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Effects of Ovarian Steroids on Luteal Function: Prevention of Luteolysis following LH-Neutralization in the Pseudopregnant Rat[1] (41436)

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Abstract. The ability of estradiol (E) and progesterone (P) to act as luteotrophins in place of LH was investigated in pseudopregnant (PSP) rats. The length of PSP was extended by either hysterectomy (hyst.) or by the experimental induction of decidual tissue (DT) on Day 5 (Day 1 = ovulation). On Day 9, LH was removed from circulation by a sc injection of a specific LH antiserum (LHAS) and each rat was simultaneously treated with various dosages of E and/or P. Control rats were treated with normal horse serum (NHS) and/or oil vehicle. Blood samples were collected at 0, 24, and 72 hr post-treatment for the estimation of serum P levels. Day 5 hyst. rats treated with any dose (0.1–10.0 μg) of E or P (2 mg), alone or in combination, and simultaneously injected with NHS or LHAS exhibited elevated P levels through Day 12 and an extended diestrous cycle. In contrast, oil-treated, LHAS-injected rats underwent immediate luteolysis. DT-bearing PSP rats treated with 10 μg of E failed to undergo luteolysis following LHAS treatment; however, neither the lower E dosages nor P were able to prevent corpus luteum regression in these rats. The placement of estradiol (5 μg) pellets under the ovarian bursa maintained luteal function in both hyst. and DT-bearing PSP rats after the Day 9 LHAS injection. To determine if E prevented or merely delayed LH-dependent P secretion, 10.0 μg of E was administered on Day 9 and LHAS injected on Day 12. The E treatment effectively overrode the requirement for LH in the hyst.-PSP rat. In contrast, the DT-bearing PSP animals underwent luteolysis following the Day 12 LHAS treatment. NHS injections had no effect on luteal function. The results of these studies suggest that both E and P can serve as direct luteotrophins, replacing the requirement for LH by the rat corpus luteum. Higher doses of E are necessary in DT-bearing PSP rats than in hyst. animals to maintain luteal function. Also, while E eliminates the need for LH in the hyst.-PSP rat, it merely delays the luteal dependency on LH in the DT-bearing rat.

Although prolactin (PRL) is recognized as an essential luteotrophin throughout pseudopregnancy (PSP) (1, 2), the rat corpus luteum (CL) also becomes crucially dependent on luteinizing hormone (LH) for the support of progesterone secretion after Day 9 (Day 1 = ovulation) (3–5). It has been demonstrated that neutralization of circulating LH at any time after Day 9 of PSP will result in luteolysis. The dependency on LH by the CL has been postulated to be necessary to increase the intraluteal luteal levels of estrogens (6) which, in turn, are responsible for the support of progesterone secretion (4, 7, 8). Chronic estrogen treatment of pregnant rats prevented luteolysis following LH neutralization with a specific LH antiserum (LHAS) (7). However, it was not clear whether estrogen was acting directly on the CL or through the placental unit to support luteal function. Preliminary studies in this laboratory suggested that ovarian steroid treatment of PSP rats could

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also prevent LHAS-induced luteolysis, in either the absence or presence of the uterus. The present studies were undertaken in order to define the luteotrophic effectiveness of estrogen and progesterone treatment in the PSP rat in which the length of PSP was extended by either hysterectomy (9) or decidual tissue (DT) induction (10).

Materials and Methods. *Animals.* Adult female rats (Holtzman Co., Sprague–Dawley) weighing between 200 and 300 g were used. Rats were housed under a controlled photoperiod of 14 hr light/day (lights on at 0500 hr) and temperature (23°) with food and water available *ad libitum*. All animals exhibited at least two normal estrous cycles prior to use. Pseudopregnancy was induced by cervical stimulation with a glass rod on the afternoon of proestrus and morning of estrus. The last day of vaginal cornification was denoted Day 1 of PSP.

