

PROLONGATION OF PSEUDOPREGNANCY BY GONADOTROPINS
IMPLANTED IN THE OVARIAN BURSAE OF RATS

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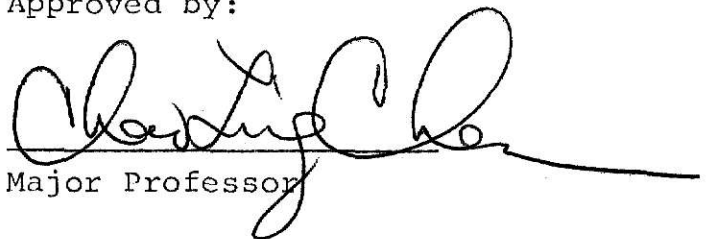
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INTRODUCTION

The functional life of the corpus luteum of ovulation can be extended in rats by pregnancy, pseudopregnancy, or lactation (1). Hypophysectomy of pseudopregnant rats causes rapid regression of the corpora lutea (2) and abolishes the secretion of progestational steroids (3, 4), suggesting that pituitary hormone(s) stimulate luteal growth and function. Because of its ability to induce pseudopregnancy (5) and maintain both the decidual reaction and pregnancy in hypophysectomized rats (3, 6, 7), several groups consider prolactin the sole luteotropin of the rat. However, prolactin, unlike LH, does not exert a direct stimulatory action on progesterone secretion (8, 9). In addition, prolactin does not support a maximal decidual response in hypophysectomized, pseudopregnant rats unless hormones with LH or estrogenic activity are supplied (2, 10), and both LH and FSH are needed along with highly purified prolactin to maintain pregnancy in rats hypophysectomized during early pregnancy (11). Therefore, probably a luteotropic complex, instead of a single pituitary hormone, maintains luteal function during pregnancy and pseudopregnancy.

The present study attempts to determine the effect of pituitary gonadotropins, either singly or combined, on the life span of the corpus luteum by measuring the duration of pseudopregnancy in rats. Hormones were implanted in the ovarian bursae to avoid the effects of acute systemic administration on endogenous gonadotropin secretion (12).

MATERIALS AND METHODS

Animals. Four experiments were conducted with 541 female Sprague-Dawley rats weighing 200-300 g. Rats were housed in a light-controlled (light on from 5 A.M. to 7 P.M. daily), air-conditioned room. Food and water were available ad libitum. Vaginal smears were taken by lavage technique every morning for at least 2 consecutive estrous cycles, and only rats undergoing regular 4- or 5-day cycles were used. Pseudopregnancy was induced by stimulating the cervix with a glass rod on the day(s) of vaginal cornification. The appearance of leukocytes in the vaginal smears marked day 1 of PSP; the first proestrus smear thereafter marked the terminal day (13).

Hormones, cholesterol and stainless-steel tubing. Ovine prolactin (NIH-P-S9), LH (NIH-LH-S18), and FSH (NIH-FSH-S9) were gifts of the Endocrine Study Section, National Institutes of Health, Bethesda, Md. Cholesterol was purchased from Sigma Chemical Co., St. Louis, Mo. Stainless-steel tubing (hypodermic-type 304) was purchased from Small Parts Inc., Miami, Fl.

Implantation of hormone in the ovarian bursae. Prolactin, LH, or FSH was ground in a jade grinder with or without cholesterol. In most hormone-cholesterol implants, the ratio of hormone to cholesterol was 1:1 (w/w), except the 3 μ g dose, where the ratio was 1:9. Twenty- (ID, 0.023") or 22-gauge (ID, 0.016") stainless-

steel tubes were tamped a known number of times into hormone or hormone-cholesterol mixture. A plunger was used to eject the hormone pellet for weighing. This procedure was done 5 times; weights were averaged to arrive at the dosage of each hormone. Rats were anesthetized lightly with ether, and dorsolateral incisions were made to expose the ovaries. A small hole was made in the ovarian bursae with ophthalmic scissors, and the hormone pellet was ejected into place with the plunger. Gonadotropins were implanted to determine whether they could: (a) reinforce the growing corpus luteum (day 4); (b) prevent regression of the corpus luteum (day 7); and (c) reactivate the regressing corpus luteum (day 9) (14, 15). Gonadotropins were also implanted on 3 consecutive days (days 4-6) to determine whether the effect of a single implant (day 4) could be enhanced.

