Influence of Various Doses of LH on Fetal Survival in Hypophysectomized Rats[1] (36574)

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Hypophysectomy before Day 11 of pregnancy results in resorption of embryos in the rat (1) whereas luteinizing hormone (LH) will maintain pregnancy following hypophysectomy performed as early as Day 8 of pregnancy (2). This does not necessarily mean that LH is the only luteotrophin during this phase of pregnancy since the conceptus has been shown to have a partial luteotrophic role before Day 12 of pregnancy (3, 4), and the placenta has a principal role thereafter (1).

For further elucidation of the luteotrophic complex during early pregnancy in the rat, we studied the effect of various doses of LH on the maintenance of pregnancy in hypophysectomized rats. The effective dose for this purpose was then employed in an attempt to maintain luteal function in hypophysectomized pseudopregnant rats.

Materials and Methods. Adult virgin Charles River CD rats were placed with fertile males on the day of proestrus. The following morning those rats which had spermatozoa in their vaginal smears were designated as in Day 1 of pregnancy. The animals were housed in a room with constant temperature and 14 hr of light and 10 hr of darkness. On the morning of Day 9 the rats were hypophysectomized by the parapharyngeal approach, allocated to one of 6 groups and given 0, 5, 10, 25, 50 or 200 μg of NIH-LH-S12. These dosages of LH were emulsified in 0.2 ml of a mixture of 95% sesame oil and 5% beeswax and injected subcutaneously daily from Days 9 to 15 of pregnancy. On Day 15 the rats were laparotomized and the number of fetuses (or placental scars) were recorded. Survival of fetuses was determined by observing their heart beats. The sellae turcicae of the mothers were examined by use of loupes for pituitary remnants and those rats with remnants were excluded from the experiment.

Another group of female rats was made pseudopregnant by mechanical stimulations of the uterine cervix with a glass rod on the day of estrus. On the following morning when the vaginal smear was characterized by the appearance of leukocytes, the rats were hypophysectomized and injected with 25 μg of LH in 0.2 ml sesame oil–beeswax subcutaneously daily for 10 days. During the last 3 days of LH treatment, the rats also received 10 μg of estradiol in 0.1 ml sesame oil subcutaneously. The vaginal smear was examined each day during the treatment.

Results. No embryos were maintained in the hypophysectomized animals treated with the vehicle (sesame oil–beeswax) only. On the other hand, those animals treated with LH showed various degrees of fetal maintenance depending on the dose of LH (Table I). Pregnancy was maintained in 2 of 8 rats treated daily with 5 μg of LH. These 2 rats had a total of 19 fetuses surviving out of 21. In the remaining 6 rats a total of 66 degenerating fetuses or placental scars were observed. However, pregnancy was maintained in all 6 rats treated daily with 10 μg and in 6 of 7 rats when treated daily with 25 μg of LH. In the rats treated with 10 μg LH, 95% of embryos were surviving at autopsy and in 6 rats treated with 25 μg LH, 60 of 62 embryos were surviving. The remaining rat had only 3 degenerating fetuses. Daily treatment of hypophysectomized rats with 50 or

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TABLE I. Maintenance of Pregnancy by LH in Hypophysectomized Rats.

Treatment daily dose of LH[a] (μg)	No. of rats[b]	No. of fetuses on Day 15			Fetuses surviving (Day 15)		Rats maintaining pregnancy	
		Normal	Absorbed	Total	No.	%	No.	%
0	7	0	82	82	0/82	0	0/7	0
5	8	19	66	85	19/85	22	2/8	25
10	6	53	3	56	53/56	95	6/6	100
25	7	60	5	65	60/65	92	6/7	86
50	6	29	46	75	29/75	39	4/6	67
200	6	24	33	57	24/57	42	4/6	67

[a] The amount of NIH-LH-S12 in 0.2 ml sesame oil plus 5% beeswax from Days 9 through 15 of pregnancy.
[b] Hypophysectomized on Day 9 of pregnancy.

200 μg LH maintained pregnancy in 67% of the rats in both groups, but was not as effective as 10 or 25 μg of LH. Although pregnancy was maintained in 67% of the rats treated with 50 and 200 μg LH, the proportions of embryos surviving were 39 and 42% respectively, and were significantly ($p < .01$, χ^2 test) less than those in the rats treated with 10 and 25 μg LH (95 and 92%, respectively).