Surgery. Hysterectomy or the experimental induction of decidual tissue on Day 5 were performed via a midventral laparotomy in lightly etherized rats using clean surgical techniques as previously described (9, 11). The experimental induction of DT was performed by traumatizing each horn of the uterus with a burred needle. In selected experiments, the ovarian bursa was exposed through bilateral dorsal flank incisions for cholesterol or estrogen pellet placement.

Hormone and antiserum treatment. 17β-Estradiol (E) and progesterone (P) were dissolved in sesame oil at the various doses (E:0.1, 1.0 and 10 μg/0.2 ml volume; P: 2.0 mg/0.2 ml volume) used in these experiments. Oil vehicle alone served for all the control injections. An antiserum (equine anti-bovine) to luteinizing hormone (LHAS) was injected sc in a 0.5-ml volume to test for LH dependency. The specificity and characteristics of the LHAS have been previously described (12–14). Normal horse serum (NHS) served as the vehicle control.

Cholesterol/estradiol pellets containing 5 μg of E (E5) were used in selected experiments to induce a high, intraluteal level of E. The pellets were made by mixing together finely ground cholesterol and estradiol moistened with acetone. The resultant paste was rolled into cylinders approximately 1 mm in diameter. After drying overnight, small pellets were cut from the cholesterol cylinders, each weighing approximately 1 mg and containing the desired E concentration.

Blood collection and hormone assay. Blood samples (0.5 ml) were collected by direct jugular puncture from rats held under ether anesthesia. Serum was collected and stored at −20° until assayed.

Serum P levels were estimated by radioimmunoassay as previously described and validated (15). Assay linearity ranged from 5 to 200 ng/ml serum and all values are expressed uncorrected for procedural loss (≤7%).

Experimental protocol. On Day 5 of PSP, rats were either hysterectomized or subjected to DT induction. Subsequently, on Day 9, each rat received a single sc injection of either 0.1 μg E, 1.0 μg E, 10 μg E, 2 mg P, 2 mg P plus 1 μg E, or oil. In selected experiments, 5-μg pellets of estradiol (E5) or cholesterol control pellets were placed under each ovarian bursa. Immediately following hormone treatment, each rat received a sc injection of LHAS or NHS. Blood samples were collected on Days 9, 10, and 12 of PSP for serum P determinations and the PSP length was monitored by vaginal smears.

Statistics. All values are expressed as the mean ± SEM. Intergroup differences were assessed by the Student's *t* test or Newman–Keuls exam where appropriate with a significance level set at $P \leq 0.05$.

Results. *Hysterectomized-PSP rats.* All rats hysterectomized on Day 5 of PSP and treated with oil, or any dose of E, P, or P plus E and subsequently injected with NHS maintained elevated serum P levels (Fig. 1) and exhibited an extended PSP length (Table I). In contrast, oil-treated, LHAS-injected rats underwent luteolysis within 24 hr as evidenced by the dramatic fall in serum P levels (Fig. 1) and a shortened diestrus length (Table I). However, simultaneous treatment of Day 5 hysterectomized rats with either 0.1 μg E, 1 μg E,

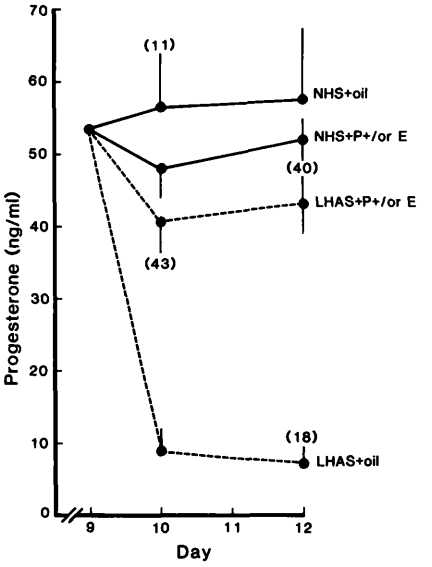

FIG. 1. Summary of the effects of a single injection of estradiol (E: 0.1–10.0 μg) progesterone (P: 2 mg) or combined (1.0 μg E plus 2.0 mg P) on serum P levels in Day 5 hysterectomized PSP rats treated with LHAS or NHS on Day 9. All values are represented as group means (±SEM), numbers denote animals used per group. No significant differences were found between Day 12 serum P levels except for LHAS/oil-treated rats ($P \leq 0.001$).