In experiment 1, pseudopregnant rats received on day 7 of PSP one of several doses of prolactin, LH, or FSH. In experiment 2, pseudopregnant rats received on day 4, 7, or 9 of PSP or consecutively on days 4, 5, and 6 one of several doses of each gonadotropin-cholesterol mixture. Prolactin-, LH-, and/or FSH-cholesterol mixtures were implanted simultaneously on day 7 or 9 of PSP in experiment 3. In this experiment, each rat received equal amounts of either 2 or 3 different hormones (i.e., prolactin and LH or FSH; LH and FSH; or prolactin, LH, and FSH). In experiment 4, each of 15 rats received on day 7 of PSP 17 μ g of LH or FSH mixed with cholesterol or 68 μ g of LH alone. Twenty-four hr later, rats were sacrificed, and their oviducts were

removed, mounted on a glass slide, and examined microscopically for ova.

Statistical analysis. Factorial design was used for the analysis of variance to determine the effects of hormone, dosage, and time of treatment. Duncan's New Multiple Range Test (16) was used to determine significant differences among treatments, dosages, and times of treatment. Differences were considered significant when p values were less than 0.05.

RESULTS

Hormones implanted without cholesterol (Table 1). Of the 5 doses of prolactin implanted, none affected the duration of PSP. LH at doses of 34 and 67 μ g significantly prolonged PSP to 16.3 and 20.7 days, respectively. FSH at a dose of 34 μ g had no effect on PSP, but 67 μ g significantly prolonged PSP to 18.0 days.

Hormone-cholesterol implantation (Table 2). In this experiment, cholesterol was used as a diluent to permit implantation of small doses of hormone. Implantation of cholesterol had no effect on PSP. Prolactin likewise had no effect on the duration of PSP regardless of dosage or day implanted. These results are similar to those of previous experiment, where no cholesterol was added (Table 1). Three μ g of LH implanted on day 7 had no effect on PSP, but when the dose was increased to 17 μ g, PSP was significantly prolonged to 18.3 days. Increasing the dose above 17 μ g did not further increase the efficacy of LH (Table 2). Results obtained from implanting the cholesterol mixture were not significantly different from those when LH was implanted alone. Various doses of LH implanted on day 4 significantly prolonged PSP as did implantation of LH on days 4, 5, and 6. However, implantations on days 5 and 6 did not augment the effect of implantation on day 4. Fifty-six and 156 μ g of LH were more effective in prolonging PSP when implanted on day 7 than on day 4. On the other hand, even 156 μ g of LH, the highest dose implanted on day 4 or

7, had no effect on PSP when implanted on day 9.

Effects of FSH implantation were similar to those of LH implantation: 3 μ g of FSH implanted on day 7 had no effect on PSP, but at a dose of 17 μ g or more, FSH consistently prolonged PSP whether it was implanted on day 4 or day 7, or consecutively on days 4, 5, and 6. Moreover, 156 μ g of FSH implanted on day 9 had no effect on PSP. However, lower doses of FSH (17 and 56 μ g) implanted on day 7 were less effective than comparable doses of LH. The effect of FSH implanted on day 7 increased progressively with dose: PSP was prolonged from 12.2 to 14.3 days with 17 μ g and to 17.6 days with 112 μ g.

Simultaneous implantation of gonadotropic hormones (Table 3).

This experiment was designed to determine whether simultaneous implantation of equal amounts of prolactin, LH, and/or FSH would enhance the effect of a single LH or FSH implant. No synergism on day 7 of PSP among the gonadotropins was observed. Simultaneous implantation of 3 μ g each of prolactin, LH, and/or FSH had no effect on the duration of PSP. That was also true when the hormones were implanted alone (Table 2). However, at doses of 40, 56, or 80 μ g, both LH and FSH significantly prolonged PSP whether implanted alone (Table 2) or together (Table 3). The effect of simultaneous implantation of LH with prolactin and/or FSH were not significantly greater than LH implanted alone, and FSH implanted with prolactin was not significantly different from FSH implanted alone. Simultaneous implantation of prolactin, LH,

and/or FSH on day 9 had no effect on PSP (Table 3) as well as those hormones were implanted alone (Table 2).

