Hormonal Maintenance of Pregnancy in Hypophysectomized Rats[1] (36987)

WM. R. LYONS AND NAZIR AHMAD

Departments of Anatomy, University of California, San Francisco, California 94122; and University of Southern California, Los Angeles 90033

The maintenance of an appreciable number of implanted embryos in hypophysectomized rats injected with large doses of ovine luteinizing hormone (LH or ICSH) was reported by Alloiteau and Bouhours (1) and by Moudgal (2). The former authors suggested that the LH (presumably contaminated with FSH) was responsible for the necessary synergistic estrogen secretion, and that the placental luteotropic hormone (3–5) activated the equally important progestogen secretion. Moudgal, however, disregarded the placental contribution; and concluded that "LH promotes both progesterone *and* oestrogen synthesis."

Several reports from our laboratory on experiments seeking to imitate the hormonal milieu of pregnancy by supplying exogenous hormones from the pituitary, ovary and placenta to hypophysectomized and hypophysectomized–oophorectomized rats are in agreement with the interpretation of Alloiteau and Bouhours (1). Moudgal's contention led to an attempt to duplicate his experiments. We had shown that the placental or hypophyseal lactogenic hormones are capable of stimulating adequate levels of progestogen secretion by the normal quota of corpora lutea of pregnancy; and that combinations of hypophyseal FSH and ICSH stimulated the ovaries of pregnancy to secrete the daily equivalent of 0.5–1.0 μg of estrone necessary during the first half of pregnancy. In contrast, the Moudgal regimen (2) resulted in hyperluteinization of the ovaries; and it was only initiated *after* one would expect a gradually increasing secretion of luteotropin by his average of 9 already nidated placentas in 6 experimental rats.

Methods. In some of the following experiments, the protocol of Moudgal was followed as closely as possible. Sexually mature, nulliparous rats from the same Long-Evans colony were caged with males of known fertility on the afternoon in early proestrus. On the following morning if sperm were detected by vaginal smear the rat was included in the experiment. Most of the rats were hypophysectomized by Mr. J. D. Nelson of the Hormone Research Laboratory who performed all of the operations in the Moudgal experiment; and others were hypophysectomized by one of us (N.A.) without alteration of the results. Knowing that less than 10% of the previously nulliparous rats bred in this colony fail to retain an average complement of 10–12 living fetuses by day 12, made it seem unnecessary to laparotomize the animals on the day of hypophysectomy to confirm pregnancy. The ovine ICSH provided by Dr. Harold Papkoff, was the same as that used by Moudgal; and was prepared in gelatin by the latters' procedure (2). At the high daily dose of 100 μg in gelatin this preparation also showed some follicle stimulation as evidenced by follicular development to the antrum stage, and large theca-luteal bodies. Rats that received this excessive dose of ICSH and did not maintain their concepti failed to show the luteal cell hypertrophy typical of pregnancy or of the nongravid rat response to hypophyseal and placental luteotropin. This is mentioned as evidence against any detectable hypophyseal LTH activity in the dose of ICSH used. The Moudgal regimen of injecting 100 μg of ICSH from Days 8 through 12 was followed in group 1A, using 14 rats. In group 1B, 6 rats on the same ICSH

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regimen also received autografts of 1/8–1/4 of an anterior pituitary under the renal capsule, at the time of hypophysectomy in order to determine whether undetected, small remnants of pituitary lodged in a blood clot at the operative site or in the burr hole might contribute significant LTH activity in this situation as grafts are known to do in others (11). In group 1C, 9 rats were injected only with 1 μg of estrone daily over the same 8–12 day period; and in group 1D, 6 rats received no injections. All of these rats were inspected under light ether anesthesia before sacrificing on Day 13. This procedure permits one to determine the number of living fetuses by observing the beating heart. The number of dead fetuses, and the various degrees of resorption of the concepti were listed together in Table I as "Resorption sites."

In group 2, 10 rats hypophysectomized on Day 7 and necropsied in Day 13 of pregnancy were treated daily from Days 7–12 as follows: 4 received 100 μg of ICSH (group 2A); 3 were injected with 1 μg of estrone (group 2B); and 3 were untreated (group 2C). In group 3, 12 rats hypophysectomized on Day 9 were treated daily from Days 9–13 as follows: 5 received 100 μg of ICSH (group 3A); 4 were injected with 1 μg of estrone (group 3B); 3 were untreated (group 3C).

Results. The data obtained at necropsies and after microscopic examination of the tissue from 57 rats are arranged in Table I. There were 3 main groups namely those rats hypophysectomized on Day 7, 8 or 9, respectively. The 12 rats of subgroups (1D, 2C and 3C) that received no treatment were of interest because they provided an ovarian histologic picture in striking contrast to that induced by the unphysiologic dose of ICSH used. One rat in each of these untreated control groups hypophysectomized on Days 7 and 8, and 3 hypophysectomized on Day 9 showed small placental residues at necropsy. None showed stimulated ovarian interstitial tissue, follicles or corpora lutea.

