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The Progesterone-Sensitive Period of Rat Pregnancy: Some Effects of LHRH and Ovariectomy (39933)

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Administration of LHRH (500 μg at 0800 and 1600 hr) to pregnant rats terminated gestation when given on Day 9, 10, or 11, while no significant effects were observed when the hormone was given on any single day from 6 to 8 or 12 to 16 (1). LHRH treatment on Day 9 was followed by a marked fall in circulating progesterone on Day 10; progesterone was still depressed on Day 11, but it appeared to be returning toward normal pregnancy levels. Progesterone levels after LHRH administered on Days 6, 7, or 8 or on Day 12 or later were not investigated in the earlier study. We have now found that circulating progesterone was also sharply depressed following administration of LHRH on Day 7, which suggests differential progesterone requirements on Days 7 and 9. Also a slight but significant depression has been found after treatment on Day 13. We have, therefore, studied the effects of altering the time of initiation of progesterone support in rats spayed on Days 7 or 10 of gestation.

Materials and methods. Pregnant Charles River CD rats (200-250 g) received LHRH, 500 μg sc, in 0.1 ml of corn oil, at 0800 and 1600 hr on Day 7 of pregnancy (Day 1 = the day vaginal smears were sperm positive). Controls received the corn oil only. All rats were bled by cardiac puncture under light ether anesthesia just prior to the first injection. One group of LHRH-treated rats and one group of controls were again bled 24 hr later (Day 8); two additional groups were bled at 48 hr (Day 9); and the remaining two groups were bled on Day 10, 72 hr post-treatment. Pregnancy was confirmed at autopsy on Day 21. Serum progesterone levels were assessed by the progesterone-binding globulin (PBG) method of Attal and Engels (2), as modified by us (1). Further groups of rats (10 each) were treated on Day 13 of pregnancy after an initial bleeding. Blood samples were again taken on Days 14, 15, and 16 of pregnancy and autopsy was performed on Day 21.

In the second series of experiments, gravid female rats were spayed at 0900 hr on Day 7 or 10 of pregnancy. Replacement therapy, progesterone at 10 mg/day sc in 0.1 of peanut oil, was initiated immediately (0 hr) or at 6, 12, or 24 hr after surgery. Daily injections were continued until sacrifice on Day 20. At autopsy, number of fetuses and weights of the fetal/placental/uterine units were recorded.

Results. I. Effects of LHRH. The administration of LHRH on Day 7 of pregnancy had no statistically significant effect on the outcome of the pregnancy, although the overall pregnancy rate (40/60 rats at term) was somewhat less than that we normally see in rats selected on the day following coitus (Table I). None of the individual groups differed from controls and, when pooled, 21 of 30 control animals were pregnant and 19 of 30 were pregnant in the groups that received LHRH.

None of the initial (Day 7) pairs of bleeding show statistically significant differences in serum progesterone levels between the controls or experimentals, although a high level of variability is evident (Table II). Only data from pregnant animals were analyzed. In the control rats, no significant changes in serum progesterone were recorded from Day 7 to Day 8 or from Day 7 to Day 9. Controls bled on Days 7 and 10 showed a significant decrease, which represented a fall from a high level to a level well within the expected control range of variation. In contradistinction, the administration of LHRH on Day 7 was associated with a marked drop of progesterone on Day 8 (actually, serum progesterone was below detectable levels in six of seven pregnant rats) and on Day 9 (undetectable in four of

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TABLE I. LACK OF EFFECTS ON PREGNANCY OF LHRH INJECTIONS TO FEMALE RATS ON DAY 7 of GESTATION.[a]

	Days of bleeding					
	7 and 8		7 and 9		7 and 10	
Treatment (Day 7)	*N*	*N* Pregnant[b]	*N*	*N* Pregnant[b]	*N*	*N* Pregnant[b]
Controls (corn oil)	10	5	10	8	10	8
LHRH (0.5 mg × 2)	10	7	10	7	10	5

[a] All rats were sacrificed for autopsy on Day 21. Days of bleeding define groups employed for progesterone evaluation.

[b] No significant differences by fourfold contingency tables of Mainland and Murray (12).

TABLE II. EFFECTS OF LHRH ADMINISTRATION ON DAY 7 OF PREGNANCY ON CIRCULATING PROGESTERONE IN RATS.

		Serum progesterone (Mean ng/ml ± SE)			
	N	Day 7	Day 8	Day 9	Day 10
Controls	5	59.8 ± 5.1	55.4 ± 15.2		
LHRH	7	64.6 ± 6.5	1.7 ± 1.7*, **		
Controls	8	58.2 ± 5.4		48.8 ± 9.3	
LHRH	7	87.0 ± 14.2		5.0 ± 2.7*, **	
Controls	8	110.9 ± 9.6			71.4 ± 15.5*
LHRH	5	83.6 ± 10.3			39.8 ± 3.8*

* Significantly different from Day 7 value, with $P < 0.05$, t test.