Ovulatory response after LH or FSH implantation. No ova were found in the oviducts of 15 pseudopregnant rats 24 hr after implanting on day 7 of PSP 17 μ g of LH, 17 μ g of FSH, or 68 μ g of LH. These results suggest that the stimulatory effect of LH or FSH on PSP is not due to ovulation and formation of new corpora lutea.

DISCUSSION

This study demonstrates that implantation in the ovarian bursae of LH or FSH, but not prolactin, prolongs PSP. Similar findings have been reported by Shaikh and Yoshinaga (17), who showed that iv or ip injection of 25 μ g LH on day 4 or on day 6 prolongs PSP to 16.2 and 16.8 days, respectively. From these observations it can be inferred that LH or FSH extends the life span of the corpus luteum in pseudopregnant rats. When implanted on day 4 or on day 7 of PSP, either LH or FSH prolongs PSP, suggesting that both hormones can stimulate the developing corpus luteum or reactivate the early regressing corpus luteum. Toyoda (18) showed that the size of the corpus luteum first exceeds that of the corpus luteum of ovulation on day 4 of PSP. Also on day 4, the ratio of progesterone to 20 α -hydroxypregn-4-en-3-one shifts sharply in favor of progesterone (14). On the other hand, changes in the level of progesterone in ovarian venous blood suggest that regression in the corpus luteum of PSP begins on day 7 (15). Implantation of LH or FSH on day 9 did not affect the duration of PSP. Therefore, apparently once the regression of the corpus luteum has progressed 2 days, even high doses of exogenous LH or FSH cannot reverse the process.

Although prolactin has been considered the only luteotropin in rats, recent studies show that LH is also essential for luteal function (10, 19, 20, 21, 22). LH increases progesterone synthesis by luteinized ovarian tissue in vitro. The steroidogenic

effect of LH also has been confirmed in vivo by an elevation in the level of progesterone in ovarian venous blood after a single iv injection of LH (8, 9). In addition, anti-LH serum administered to rats during the first half of pregnancy terminates pregnancy (23) and reduces progesterone secretion 80% within 24 hr (24). These data, which demonstrate that functional corpora lutea depend on LH to maintain the high progesterone secretion needed during gestation, are consistent with the finding that LH implanted in the ovarian bursae can prolong PSP.

The results also show that 17 μ g or more of FSH prolongs PSP. Greenwald and Johnson (25) reported that 200 μ g of FSH administered by sc injection in conjunction with prolactin maintained the normal weight of embryonic swellings in rats hypophysectomized on day 6 of pregnancy. They attributed the efficacy of 200 μ g of FSH to LH contamination. However, in the present study, the effects of FSH cannot be attributed to LH contamination, for NIH-FSH-S9 contains less than 1% LH. Therefore, within the effective dose range (17-156 μ g FSH), LH content was less than 3 μ g, an amount that did not prolong PSP. Ahmad, Lyons and Papkoff (11) have shown that the combination of highly purified prolactin and LH is incapable of maintaining pregnancy in rats hypophysectomized during the first half of pregnancy unless FSH is administered, indicating that FSH is also involved in regulating luteal function.

Exogenous prolactin appears unable either to prolong the life span of the corpus luteum or to enhance the presumed luteo-

tropic effect of LH or FSH (Table 3). However, these results alone do not preclude a possible luteotropic role for prolactin. It is likely that endogenous prolactin secretion is sufficient to permit synergism with implanted gonadotropins, for in the presence of a low level of prolactin and rising serum LH PSP can be induced and maintained (26). After PSP is initiated, the daily nocturnal surge of prolactin is probably sufficient to maintain PSP (27). Freeman and Neill (27) reported that, toward the end of PSP, prolactin still has high daily nocturnal surges, suggesting that regression of the corpus luteum is not due to lack of endogenous prolactin support. In view of these data, exogenous prolactin would not be expected to prolong PSP.

