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Maintenance of Pregnancy in the Rat in the Absence of LH[1] (40366)

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The significant role of LH in pregnancy in the rat was revealed by studies utilizing specific antisera to LH (LHAS) (1, 2). Madhwa Raj and Moudgal (2) demonstrated that LHAS was capable of delaying implantation if given before day 4. This study also showed that LHAS terminated pregnancy when given on days 8 through 11, further identifying the need for LH during this period (3–5). A subsequent study (6) also confirmed the LH requirement and showed further that inhibition of prolactin secretion by ergocornine caused resorption of the conceptuses when injected on days 6 and 7 but not if given after day 7. Their study also suggested that a placental luteotropin could substitute for pituitary prolactin after day 7 and this has been successfully proven (7). Thus, it appears that there are changing requirements for LH and prolactin during pregnancy and this may also be true of the steroids.

Fetal resorption as a consequence of LHAS administration has been correlated with reduction of progesterone secretion in many animal models (8, 9). However, fetal resorption in response to LHAS may also be related to reduced estrogen secretion during days 8 through 11 (10).

Rat blastocysts survive in utero beyond the usual time of implantation if two conditions are met. First, that sufficient progesterone is available to insure their survival and second, that there is no estrogen available to cause implantation. In this state implantation can be initiated by the provision of small amounts of estrogen.

The two conditions causing delay of implantation can be met by autografting the anterior pituitary beneath the kidney capsule before implantation. This experimental model has adequate prolactin from the graft to support sufficient progesterone secretion to insure blastocyst viability. Conversely, there is insufficient gonadotropin secretion to induce estrogen secretion to initiate implantation. Thus, the blastocysts remain unimplanted until estrogen is administered.

The day estrogen administration begins in rats experiencing delay of implantation becomes equivalent to day 4 of normal pregnancy. In the pituitary autografted model estrogen must be given for 9 consecutive days, equivalent to days 4 through 12 of normal pregnancy. Estrogen induces implantation and insures fetal survival beyond day 16 equivalent (7). This experimental model provides the opportunity to test if specific LHAS is capable of acting at any site other than by neutralizing maternal pituitary LH.

Methods and materials. Sprague-Dawley rats used in these experiments were housed in a temperature, humidity and light (14L-10D) controlled room. They were provided food and water *ad libitum.* Females were caged with experienced males and day 1 of pregnancy was the day sperm were found in the vaginal lavage. Parapharyngeal hypophysectomies were performed on day 2 and were later confirmed complete at autopsy. The anterior pituitary gland of each female was autografted (APtr) beneath the kidney capsule (7). Successive laparotomies were performed on days 8, 12, 16, and 20. No fetal sites were found on day 8 because blastocyst implantation was delayed due to the absence of estrogen. Estradiol-17β (E-17β) (Sigma Chemical Co.) was administered in sesame oil (0.1 μg/day, days 8 through 12) by subcutaneous injection. This E-17β induced implantation and sites were visible on day 12. Thus, because of the 4 day delay in implantation days 8, 12, 16 and 20 became equivalent to day 4, 8, 12 and 16 of a normal pregnancy.

Antiserum to LH (LHAS) was prepared by

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immunizing rabbits with Papkoff ovine LH in Freund's complete adjuvant. The antiserum was rendered "monospecific" to LH by absorption with dilute normal sheep serum. The specificity of this material was assessed using the Ouchterlony diffusion technique. This test determined there was no cross reactivity with dilute normal sheep serum, ovine liver extract, NIH-FSH or NIH prolactin. However, a single clear precipitin band developed relative to Papkoff-LH with no spurs indicating no cross reactivity with the tissue preparations or the other pituitary hormones (2). The LH neutralizing capacity of this antiserum in our animals was assessed by determining the amount required to induce abortion. When given as one subcutaneous injection on day 8 of pregnancy, 0.7 ml of the antiserum induced total resorption. The antiserum dose used in the present experiments was one ml or 1.4 times the dose producing 100% resorption.

Results. LHAS given the day before the implantation-inducing E-17β treatment failed to delay nidation or to cause later resorption of the fetal sites (Table I). Administration of single doses of LHAS on days equivalent to days 7, 8, 9, 10 or 11 also failed to cause fetal resorption. Further, individual rats given aborting doses of LHAS on days 9 and 10 equivalent, did not show signs of fetal resorption.

Discussion. Previous experiments using hypophysectomized and pituitary autografted rats have been subject to the criticism that some small amount of LH may have been available for continuing luteal function from basophilic cells on remnants of the pituitary stalk or from cells of the grafted pituitary. The current experiments were designed to neutralize any LH with LHAS in the pregnant rat, which was hypophysectomized and pituitary autografted and treated with adequate amounts of estrogen to support pregnancy. Neither blastocyst survival nor subsequent implantation was influenced by the administration of LHAS. Most importantly, LHAS did not cause fetal resorption when given on days equivalent to days 8 through 11 of pregnancy. Thus, the corpus luteum can function at a physiological level in the absence of LH.

