

## Prolactin and Hysterectomy Delay Rather Than Prevent the Critical Need for LH (LH Dependency) in the Luteotrophic Process of the Rat (40865)<sup>1</sup>

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**Abstract.** The corpus luteum (CL) of the pseudopregnant rat normally becomes dependent on LH by Day 9 for the maintenance of progesterone production. The effects of hysterectomy or treatment with prolactin on the development of LH dependency by rat corpora lutea (CL) was studied by subjecting rats to one of the following procedures: hysterectomy before the induction of pseudopregnancy ("chronic hysterectomy"); hysterectomy on Day 2 of pseudopregnancy; hysterectomy on Day 5 of pseudopregnancy, combined with prolactin treatment by homotransplantation of a single donor pituitary on Days 2 or 5; hysterectomy on Day 2 of pseudopregnancy combined with homotransplantation of one or four pituitaries on Day 2. In each of these conditions, treatment with an antiserum to LH (LHAS) on Day 9 does not result in luteolysis (5). However, treatment with LHAS on Day 12 was able to cause luteolysis. Early hysterectomy and prolactin, therefore, act similarly to delay rather than prevent the dependency of the rat's corpora lutea on LH as a luteotrophin.

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The corpus luteum (CL) of the rat becomes critically dependent on LH for the maintenance of progesterone production. A single dose of an antiserum to LH (LHAS) causes luteolysis in pregnant rats between Days 8 and 12 (1, 2) and in intact pseudopregnant rats from Day 9 until the end of the luteal lifespan (Day 1 = day of ovulation). Rats subjected to decidual tissue induction or hysterectomy on Day 5 of pseudopregnancy responded similarly to LHAS treatment (3-5). However, if rats were hysterectomized before the induction of pseudopregnancy, LHAS treatment on Days 9 or 11 did not induce luteolysis (3). Prolactin treatment also prevented the luteolytic effect of LHAS on Day 9 (4).

These findings suggested that LH did not become an essential luteotrophin until

about Day 8 of pregnancy or Day 9 of pseudopregnancy (1, 3). Since both prolactin and chronic hysterectomy (i.e., hysterectomy before the induction of pseudopregnancy) could prevent the luteolytic effect of LHAS on Day 9 (3, 4), these treatments may have either abolished the requirement for LH or delayed its onset. Ford and Yoshinaga have shown that LHAS treatment of chronically hysterectomized rats on Day 15 of pseudopregnancy did induce luteolysis (6). This finding suggested to us that hysterectomy probably delayed rather than prevented the dependency of the corpora lutea on LH. We decided to test this possibility and to compare the effect of hysterectomy on LH dependency with that of prolactin.

**Materials and methods. Animals.** Adult female Holtzman (Sprague-Dawley strain) rats weighing 200-300 g were housed at 23°C under a controlled photoperiod of 14-hr light/day (lights on at 0500). Two consecutive estrous cycles were observed before any rat was included in an experiment. Pseudopregnancy was induced by mechanical stimulation of the cervix during proestrus and estrus; the last day of vaginal cornification (day of ovulation) was defined as Day 1 of pseudopregnancy.

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**Hysterectomy and pituitary homotransplantation.** All operations were done with ether as anesthesia and a clean but not aseptic technique. Hysterectomy was done as described previously (7); sham hysterectomy consisted of laparotomy without uterine manipulation. Homotransplanted pituitaries served as the means of prolactin treatment (4, 8–12). The donor pituitaries were inserted under the left kidney capsule of the host immediately after being removed from cyclic rats decapitated during diestrus. When a rat received more than two pituitary transplants, both kidneys were used (4).

**Blood sampling and assay of the serum progesterone level.** Blood samples, usually about 0.5 ml, were drawn by jugular venipuncture with the rat held under light ether anesthesia. The blood was allowed to clot and was centrifuged at 4°C, and the serum stored at –20°C until assay. The serum progesterone level was determined on hexane-extracted serum by radioimmunoassay (13).