The present study suggests that LH and FSH are also involved in the regulation of luteal function in pseudopregnant rats. How LH and FSH exert their luteotropic effects remains to be demonstrated, but the effect of these two hormones implanted in the ovarian bursae is not due to ovulation and subsequent formation of corpora lutea. Studies to determine the serum levels of estrogen and progesterone after implantation of LH or FSH in the ovarian bursae are in progress.

TABLE 1. Effects of prolactin, LH, or FSH implanted in the ovarian bursae on the duration of pseudopregnancy¹

Treatment	Duration of pseudopregnancy
	$\bar{x}$ days $\pm$ SE (<u>n</u>)
Sham operation	12.10 $\pm$ 0.29 (10)
Prolactin	
67 μ g	12.33 $\pm$ 0.49 (6)
105 μ g	12.75 $\pm$ 0.25 (4)
215 μ g	13.67 $\pm$ 1.17 (6)
321 μ g	12.80 $\pm$ 0.55 (10)
642 μ g	12.90 $\pm$ 0.38 (10)
LH	
34 μ g	16.33 $\pm$ 1.89 (6)*
67 μ g	20.67 $\pm$ 0.88 (6)*
FSH	
34 μ g	11.67 $\pm$ 0.21 (6)
67 μ g	18.00 $\pm$ 1.44 (6)*

¹Hormones were implanted on day 7 of pseudopregnancy.

*Significantly ($p < 0.01$) different from sham-operated control.

TABLE 2. Effects of prolactin, LH, or FSH implanted in the ovarian bursae on the duration of pseudopregnancy

Day of PSP hormone implanted	Amount ¹ of hormone implanted (µg)	Duration of pseudopregnancy $\bar{x}$ days $\pm$ SE (n)		
		Prolactin	LH	FSH
4	---	---	---	---
	56	---	15.40 $\pm$ 0.75 (5) ^a	14.20 $\pm$ 1.20 (5) ^a
	112	---	17.75 $\pm$ 1.33 (8) ^a	15.43 $\pm$ 1.09 (7) ^b
	156	12.32 $\pm$ 0.30 (19) ^c	15.80 $\pm$ 0.46 (20) ^a	16.17 $\pm$ 0.54 (18) ^a
4	156	12.50 $\pm$ 0.34 (6) ^c	14.33 $\pm$ 1.93 (6) ^a	15.60 $\pm$ 1.44 (5) ^a
5	112			12.36 $\pm$ 0.19 (33) ^c
6	112			---
7	---	---	---	---
	3	12.83 $\pm$ 0.48 (6) ^c	12.17 $\pm$ 0.31 (6) ^c	12.17 $\pm$ 0.17 (6) ^c
	17	12.80 $\pm$ 0.37 (5) ^c	18.33 $\pm$ 1.41 (6) ^a	14.33 $\pm$ 0.84 (6) ^b
	34	12.00 $\pm$ 0.32 (5) ^c	16.00 $\pm$ 1.29 (6) ^a	14.83 $\pm$ 1.54 (6) ^a
	56	12.60 $\pm$ 0.93 (5) ^c	19.17 $\pm$ 0.83 (6) ^a	14.67 $\pm$ 0.49 (6) ^b
	80	14.14 $\pm$ 0.59 (7) ^c	17.67 $\pm$ 0.61 (6) ^a	16.17 $\pm$ 0.98 (6) ^a
	112	13.75 $\pm$ 0.59 (8) ^c	18.18 $\pm$ 0.57 (11) ^a	17.60 $\pm$ 0.74 (10) ^a
	156	12.14 $\pm$ 0.60 (7) ^c	18.13 $\pm$ 0.74 (8) ^a	17.38 $\pm$ 0.98 (8) ^a
	321	12.40 $\pm$ 0.51 (5) ^c	---	---
	---	---	---	---
9	---	---	---	---
	80	13.33 $\pm$ 0.33 (6) ^c	13.17 $\pm$ 0.40 (6) ^c	13.00 $\pm$ 0.40 (6) ^c
	156	12.67 $\pm$ 0.33 (6) ^c		12.47 $\pm$ 0.18 (45) ^c

¹ Hormones were mixed with equal amounts of cholesterol except for the 3 µg dose, where the ratio of hormone to cholesterol was 1:9 (w/w). Hormone-cholesterol mixtures were implanted in both ovarian bursae.