10 μg E, 2 mg P, or 2 mg P plus 1 μg E in combination, effectively prevented LHAS-induced luteolysis (Fig. 1, Table I).

DT-PSP rats. Rats bearing a decidualized uterus exhibited serum P levels and extended PSP lengths comparable to those of controls following NHS treatment, regardless of the concomitant steroid treatment (Fig. 2, Table II). In contrast, LHAS treatment induced luteolysis in all DT-PSP rats receiving either oil, P or E injections, unless the rats were treated with 10 μg of E. In the later group, serum P levels were maintained and PSP lengths were extended similar to those of controls (Fig. 2).

Effects of intrabursal placement of estrogen pellets on luteal function. In an attempt to directly increase the intraluteal concentration of E, DT-PSP and Day 5 hysterectomized rats received cholesterol or E5 pellets under each ovarian bursa on Day 9 of PSP. Rats were subsequently treated with either NHS or LHAS on Day 9 of PSP. Cholesterol pellet-bearing rats (both DT and hysterectomized) treated with NHS maintained elevated P levels through Day 13 (Table III). In both groups, LHAS induced luteolysis unless estradiol pellets were present. In these groups, serum P levels were maintained at control levels in the hysterectomized group while in the DT-PSP rats, a decline in serum P levels was observed (Table III), but the drop was less severe than that exhibited by the corresponding cholesterol pellet group.

Effects of estradiol treatment on the delayed appearance of LH dependency. To clarify further the protective role of estradiol in preventing LHAS-induced luteolysis, Day 5 hysterectomized and DT/PSP rats received a single sc injection of E on Day 9 and either a LHAS or NHS injection on Day 12 to determine if E treatment actually substituted for LH or merely delayed the timing of the requirement for LH.

Treatment with 10 μg of E maintained normal serum P levels in both hysterectomized and DT-PSP rats treated with NHS on Day 12 (Table IV). In hysterectomized rats, this treatment effectively prevented Day 12 LHAS-induced luteolysis, maintaining serum P and diestrus length at control values. In contrast, LHAS induced luteolysis and shortened diestrus length in DT-PSP rats previously treated with E10. Thus, while E10 effectively overrode the requirement for LH in the hysterectomized rats, it merely delayed the requirement for LH in the DT-PSP rat.

Discussion. The results of this study indicate that ovarian steroids can substitute for LH and serve as luteotrophins in the PSP rat. While physiological doses of E (0.1 μg) effectively prevented LHAS-induced luteolysis in the hysterectomized rat, a much higher dose (10.0 μg E) was necessary to achieve similar results in rats bearing a decidualized uterus. In addition, while P alone (Fig. 1, Table I), or in combination with E, maintained luteal function in LHAS-treated hysterectomized rats, the same hormone regimes were ineffective in the DT-PSP rat (Table II). Thus, it appears that the continued presence of the uterus

TABLE I. EFFECTS OF STEROID HORMONES ON THE MAINTENANCE OF LUTEAL FUNCTION IN THE DAY 5 HYSTERECTOMIZED PSP RAT TREATED WITH LHAS OR NHS