**Test for LH dependency.** A single dose of 0.5 ml of a specific horse antiserum to bovine LH (LHAS) was injected sc on Day 12; control treatment was the sc injection of 0.5 ml of normal horse serum (NHS). The immunologic characteristics and biologic effects of the LHAS have been described (1, 14). The primary criterion of a luteolytic effect was a marked and permanent fall in the secretion of progesterone, determined by assay of the serum progesterone level at the time of LHAS injection (0 time) and 24 and 72 hr later (in the rat, changes in the serum concentration of progesterone are directly related to changes in the rate of its secretion (15)). Supporting evidence for luteolysis was the duration of the vaginal diestrus.

**Experimental plan.** The test for LH dependency was carried out on Day 12 of pseudopregnancy in groups of rats subjected to one of the following procedures: hysterectomy 6 weeks or more before the induction of pseudopregnancy ("chronic

TABLE I. THE EFFECT OF HYSTERECTOMY, AND OF PROLACTIN TREATMENT BY MEANS OF PITUITARY HOMOTRANSPLANTATION, ON THE LUTEOLYTIC EFFECT OF A HORSE ANTISERUM TO BOVINE LH (LHAS), INJECTED ON DAY 12 OF PSEUDOPREGNANCY<sup>a</sup>

Experimental conditions	Treatment with LHAS or NHS	N	Serum progesterone <sup>b</sup> (ng/ml) on:			Vaginal diestrus (days) <sup>c</sup>	
			Day 12	Day 13	Day 15		
1. "Chronic" hysterectomy <sup>d</sup>	NHS	5	44.1 ± 12.2	35.4 ± 9.1	29.4 ± 4.0	NO	
	LHAS	5	40.8 ± 2.80	17.4 ± 4.5	7.7 ± 2.8	NO	
2. Hysterectomy on Day 2	NHS	8	41.4 ± 4.0	36.6 ± 4.0	37.8 ± 4.0	22.9 ± 0.6	
	LHAS	7	39.0 ± 14.0	5.3 ± 1.6	7.3 ± 3.0	17.6 ± 0.3	
3. Hysterectomy on Day 5; pituitary homotransplantation on Days 2 or 5 <sup>e</sup>	NHS	16	54.8 ± 3.7	51.4 ± 4.9	42.6 ± 3.9	21.5 ± 0.9	
	LHAS	15	61.6 ± 8.5	13.5 ± 4.0	12.0 ± 3.7	17.9 ± 1.0	
4. Hysterectomy plus pituitary homotransplantation on Day 2:	1. Pituitary	NHS	4	45.1 ± 7.3	70.4 ± 13.4	35.8 ± 5.1	21.3 ± 0.3
		LHAS	3	57.1 ± 11.5	5.8 ± 0.9	4.2 ± 1.0	15.7 ± 0.3
	4 Pituitaries	NHS	3	59.2 ± 5.4	46.9 ± 1.6	39.1 ± 1.4	25.3 ± 2.9
		LHAS	5	81.7 ± 13.4	28.8 ± 10.7	6.1 ± 2.1	20.6 ± 0.5
	(One or four pituitaries combined)	NHS	7	51.2 ± 5.3	60.3 ± 8.6	37.2 ± 2.8	23.0 ± 1.4
		LHAS	8	72.5 ± 9.9	20.1 ± 7.6	5.4 ± 1.3	18.8 ± 1.0

<sup>a</sup> Values are means ± SEM. Day 1 of pseudopregnancy was the last day of vaginal estrus (day of ovulation). LHAS or normal horse serum (NHS), 0.5 ml, was injected sc between 0900 and 1200 hr.

<sup>b</sup> All the Day 13 values in the LHAS-treated groups were significantly different ( $P = < 0.01$  to  $< 0.001$ ) from the Day 13 values in the NHS-treated groups, except for Group 1 and Group 4, four pituitaries. Differences in serum progesterone between LHAS- and NHS-treated groups on Day 15 ( $P = < 0.01$  to  $< 0.001$ ) and in length of diestrus ( $P = < 0.05$  to  $< 0.001$ ) were significantly different in all the groups.