² Includes unoperated, sham-operated, and cholesterol-implanted rats.

⁺ p<0.05 vs. corresponding doses of the same hormone implanted on day 4.

⁺⁺ p<0.05 vs. 17, 34, and 56 µg of FSH implanted on day 7.

As determined by Duncan's New Multiple Range Test, groups sharing common superscripts are not significantly (P>0.05) different.

TABLE 3. Effect on the duration of pseudopregnancy of equal amounts of gonadotropins implanted simultaneously in the ovarian bursae

Day of PSP Amount ¹ of hormone implanted (μ g)	Duration of pseudopregnancy $\bar{x}$ days $\pm$ SE (n)			
	Prolactin-LH	Prolactin-FSH	LH-FSH	Prolactin-LH-FSH Control ²
7	---	---	---	12.40 $\pm$ 0.18 (45)
3	12.33 $\pm$ 0.42 (6)	13.17 $\pm$ 0.75 (6)	11.17 $\pm$ 0.40 (6)	13.83 $\pm$ 0.54 (6) ---
40	16.80 $\pm$ 0.37 (5)*	14.80 $\pm$ 0.73 (5)*	18.17 $\pm$ 0.79 (6)*	19.67 $\pm$ 1.48 (6)* ---
56	15.60 $\pm$ 1.36 (5)*	15.83 $\pm$ 0.75 (6)*	19.20 $\pm$ 0.49 (5)*	18.83 $\pm$ 0.91 (6)* ---
80	18.20 $\pm$ 0.80 (10)*	17.70 $\pm$ 0.47 (10)*	19.00 $\pm$ 0.33 (10)*	19.60 $\pm$ 0.62 (10)* ---
9	14.22 $\pm$ 1.34 (9)	13.29 $\pm$ 1.13 (7)	14.00 $\pm$ 1.00 (6)	12.60 $\pm$ 0.25 (5) 12.47 $\pm$ 0.18 (45)

¹ Hormones were mixed with equal amounts of cholesterol except for the 3 μ g dose where the ratio of hormone to cholesterol was 1:9 (w/w). Hormone-cholesterol mixtures were implanted in both ovarian bursae.

² Includes unoperated, sham-operated and cholesterol-implanted rats.

*Significantly ($P < 0.01$) greater than control.

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PROLONGATION OF PSEUDOPREGNANCY BY GONADOTROPINS
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AN ABSTRACT OF A MASTER'S THESIS

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The effect on the duration of pseudopregnancy (PSP) of prolactin, LH, FSH, or various combinations of those hormones was studied in female rats. Hormones were implanted in the ovarian bursae on day 4, 7, or 9 of PSP or consecutively on days 4, 5, and 6. Prolactin, in doses ranging from 3 to 642 μg , and LH or FSH, at a dose of 3 μg , had no effect on PSP. However, 17 μg or more of LH implanted on day 4 or 7 significantly ($p < 0.01$) prolonged PSP to 16 and 18 days, respectively. High doses of FSH ($> 80 \mu\text{g}$) likewise prolonged PSP, but lower doses (17 and 56 μg) implanted on day 7 were less effective. The effect of consecutive (days 4-6) implantation of LH or FSH was similar to that of a single (day 4) LH or FSH implant. There was no synergism among prolactin, LH, and/or FSH when equal amounts of those hormones were implanted simultaneously on day 7 or 9. Neither LH nor FSH, when implanted on day 9, affected PSP. Ova were not found in 15 rats 24 hr after implantation on day 7 of 17 μg or 68 μg of LH or 17 μg of FSH, suggesting that LH- or FSH-induced prolongation of PSP was not due to the formation of corpora lutea. Results of this study show that LH or FSH, but not prolactin, when implanted in the ovarian bursae, prolongs pseudopregnancy.