF_{2\alpha}$-treated hamsters and rats (3, 8).

It is also known that exogenous estrogen is luteotropic in the rat (26, 27) and will maintain pseudopregnancy 2–3 times the duration of a "normal" pseudopregnancy (28). These high levels of estrogen apparently act at the hypothalamic level, allowing prolactin release. Perhaps, as was seen in the rabbit, estrogen is also in some way antagonizing a prostaglandin ($PGF_{2\alpha}$), which may be the natural regulator of the corpus luteum life span. Recent studies measuring the concentration of $PGF_{2\alpha}$ in uterine endometrium have suggested a correlation between luteal demise and elevated levels of this prostaglandin (29).

In rats and rabbits, strong evidence argues against the involvement of the hypothalamic–pituitary axis in the mediation of the $PGF_{2\alpha}$ effect (30). However, a common factor for progesterone, estrogen, and the luteotropins (prolactin and FSH) is their capability of providing a hyperemic condition in reproductive tissues. In the nonpregnant ewe, exogenous estrogen was shown to increase blood flow in the uterus as much as five fold within 2 hr after administration (31). Since estrogen receptor is present in the ovary, estrogen may also enhance ovarian blood flow.

It has been hypothesized that $PGF_{2\alpha}$ acts as a venoconstrictor retarding ovarian or utero-ovarian blood flow, causing altered steroidogenesis and luteal regression (32). Studies in rats and rabbits on the effect of $PGF_{2\alpha}$ on ovarian blood flow demonstrated a reduced utero-ovarian flow rate (33) and, in rabbits, simultaneous luteal regression (13). In this study the hyperemic properties of estrogen may have partially counteracted the effects of altered blood perfusion on ovarian and luteal tissue brought about by $PGF_{2\alpha}$.

Summary. Administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or luteinizing hormone (LH) induces luteolysis in the rabbit. The present study was designed to compare the effect of estrogen treatment on the luteolytic action of

$PGF_{2\alpha}$ and LH in pseudopregnant Dutch Belted rabbits. $PGF_{2\alpha}$ (500 μg administered subcutaneously twice a day on days 9 through 12 of pseudopregnancy) reduced ($p < .001$) corpora lutea weights by day 14 and lowered peripheral plasma progestin levels on days 11 ($p < .001$) and 14 ($p < .05$). LH (50 μg intravenously on day 9) comparably reduced corpora lutea weights although plasma progestin levels increased due to LH-induced ovulations. In animals receiving 17β-estradiol (20 μg/day subcutaneously on days 9 through 12) plus LH, corpora lutea weights did not differ significantly from controls. Corpora lutea in rabbits receiving 17β-estradiol plus $PGF_{2\alpha}$ were heavier ($p < .05$) than in animals receiving $PGF_{2\alpha}$ alone but less ($p < .05$) than controls. This luteotropic effect of estradiol was also reflected by the presence of significant ($p < .05$) plasma progestin concentrations on days 11 and 14. A 5-fold increase in the dose of $PGF_{2\alpha}$ did not completely overcome the luteotropic effect of 17β-estradiol.

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