Steroid treatment[a]	LHAS/NHS[b]	N	Serum progesterone (ng/ml)[c]			Diestrus length[d] (days)
			Day 9	Day 10	Day 12	
None	NHS	7	45.1 ± 3.0	46.0 ± 5.9	43.4 ± 9.8	21.0 ± 1.2
Oil	NHS	4	69.9 ± 9.0	74.9 ± 11.7	81.7 ± 21.5	20.8 ± 0.8
None	LHAS	12	56.8 ± 4.2	11.3 ± 1.7	6.5 ± 1.0	13.3 ± 0.1
Oil	LHAS	6	58.8 ± 4.8	6.1 ± 0.7*	9.5 ± 0.5	15.0 ± 0.6*
Estradiol (0.1 μg)	NHS	8	56.2 ± 4.9	49.8 ± 4.2	48.2 ± 4.1	21.3 ± 0.7
	LHAS	7	42.7 ± 5.7	39.0 ± 5.0	33.8 ± 4.8	20.1 ± 1.7
Estradiol (1.0 μg)	NHS	8	47.5 ± 5.3	43.5 ± 5.1	60.4 ± 6.9	23.9 ± 1.7
	LHAS	8	49.0 ± 6.5	31.2 ± 5.3	49.4 ± 7.1	22.6 ± 1.0
Estradiol (10 μg)	NHS	8	55.6 ± 3.9	38.2 ± 4.0	47.2 ± 7.2	21.1 ± 1.2
	LHAS	8	63.9 ± 8.5	32.1 ± 5.6	44.9 ± 7.5	20.9 ± 1.8
Progesterone (2 mg)	NHS	8	44.4 ± 4.8	55.9 ± 7.9	44.2 ± 5.3	21.3 ± 1.0
	LHAS	10	53.3 ± 5.8	47.3 ± 7.3	33.9 ± 5.5*	19.6 ± 1.4
Progesterone (2 mg) plus estradiol (1.0 μg)	NHS	10	53.8 ± 5.0	47.7 ± 6.1	60.3 ± 4.9	23.1 ± 1.4
	LHAS	8	50.3 ± 4.3	47.4 ± 6.8	50.4 ± 7.5	23.0 ± 1.7

[a] Rats were treated with a single sc injection of oil vehicle, estradiol (0.1–10.0 μg), progesterone (2.0 mg), or a combination of progesterone and estradiol on Day 9 of PSP.

[b] LHAS and NHS (0.5 ml) were administered sc on Day 9 of PSP.

[c] All values are represented as group means (±SEM). Intergroup differences are denoted as $*P \leq 0.05$.

[d] Diestrus length (days) was counted as the number of days from Day 1 (ovulation) to, but not including, the subsequent estrus period.

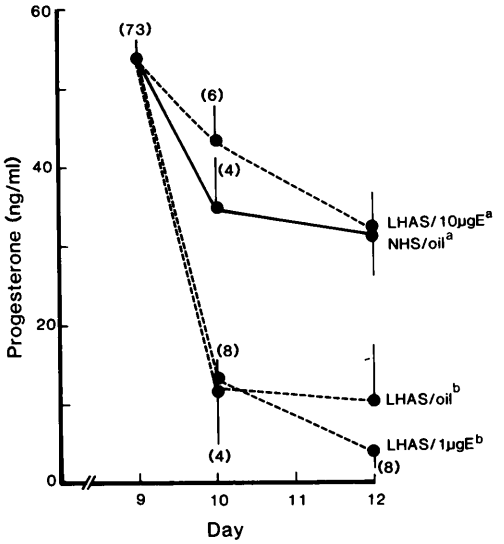

FIG. 2. Effects of estradiol injections (1.0 and 10.0 μg) on serum progesterone levels in NHS- and LHAS-treated, DT-bearing, PSP rats. Both LHAS/NHS and estradiol injections were given on Day 9. All values are represented as group means (±SEM); numbers denote rats per group. Statistical differences on Day 12 are represented as a vs b, $P \leq 0.01$.

(or DT) alters the responsivity of the rat CL to LH neutralization. The results also further the concept that E can serve as a luteotrophin in the rat (7, 8, 16).