In addition to the animals listed in Table I, a total of 6 rats, 2 rats each in 10, 50 and 200 μg LH groups, were found to be nonpregnant, judged by absence of placental scars on the mesometrial side of the uterine horns. Water imbibition of the uterus was not observed in these rats treated with 10 μg LH but it was seen in one of the rats treated with 50 μg and in both of the rats treated with 200 μg. The vaginal smears of these nonpregnant rats treated with 50 or 200 μg LH were cornified. The ovaries of these rats treated with high doses of LH were mostly composed of 2 or 3 sets of corpora lutea and no follicles were visible.

Daily treatment of hypophysectomized-pseudopregnant rats with 25 μg of LH did not maintain leukocytic smear. Four of 6 rats thus treated showed vaginal cornification after 5 to 6 days of treatment. In the remaining 2 rats cornified epithelial cells appeared in the vaginal smear 1 day after the first injection of 10 μg estradiol.

Discussion. The present results confirm previous reports showing that LH is capable of maintaining early pregnancy following removal of the hypophysis (2, 5). Moreover, our data indicate that the lowest and the higher doses of LH used in this experiment were less effective in maintaining pregnancy. Since small doses of estrogen have been shown to substitute for LH in maintaining pregnancy after hypophysectomy (6), it appears that the effect of LH in maintaining pregnancy may be at least partially due to its ability to stimulate estrogen secretion. Macdonald, Armstrong and Greep (7) demonstrated that LH would induce implantation of blastocysts in hypophysectomized pregnant rats, whereas Madhwa Raj, Sairam and Moudgal (8), using antisera to LH were able to prevent ovi-implantation. Therefore, estrogen secretion should be included in the mode of action of LH.

On the other hand, Madhwa Raj and Moudgal (9) showed that LH antiserum terminated pregnancy when given before Day 12 of pregnancy and only concomitant administration of progesterone overcame the effect of LH antiserum, suggesting that LH is also involved in progesterone secretion during early pregnancy.

The present data showing that higher doses of LH were less effective in maintaining pregnancy than lower doses, cannot be explained solely by the effect of LH on progesterone secretion. It is more likely that the higher doses elevated the estrogen output relative to that of progesterone and resulted in the failure of pregnancy. High levels of estrogen secretion stimulated by higher doses of LH were noted in nonpregnant rats in our experiments. Since the ovaries of these rats did not contain any noticeable follicles, estrogen appears to be secreted by this luteinized ovary. Armstrong (10) demonstrated that progesterone synthesis was initially increased by LH administration *in vivo*, but it was diminished thereafter. This secondary effect of LH to decrease progesterone synthesis may also be involved.

The requirements of ovarian steroid hormones for maintaining pregnancy as reported by Alloiteau and Bouchours (6) differ from those obtained by Madhwa Raj and Moudgal (9). Although the former workers showed that a small dose of estrogen maintained pregnancy in hypophysectomized rats, they held that progesterone secretion was being maintained by luteotropin secreted by the young conceptus. On the other hand, the results obtained by the latter group indicated that exogenous progesterone alone could maintain pregnancy in intact rats treated with LH antiserum. The difference may be explained by the presence of the pituitary in the pregnant animals used by the latter group. Treatment with LH antiserum reduced the secretion of progesterone (11) and most likely that of estrogen but only to the degree that additional progesterone could preserve the pregnancy. The necessity of estrogen and progesterone for the maintenance of pregnancy in hypophysectomized rats was shown by Ahmad (12).

The presence of the young conceptus in the uterus appears essential for exogenous LH to maintain the luteal function. Our data, in agreement with that of Macdonald and Greep (13, 14), clearly show that LH alone could not maintain luteal function in hypophysectomized pseudopregnant rats. The presence of a luteotrophic substance in the placenta has been well documented (15, 16) and its luteotrophic action appears soon after implantation (3, 4). Further study of the roles of placental luteotrophin and pituitary gonadotrophin on the steroidogenic activity of the ovary during early pregnancy is under way.

Summary. Effect of various doses of LH on maintenance of pregnancy was examined in the rats hypophysectomized on Day 9. Daily doses of 10 and 25 μg LH were found optimal for the maintenance but doses lower or higher than these were less effective. LH did not maintain luteal function in hypophysectomized-pseudopregnant rats. These results suggest that optimal amount of LH in the presence of conceptus maintains luteal function in early pregnancy, and that high doses of LH stimulate secretion of estrogen more than that of progesterone leading to resorption of fetuses.

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