** Significantly different from control value, with $P < 0.01$, t test.

seven animals). On Day 10, progesterone was again below control levels, but a recovery toward normal was apparent (all pregnant animals had detectable levels).

Virtually all rats that had been treated on Day 13 were pregnant at autopsy on Day 21 (Table III). One rat in the LHRH groups (Day 15 bleeding) was not pregnant at autopsy and one (from Day 16 bleeding) died at the time of cardiac puncture. All pregnancies appeared normal. Control progesterone levels were essentially homogenous on Days 13 and 14; they increased steadily thereafter, with highest levels reached on Day 16. The rise from day 14 to 16 was not seen in the treated rats which remained at or below the Day 13 level; progesterone was not significantly lower than controls on Day 14. LHRH-treated rats had circulating progesterone levels that were lower than initial (Day 13) levels and significantly lower than controls on Days 15 and 16. Thus, LHRH was associated with deccreased levels of circulating progesterone in rats that carried normally to term; however, few animals had depressions of progesterone to levels less than 20 ng/ml.

II. Effects of spaying. No differences in measured parameters of the pregnancies were observed between animals sham-operated on Day 7 and those operated on Day 10 (Table IV). Similar results were obtained in rats treated immediately with progesterone and those in which the progesterone was delayed to 6 hr after operation. The most obvious, and statistically convincing data, followed delay of 12 hr in administration of progesterone. In animals so treated and spayed on Day 7, percentage of pregnancies, number of fetuses, and weights of fetal/placental/uterine units were not significantly different from those in sham-operated controls. In contrast, each of these parameters was significantly lower than control levels in animals spayed on Day 10. Although statistical comparisons are probably not possible, the data suggest a greater degree of retardation of resorption on Day 7 over Day 10, when progesterone was delayed a full 24 hr.

Discussion. Administration of a high dose of LHRH to rats on Day 7 of pregnancy

was followed by a marked decrease in circulating progesterone *in animals that carried their pregnancies normally to term.* The nadir of this depression occurred on Days 8 and/or 9, with recovery toward normal levels by Day 10. A similar depression, observed after administration of LHRH on Day 9, was believed to account for the termination of pregnancies in most rats treated on Day 9 and all those treated on Day 10 or 11 (1). This similarity of progesterone depression at times during the early postnidatory period when LHRH was effective and not effective in terminating pregnancy prompted the search for an alternate explanation, and led to the second series of experiments. These clearly indicated that rat pregnancy is more sensitive to progesterone deprivation on Day 10 than it is on Day 7. Thus, the termination of pregnancy by LHRH on Day 10 would appear to result from a more profound response to decreased progesterone than is seen on Day 7.

The decrease in circulating progesterone following LHRH administration on Day 13 seems quite moderate, and reasonably high titers are seen. One would surmise here that the functional luteolysis is inadequate to initiate resorption, i.e., there is adequate progesterone to maintain pregnancy. Thus, although a decrease in progesterone can be demonstrated, it is not critical to the pregnancy.

The development over the past decade of highly sensitive methods for evaluating circulating progesterone has led to a spate of papers describing changes in this hormone during the course of rat pregnancy (3–7). None of these reports shows marked differ-

TABLE III. EFFECTS OF LHRH ADMINISTRATION ON DAY 13 OF PREGNANCY ON CIRCULATING PROGESTERONE IN RATS

	Serum progesterone (Mean ng/ml ± SE)					*N* Pregnant Day 21
	N	Day 13	Day 14	Day 15	Day 16	
Controls	10	61.1 ± 8.6	61.3 ± 3.7			10
LHRH	10	57.7 ± 5.1	54.6 ± 5.4			10
Controls	10	55.7 ± 4.3		77.6 ± 3.1*		10
LHRH	10	61.1 ± 6.3		45.3 ± 7.3**		9
Controls	10	65.7 ± 5.0			85.5 ± 8.4*	10
LHRH	10	65.5 ± 5.8			44.9 ± 5.5*,**	9[a]

[a] One rat died from cardiac puncture following Day 16 blood sample.
* Significantly different from Day 13 value, with $P < 0.05$, *t* test.
** Significantly different from control value, with $P < 0.001$, *t* test.

TABLE IV. EFFECTS OF DELAYED INITIATION OF PROGESTERONE SUPPORT FOLLOWING OVARIECTOMY IN RATS SPAYED ON DAY 7 OR 10 OF PREGNANCY.