Exactly how the employed steroid treatments prevented the luteolytic effects of LHAS is unclear. Progesterone treatment may have been effective in the hysterectomized animal by its aromatization to estrogens (which, in turn, were ultimately responsible for luteal maintenance) or by having a direct effect on CL function. The recent evidence that P can maintain PRL surges (17), and that elevated PRL levels can effectively prevent LHAS-induced luteolysis, may also explain these effects (4, 5). The 100-fold more estrogen required to overcome the luteolytic effects of LHAS in the DT rat, as compared to the hysterectomized rat may be due to uterine or DT retention of the steroid. The fact that the dissolution of the DT (i.e., vaginal bleeding as a result of DT regression) occurred in rats receiving the 10-μg dose of E indicated that the steroid was having some type of

TABLE II. EFFECT OF OVARIAN STEROID HORMONE TREATMENT ON LUTEAL FUNCTION IN THE DT-BEARING PSP RAT FOLLOWING LHAS OR NHS TREATMENT

Steroid treatment	LHAS/NHS	N	Serum progesterone (ng/ml) Day 9	Day 10	Day 12	Diestrus length (days)
Oil	NHS	4	57.5 ± 9.5	34.5 ± 12.3	32.6 ± 10.7	19.5 ± 2.5
	LHAS	4	60.3 ± 7.7	12.0 ± 7.9**	10.7 ± 6.2**	13.3 ± 0.2***
Progesterone (2.0 mg)	NHS	4	89.8 ± 17.1	78.7 ± 13.9	41.5 ± 6.1	19.5 ± 2.0
	LHAS	8	76.9 ± 9.9	74.3 ± 12.1	6.0 ± 1.9***	14.4 ± 1.0**
Progesterone (2.0 mg) plus estradiol (1.0 μg)	NHS	10	48.7 ± 5.6	55.7 ± 6.9	34.4 ± 5.0	18.3 ± 1.3
	LHAS	13	68.3 ± 7.6	54.5 ± 5.7	4.3 ± 0.8***	15.2 ± 0.6**

Note. All values are represented as the group mean (±SEM). Intergroup differences are denoted as $^{**}P \leq 0.01$; $^{***}P \leq 0.001$. See Table I for description of groups.

TABLE III. EFFECTS OF E5 PELLETS ON SERUM PROGESTERONE LEVELS IN DT-BEARING AND HYSTERECTOMIZED PSP RATS TREATED WITH NHS OR LHAS

Group	Treatment	N	Progesterone (ng/ml)			
			Day 9	Day 10	Day 11	Day 13
DT	Chol/NHS	5	72.9 ± 13.3	60.1 ± 8.7	50.2 ± 3.8	41.5 ± 9.3
	Chol/LHAS	6	80.1 ± 6.0	4.7 ± 0.9***	5.2 ± 1.0***	5.8 ± 0.9***
	E5/NHS	6	68.8 ± 6.3	78.2 ± 4.6	29.0 ± 3.9	57.8 ± 5.7
	E5/LHAS	6	80.2 ± 6.2	25.6 ± 7.7**	29.9 ± 8.2	31.4 ± 7.3**
Hyst	Chol/NHS	5	53.9 ± 3.2	56.8 ± 7.5	58.9 ± 10.3	48.7 ± 6.7
	Chol/LHAS	6	59.7 ± 9.3	11.5 ± 4.1***	14.9 ± 7.8***	11.9 ± 6.7***
	E5/NHS	6	88.0 ± 8.6	79.4 ± 9.5	44.7 ± 8.0	61.8 ± 11.8
	E5/LHAS	6	66.0 ± 6.8	55.4 ± 10.7*	35.1 ± 5.4*	49.3 ± 10.6

Note. DT and hysterectomized rats received an intrabursal cholesterol or E5 pellet (total of 10.0 μg of E) on Day 9 and were simultaneously treated with LHAS or NHS. All serum progesterone values are represented as group means (±SEM). Intergroup differences are demonstrated as $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

effect on the uterus. However, this treatment did not cause a shortening of the PSP length and DT bleeding did not occur in rats bearing E5 pellets. Thus, E may have a direct luteotrophic effect on the rat CL, and higher E doses may be needed to observe this response in animals bearing active, progestational uteri. The uterus may remove E from the circulation which would reduce its luteotrophic effectiveness by lowering the amount which could be concentrated intraluteally.

