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Involvement of Luteinizing Hormone as a Luteotropin in the Golden Hamster[1] (37776)

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Pregnancy and parameters of luteal function can be maintained until Day 8 with daily injections of 200 μg FSH plus 1 mg prolactin in hamsters hypophysectomized in Day 4 (1–3). However, LH is obligatory for maintenance of pregnancy in the intact hamster between Days 6 and 12 as antiserum to LH induces abortion (4). The present study was undertaken to determine the need for gonadotropins during Days 8–12 in the hypophysectomized hamster as no such data were available.

Materials and Methods. Adult female Lakeview (Charles River) hamsters arrived at our laboratory on Day 6 of pregnancy or bred in our animal house (Day 1 = day of sperm detection). They were housed in rooms with constant temperature and light (14 light–10 dark). On Day 8 the animals were laparotomized and those with implantation sites were hypophysectomized on either Day 8 or 12 by the parapharyngeal approach under ether anesthesia. Ovine prolactin (NIH-P-S9) and ovine FSH (NIH-FSH-S8,S9) were administered subcutaneously in saline twice daily. Ovine LH (NIH-LH-S17,S18) was given as a single daily injection suspended in sesame oil–5% beeswax. The treatment was started within 2 hr of hypophysectomy. FSH and prolactin were premixed but LH and prolactin were injected at separate sites. Rabbit anti-ovine LH serum was administered to intact hamsters on Day 8 of pregnancy as a single subcutaneous injection. The antiserum was prepared and thoroughly characterized as described earlier (5). The absorbed antiserum was devoid of antibodies to other contaminating pituitary gonadotropins or organ and serum specific proteins. The animals were observed twice daily for general condition and any external vaginal bleeding. Autopsy was performed on Day 14 or 15 at which time the nature and number of implantation sites were noted. The ovaries were dissected of extraneous tissue, weighed and fixed in Bouin's fluid. Paraffin sections were stained in hematoxylin–eosin for histological observations. Completeness of hypophysectomy was ascertained at autopsy by examining the sellae turcicae under a dissecting microscope and only animals lacking remnants were included in the present report.

Results. Ablation of the pituitary on Day 8 of pregnancy resulted in vaginal bleeding within 48–72 hr and subsequent resorption of fetuses as seen at autopsy on Day 14 (Table I). While daily treatment with 5 or 10 μg of LH was not effective, 25 μg LH delayed the onset of external bleeding and fetal resorption. At the level of 50 μg, LH could almost totally reverse the effects of hypophysectomy; there was 82% live sites. However, further increase in dose of LH was deleterious causing a reduction in the number of embryos surviving. Treatment of intact hamsters with 0.5 ml of a well-characterized antiserum to ovine LH on Day 8 also resulted in termination of pregnancy, preceded by the onset of external bleeding by 48 to 72 hr.

In contrast to the ineffectiveness of 25 μg LH alone, a combination of this dose of LH

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TABLE I. Demonstration of Involvement of LH in Maintenance of Pregnancy Between Days 8–12 in Intact or Hypophysectomized Hamsters.

Treatment[a]	Ovarian wt (mg ± SE)	N/Ns[b]	Sites (mean ± SE) Live	Sites (mean ± SE) Resorbed	% Live sites
Nonoperated control	55.5 ± 1.6	10/10	12.3 ± 0.7	0.8 ± 0.3	94
Anti-LH serum[c]	29.0 ± 2.4	6/0	0	All	0
PX	17.2 ± 1.3	4/0	0	All	0[d]
PX + 5 μg LH	17.4 ± 3.1	5/0	0	All	0[d]
10 μg LH	21.5 ± 0.5	4/0	0	All	0[d]
25 μg LH	19.2 ± 0.6	6/0	0	10.4 ± 1.0	0[e]
50 μg LH	48.2 ± 3.2	7/7	10.4 ± 1.4	2.3 ± 0.9	82
100 μg LH	47.3 ± 2.8	6/5	5.8 ± 1.7	5.7 ± 2.0	50

[a]PX = hypophysectomy on Day 8, NIH-LH-S18 given in 0.2 ml sesame oil–beeswax daily from Days 8 to 13.
[b]Ns = number of animals with live sites. N = total number of animals.
[c]Animals not hypophysectomized but given A/S to LH only on Day 8.
[d]Onset of vaginal bleeding noted on Day 10.
[e]Onset of vaginal bleeding noted on Day 11 or 12.

with 2 mg prolactin was highly effective in maintaining gestation (Table II). Lower dose of 10 μg LH was not efficacious even when given with prolactin. The combination of 200 μg FSH and 2 mg prolactin given as two daily injections resulted in pregnancy maintenance in 4 of 15 animals with an average of 31% live sites.

The effects of hypophysectomy on Day 12 were more variable and less drastic than those observed on Day 8. Six of nine animals retained live fetuses (12.5 ± 0.8) and showed a normal complement of resorbing sites as compared to unoperated controls. In the other three animals, expulsion of fetuses (av 8.0/rat) on Day 14, 15 or both was noted. Only one of these rats had 6 live fetuses retained.