Gestational age at ovariectomy (days)	Progesterone delay (hr)	*N*	% Normal pregnancies	*N* Fetuses (mean ± SE)	Weight of uterus and conceptus (mean g ± SE)
7	Sham operation	10	90	11.3 ± 1.1	46.6 ± 6.2
	0	10	100	9.7 ± 0.8	52.1 ± 4.1
	6	10	90	10.0 ± 0.8	50.2 ± 7.0
	12	10	80	8.3 ± 1.3	34.9 ± 7.6
	24	5	40	3.0 ± 2.0*	6.9 ± 5.5*
10	Sham operation	17	100	11.4 ± 1.0	44.6 ± 3.4
	0	9	100	9.8 ± 0.9	51.7 ± 5.1
	6	10	90	9.7 ± 1.5	48.8 ± 9.5
	12	19	21**	6.8 ± 1.3*	8.1 ± 2.3*
	24	8	0**	–	0.6 ± 0.1*

* $P < 0.05$, by *t* test, versus appropriate controls.
** $P < 0.01$, by fourfold contingency tables (12), versus appropriate controls.

ences in progesterone levels on Days 7 and 9. The reason for a differential sensitivity thus would appear to reside in the developing conceptus or uterus.

Remarkably similar effects have recently been published following other kinds of treatments. Antibodies to progesterone resulted in complete resorption of implantation sites when administered on Day 11, but not on Days 7, 15, or 19 of pregnancy (8). In our earlier paper (1) we showed that LHRH at 0.5 mg × 2 was effective on Days 9, 10, and 11, but not on Days 7, 8, 12, or 13–16 (0.5 mg bid); unpublished data from this laboratory show no effect of the same dose on Days 5 or 6 of pregnancy. Csapo and associates (9, 10) observed a marked reduction in progesterone following treatment of animals with progesterone antibodies on Day 5 or 9 of gestation [by our designation; they consider the day of sperm-positive vaginal smear as Day 0 (11)]; however, their data do not suggest so profound a depression following Day 5 treatment as following injection on Day 9. Further, their data suggest that normal circulating levels of progesterone are higher on Day 5 than 9, and they propose that depression below critical levels for pregnancy maintenance may be more difficult to achieve on Day 5. Although this is likely to be correct with a quantitative binder of progesterone such as an antibody, our data suggest profound depressions of progesterone following LHRH on both Days 7 and 9 of pregnancy.

Especially significant is the fact that six of seven rats treated on Day 7 had progesterone levels below the detection limit of our assay (<3 ng/ml) when sampled on Day 8, yet gestation continued normally to autopsy just prior to term. These data, coupled with the greater sensitivity to progesterone deprivation of animals spayed on Day 10 than on Day 7, suggest to us that the effect may reside more in the responsiveness of the fetal/placental/uterine unit than in absolute changes in progesterone titers, while on Day 13 the functional luteolysis appears simply insufficient to induce resorption.

Summary. LHRH (0.5 mg × 2) on Day 7 of pregnancy is followed by a profound, ephemeral reduction in circulating progesterone, but not by fetal resorption; a similar decrease in progesterone follows the same treatment on Day 9, but it is associated with termination of pregnancy in most animals. These effects appear to result from differential sensitivity to progesterone deprivation, since delay of progestational support for varying periods following ovariectomy on Day 7 produces fewer indications of resorptions than on Day 10. A slight, but significant, reduction in progesterone after treatment on Day 13 would appear inadequate to affect continuation of the pregnancy.

1. Humphrey, R. R., Windsor, B. L., Reel, J. R., and Edgren, R. A., Biol. Reprod. **16,** 614 (1977).
2. Attal, J., and Engels, J. A., J. Endocrinol. **60,** 703 (1971).
3. Fajer, A. B., and Barraclough, C. A., Endocrinology **81,** 617 (1967).
4. Hashimoto, I., Hendricks, D. M., Anderson, L. L., and Melampy, R. M., Endocrinology **82,** 333 (1968).
5. Morishige, W. K., Pepe, G. J., and Rothchild, I., Endocrinology **92,** 1527 (1973).
6. Wiest, W. G., Kidwell, W. R., and Balogh, K., Endocrinology **82,** (1968).
7. Wiest, W. G., Endocrinology **87,** 43 (1970).
8. Raziano, J., Ferin, M., and Vande Wiele, R. L., Endocrinology **90,** 1133 (1972).
9. Csapo, A., Dray, F., and Erdos, T., Endocrinology **97,** 603 (1975).
10. Csapo, A., and Erdos, T., Amer. J. Obstet. Gynecol. **126,** 598 (1976).
11. Csapo, A., and Wiest, W. G., Endocrinology **85,** 735 (1969).
12. Mainland, D., and Murray, I. M., Science **116,** 591 (1952).

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