<sup>c</sup> Total length of the vaginal diestrus; NO = not observed.

<sup>d</sup> Hysterectomy before the induction of pseudopregnancy.

<sup>e</sup> Each rat received a single donor pituitary. The time of transplantation had so little effect on the values that the groups were combined.

hysterectomy'); hysterectomy on Day 2 of pseudopregnancy; hysterectomy on Day 5 of pseudopregnancy, combined with homotransplantation of a single pituitary on either Days 2 or 5; hysterectomy, combined with homotransplantation of one or four pituitaries on Day 2 (Table I). In each of these conditions, LHAS treatment on Day 9 did not induce luteolysis (5).

**Results.** In a group of 17 intact pseudopregnant rats, run as a control with those subjected to the procedures just described, the mean ( $\pm$ SEM) serum progesterone levels on Days 9, 10, and 12 were  $54.0 \pm 4.3$ ,  $50.5 \pm 4.3$ , and  $22.2 \pm 4.1$ , respectively. The mean duration of their vaginal diestrus was  $12.2 \pm 0.3$  days. As expected (3), the control (NHS-treated) hysterectomized rats had higher serum progesterone levels on Day 12 and a longer pseudopregnancy than did the intact rats (Table I). The LHAS treatment induced luteolysis not only in all the groups of rats subjected to hysterectomy alone, but also in those subjected to hysterectomy combined with pituitary homotransplantation (Table I).

**Discussion.** The luteolytic response to LHAS treatment on Day 12 in the rats hysterectomized on Day 2 or earlier was not surprising. A closer look at the earlier report (3) showed, in fact, that the corpora lutea of chronically hysterectomized pseudopregnant rats were probably responding to LHAS treatment on Day 11 more than did those treated on either Days 9 or 10. The finding of Ford and Yoshinaga (6), therefore, which implied that the corpora lutea of chronically hysterectomized rats were LH dependent on Day 15, led us to expect that early hysterectomy would delay rather than prevent LH dependency. The effect of pituitary homotransplantation, however, was unexpected. Serum prolactin levels in rats bearing pituitary transplants tend to be constant throughout the day (4, 11) and directly related to the number of pituitaries transplanted (4). One transplant kept the serum prolactin concentration at a level (4) roughly equal to the peaks of the pseudopregnant rat's two daily secretion surges (16). Four transplants raised the level about fourfold (4). Enough prolactin was secreted by only one pituitary

transplant to prevent LH dependency on Day 9 in pseudopregnant rats hysterectomized on Day 5 (4), even when the pituitary was not transplanted until Day 5, or was left in place only between Days 2 and 5 (5). In the presence of the uterus this effect of prolactin was markedly reduced (4). We expected, therefore, that the prolactin secreted by four pituitaries transplanted into rats that were also hysterectomized on Day 2, would certainly postpone LH dependency indefinitely. Since this was not the case (Table I), it is obvious that neither prolactin nor hysterectomy can prevent LH dependency, although they can delay its appearance.

It has been suggested that the requirement of LH by rat corpora lutea may differ in various physiological situations (3). Among pregnant rats, LHAS almost uniformly induces luteolysis when it is injected on Day 9, while among pseudopregnant rats, between 10 and 20% of a group escape its luteolytic effect at this time. Our results imply that what varies among the several kinds of corpora lutea is not the need for LH but *when* the full need for LH is reached. Eventually the corpora lutea in all these conditions would probably become equally dependent on LH. The only exception may be the corpora lutea of the hypophysectomized, pituitary, autotransplanted rat; these may be permanently independent of LH (4, 12; unpublished findings). The *limited* ability of prolactin or hysterectomy to postpone the appearance of LH dependency, therefore, may be connected in some way with the presence of the *in situ* pituitary.

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