Blastocyst implantation and maintenance of pregnancy in the model used was dependent upon two factors, the continuing secretion of progesterone from the corpus luteum maintained by prolactin from the autografted pituitary and the provision of exogenous estrogen (estradiol-17β, 0.1 μg/day) (7). This augers toward the concept that estrogen secretion induced by LH (11, 12) may be acting on the uterus in concert with progesterone to accommodate the rapidly expanding fetuses. Estradiol may also act upon the corpus lu-

TABLE I. EFFECT OF LH ANTISERUM (LHAS) ON THE BLASTOCYTES OR IMPLANTATION SITES OF RATS BEARING PITUITARY AUTOGRAFTS.[a]

Ref Group	Treatment: LHAS RD[b]	Estradiol 0.1 μg/day: RD	CD[c]	Observations (RD): 8 ♀S/♀T[d]	8 $\bar{X}$s[e]	12 ♀S/♀T	12 $\bar{X}$s	16 ♀S/♀T	16 $\bar{X}$s
21	3	4–12	8–16	4/4	10.0 ± 1.0	3/4	10.3 ± 1.3	3/4	9.7 ± 1.2
19 and 23	7	4–12	8–16	1/1	13	1/1	13	1/1	13
	8	4–12	8–16	6/6	11.7 ± 1.8	5/6	10.6 ± 1.5	5/6	9.2 ± 1.5
	9	4–12	8–16	5/5	12.8 ± 1.2	4/5	10.8 ± 2.2	4/5	7.0 ± 1.4
	10	4–12	8–16	3/3	9.7 ± 2.7	3/3	9.7 ± 2.7	3/3	8.0 ± 2.5
	11	4–12	8–16	1/1	12	1/1	12	1/1	10
24	9, 10	4–12	8–16	3/3	10.3 ± 0.3	3/3	10.0 ± 0.6	2/2[f]	9.0 ± 0.0

[a] Anterior pituitaries grafted on day 2, the day after sperm were seen in the vaginal lavage.
[b] RD—Relative day of normal pregnancy.
[c] CD—Chronological day after day 1.
[d] ♀S/♀T—females with sites/total females observed.
[e] $\bar{X}$s—mean number of sites ± SE in females with sites.
[f] One animal died.

teum to promote progesterone secretion in the presence of anterior pituitary or placental prolactin.

It is interesting to note that Madhwa Raj and Moudgal (2) were unable to thwart the effect of LHAS by the administration of estrogen. Yet, in the model under consideration, which was reported in a previous study (7), estrogen administration was a *sine qua non* for maintenance of the fetus from day 8 to 12. Additionally, estrogen alone was not adequate to maintain pregnancy following hypophysectomy accomplished during this important phase of pregnancy. The reason for the failure or success of fetal maintenance with estrogen awaits insight derived from further studies.

Although LHAS depressed progesterone levels and caused resorption in the intact pregnant rat, these seemingly associated events may not be cause and effect. It is possible that estrogen secretion was also reduced by the administration of LHAS. The previous studies of the capacity of LHAS to cause abortion seem to amply illustrate that LH stimulates both progesterone and estrogen secretion.

In conclusion this study demonstrated that luteal function does not require the presence of LH between days 8 and 11. Previous experiments (7) showed there was an estrogen requirement for pregnancy maintenance in the rat. The current study implies that there may be a role for LH in pregnancy to maintain estrogen secretion. This work also confirms the concept, long accepted but unproven, that LHAS acts specifically against pituitary LH-like material and denies the possibility that LHAS acts upon the unimplanted blastocyst, the implanting blastocyst, or the development of the placenta.

Summary. Pregnant rats were hypophysectomized and pituitary autografted on day 2, the day after sperm were observed in the vaginal lavage. Estradiol-17β (E-17-β) was injected (0.1 μg/day) on days 8 through 16 to induce implantation and maintain pregnancy. This protocol resulted in a 4 day delay of implantation, and day 8 becomes equivalent to day 4 of normal pregnancy. A single dose of LHAS (equivalent to 1.4 times the dose necessary to cause abortion on day 8 in the normal pregnant rat) failed to prevent implantation when administered on day 7 or cause fetal resorption when administered on days 11, 12, 13, 14 or 15 (equivalent to days 4, and 7 through 11). LHAS given on the two successive days 13 and 14 (days 9 and 10 equivalent) was also without effect. These results suggest that LHAS causes abortion in the rat by acting on pituitary LH-like material and not on the ovary, developing fetus or placenta.

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