The present data extend previous observations that E can serve as a luteotrophin in the rat (7, 8, 16). Furthermore, this is the first demonstration that LHAS-induced luteolysis can be overcome by ovarian steroids, especially P, in the PSP rat. This supports the recent observations by Macdonald (18) in which the LH-dependent period of luteal function during pregnancy was maintained by E in the absence of LH. These data support the contention of Gibori *et al.* (15) that LH is needed for production (or concentration) of intraluteal estrogen which is needed to maintain progesterone secretion (8). Furthermore, the demonstration that the direct application of E to the rat CL in the form of pellets prevented the luteolytic effects of LHAS (Table III) add further support to the idea that intraluteal estrogen formation is essential for CL function after Day 9 of PSP (in the absence of the uterus) and that the intraluteal level of E is critical for continued CL function.

A systemic effect of ovarian steroids on CL function cannot be ruled out on the basis of these studies. Previous reports (15, 20) have demonstrated a direct luteotrophic effect of E on CL function in the pregnant rat. In the present study, using rats bearing an *in situ* pituitary gland, the employed doses of E may have induced an increased release of prolactin (21). It has previously been demonstrated that prolactin can block the luteolytic effects of LHAS (4, 5) by delaying the appearance of LH dependency (22) by approximately 3 days. Previous studies (23) have indicated that a single sc injection of PRL administered simultaneously with LHAS to hysterectomized, but not DT-PSP, rats prevented luteolysis. If the E or P treatments given in the hysterectomized rats in the present study induced or extended (17) the prolactin surges, this could also serve as an explanation as to how these steroid treatments were luteotrophic. However, the inability of PRL injections (Garris and Rothchild, unpublished observations) to support P secretion in the DT rat and the inability of four pituitary transplants to block LHAS-induced luteolysis in the PSP rat (5) probably indicates that both estrogen and progesterone work directly on the CL and not through the pituitary gland. The presence of a DT uterus

TABLE IV. EFFECTS OF ESTRADIOL TREATMENT ON THE TIMING OF LH DEPENDENCY IN THE DAY 5 HYSTERECTOMIZED AND DT-BEARING PSP RAT

Group	Treatment	LHAS/NHS	N	Serum progesterone (ng/ml) Day 9	Day 12	Day 13	Day 15	Diestrus length (days)
Hyst	Estradiol (10.0 μg)	NHS	8	83.6 ± 7.8	108.2 ± 3.7	78.5 ± 8.3	75.2 ± 6.1	21.4 ± 0.9
		LHAS	9	87.8 ± 6.0	74.2 ± 12.0*	56.5 ± 9.1	45.4 ± 7.0*	19.4 ± 1.4
DT	Estradiol (10.0 μg)	NHS	5	62.7 ± 7.6	49.0 ± 4.1	44.1 ± 1.2	30.5 ± 6.3	18.4 ± 1.0
		LHAS	6	56.0 ± 5.7	41.7 ± 4.6	3.6 ± 0.6***	4.2 ± 0.4**	15.7 ± 0.8*

Note. The indicated steroid treatment was administered on Day 9 and either NHS or LHAS injected on Day 12. All values are represented as group means (±SEM). Intergroup differences are indicated by $*P < 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

may necessitate that larger doses of PRL be administered to override the combined effects of the uterus and LHAS treatment on CL function.

While the results of the present study suggest that E can serve as a luteotrophin in the rat, the possibility that E treatment merely delays the appearance of LH dependency needs to be investigated further. Previous studies (5) have demonstrated that prolactin can delay the appearance of LH dependency in the PSP rat and that chronic E treatment can restore the need for LH to the proper time (22). The present studies indicate that E can substitute for LH more effectively in the hysterectomized rat than in the DT-PSP rat; however, the reason for this discrepancy is not clear beyond the points previously discussed. Thus, while several reports suggest that E can *substitute* for LH as luteotrophin in the rat (18, 19), its role as an *essential* luteotrophin remains to be clarified.

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