The ovaries of all animals which failed to maintain pregnancy showed loss of luteal bodies, degeneration of graafian follicles and interstitium by Day 14, irrespective of treatment. Concomitant with maintenance of gestation, the ovaries of animals given 50 or 100 μg LH, or 25 μg LH plus prolactin, or 200 μg FSH plus prolactin retained corpora lutea which were in moderate to good condition histologically. However, none of these animals showed graafian follicles of the quality seen in unoperated controls. Higher doses of LH caused relatively extensive interstitial growth and some thecal luteinization.

TABLE II. Effects of Replacement Therapy with Combinations of Gonadotropins on Fetal Survival After Hypophysectomy in the Hamster.

Daily treatment[a]	Ovarian wt (mg ± SE)	N/Ns[b]	Sites (mean ± SE) Live	Sites (mean ± SE) Resorbing	% Live sites
10 μg LH + 2 mg prolactin	16.1 ± 1.2	5/0	0	12.0 ± 0.6	0
25 μg LH + 2 mg prolactin	38.9 ± 3.3	10/10	9.1 ± 0.7	2.5 ± 0.8	78
200 μg FSH + 2 mg prolactin	24.2 ± 1.6	15/4	3.8 ± 1.7[c]	8.3 ± 1.4[c]	31

[a]Hypophysectomy performed on Day 8.
[b]N = total number of animals; Ns = number of animals with live sites.
[c]Based on 12 observations, the remaining 3 animals had no assessable sites.

Discussion. The termination of gestation accompanied by degeneration of various compartments of the hamster ovary as revealed by histology, which follows hypophysectomy (1) or anti-LH treatment (4), is consistent with previous observations. While the combination of FSH and prolactin has been shown to form a "minimal luteotropic complex" between Days 4 to 8 (1, 3), the present observations implicate a specific involvement of LH between Days 8 to 12. Supporting this conclusion is the observation that anti-LH-induced termination of pregnancy is only reversed by progesterone and not by prolactin plus FSH (4). The retention of well-developed luteal bodies in the LH-supplemented animals indicates that LH plays a role in the morphological integrity of the corpus luteum in the hypophysectomized hamster before Day 12 of pregnancy.

Replacement therapy with 25 μg LH + 2 mg prolactin was as effective as 50 μg alone in maintaining pregnancy. The FSH plus prolactin combination was effective in maintaining pregnancy, though to a lesser extent. These observations indicate that LH must be involved in the maintenance of gestation, acting through the corpus luteum and includes the possibility that it may act in concert with FSH, prolactin and a placental factor(s) in the normal intact animal to achieve optimal maintenance. Greenwald (3) confirmed the maintenance of luteal weights and progesterone levels with the FSH plus prolactin treatment given to hypophysectomized hamsters between Days 4 and 8 and suggested that alternative explanations may exist for the anti-LH-induced termination of pregnancy after Day 8 (4). The present authors believe that neither the results of Rao, Madhwa Raj and Moudgal *et al.* (4) nor this study can be compared to that of Greenwald, as they were done during two different periods of gestation. Currently, evidence from the rat is accumulating to indicate that hormonal requirements approximate to the time of implantation are different from those between Days 8 to 12 of gestation. Anti-LH becomes progressively effective between Days 6 to 8 (6). Further, in the hypophysectomized rat, prolactin and β subunit of LH are required before Day 8 for pregnancy maintenance but only LH-β is sufficient after Day 8 (7). These results suggest the emergence of the placenta as a source of a prolactin-like material, as early as Day 8. The observations made between Days 4–8 of pregnancy, when viewed in the perspective of present results, indicate a similar situation to be occurring in the hamster. Further, LH is more efficacious than FSH plus prolactin for progesterone production from hamster corpus luteum *in vitro* (8). This supports the concept of a luteotropic and progestational role of LH during gestation in this species. The dose of LH optimal for maintenance of luteal function seems to be critical, as in the hypophysectomized rat (9). Low amounts of LH, administered in a depot vehicle seem to promote optimal conditions without bringing about an "exhaustion" of precursor pool in the corpus luteum. Further, increased amounts of LH caused extensive interstitial stimulation which could contribute large amounts of estrogen leading to the observed lower degree of pregnancy maintenance at the 100 μg LH level.

Hypophysectomy on Day 12 was not as effective in terminating pregnancy as on Day 8. This extends the idea that embryonic survival is less dependent on hypophysial support beyond Day 12. Unlike the rat, the hamster may represent a transitional species from this viewpoint.

Summary. Hypophysectomy (PX) or anti-LH treatment resulted in vaginal bleeding followed by absortion as well as luteal and follicular degeneration in 8-day pregnant hamsters. The effects were successfully reversed by daily injections of 50 μg LH in sesame oil–beeswax (82 *vs* 94% in controls) or 25 μg LH plus 2 mg prolactin (78%). Partial reversal was observed with 100 μg LH (50%) or 200 μg FSH plus 2 mg prolactin (31%). Lower doses of LH with or without prolactin, were ineffective. Hypophysectomy on Day 12 caused expulsion of fetuses by Day 14 or 15 in 3 out of 9 animals. These results support a luteotropic and progestational role for LH in the pregnant hamster.

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