The 1 μg dose of estrone is the estrogenic equivalent necessary with progesterone to maintain pregnancy in the rat (7); but our interest in using it in three subgroups lay in the possibility that it might increase LTH production by the placental cells, and thus explain any beneficial effect due to estrogen stimulation by the the high dose of ICSH slightly contaminated with FSH as evidenced in the ovaries and shown in Table I. Estrogen by itself or without its counteracting (sometimes called synergistic) action of progestogen may act as an abortifacient in the rat. It is therefore not surprising that of 16 rats in the estrone-treated groups, only 2 retained living fetuses and another 8 showed resorption sites. As in the uninjected control rats, all of the estrone-treated rat ovaries showed regressed interstitial and follicular tissue. Only the 2 rats with live fetuses (1 in group 1C, and 1 in group 3B) showed functional luteal cell stimulation (hypertrophy) typical of LTH of either chorionic or pituitary origin. In the 3 estrone-treated groups a total of 74 resorption sites were found in 8 of the 16 rats. Also in these groups 1 rat hypophysectomized on Day 7, and 5 on Day 8 showed complete resorption at necropsy.

All of the 14 rats in groups 1A in which the Moudgal regimen was used (100 μg of ICSH in gelatin daily from Days 8 to 12) had successfully nidated their embryos. At necropsy on Day 13, 5 showed no living young whereas 9 had a total of 98 (range 5–15) live fetuses and 22 (range 1–6) resorbing concepti. The 5 rats with no living fetuses had a total of 65 (range 10–15) resorbing concepti. Group 1C in which autografts of 1/8 to 1/4 of a pituitary were placed beneath the renal capsule showed essentially the same findings as those in group 1A namely: 2 rats showed no living fetuses but had 13 and 14 resorbing concepti, respectively; whereas 4 had a total of 35 living fetuses (range 4 to 13) and 16 resorbing concepti (range 0 to 10). All of the ovaries of these 2 groups comprising 20 rats showed stimulated interstitial tissue and follicles (many thecaluteinized). The corpora lutea in all but 5 rats in group 1A and 2 rats in group 1B showed hypertrophied cells typical of LTH stimulation. These 7 without living fetuses but with placental remnants showed corpora with mixtures of stimulated, maintained and degenerating cells.

Group 3A, hypophysectomized on Day 9

TABLE I. Effects of Various Treatments on the Maintenance of Pregnancy in Hypophysectomized Rats.

Group	No. rats	Day[a] of pregnancy			Daily treatment	No. of live fetuses	No. resorb. sites	Rats without sites or fetuses	No. of rats showing stimulation[b] of		
		$\overline{H}$	Necropsy	Inject					IT	Foll.	CL
1A	14	8	13	8–12	100 μg ICSH	98 (9 rats)	87 (14 rats)	0	14	14	9[c]
1B[d]	6	8	13	8–12	100 μg ICSH	35 (4 rats)	43 (6 rats)	0	6	6	4[c]
1C	9	8	13	8–12	1 μg Estrone	12 (1 rat)	32 (2 rats)	5	0	0	1
1D	6	8	13	—	—	0	10 (1 rat)	5	0	0	0
2A	4	7	13	7–12	100 μg ICSH	0	37 (4 rats)	0	4	4	0
2B	3	7	13	7–12	1 μg Estrone	0	15 (2 rats)	1	0	0	0
2C	3	7	13	—	—	0	5 (1 rat)	2	0	0	0
3A	5	9	14	9–13	100 μg ICSH	53 (5 rats)	8 (5 rats)	0	5	5	5
3B	4	9	14	9–13	1 μg Estrone	8 (1 rat)	27 (3 rats)	0	0	0	1
3C	3	9	14	—	—	0	33 (3 rats)	0	0	0	0

[a] Day 1 = day of sperm detection in vagina.

[b] Histologic evidence of interstitial, follicular or luteal cell stimulation.

[c] The corpora of the rats without living fetuses in groups 1A and 1B were composed of a mixture of stimulated, maintained and degenerating luteal cells.

[d] These rats received 1/8 to 1/4 of a pituitary autograft under the renal capsule.

and injected with ICSH showed good maintenance of pregnancy in all of the 5 rats. Group 2A hypophysectomized on Day 7 and also injected with ICSH showed no living fetuses, but a total of 37 resorption sites (range 5–11) in all 4 rats. Interstitial cell and follicular stimulation including theca-luteinization was found in the ovaries of all rats in both of these groups, whereas luteal cell hypertrophy was found only in the 5 rats of group 3A.

Discussion. Since it had already been shown that rat chorionic mammoluteotropin (RCM) is detectable by direct test as early as Day 8 of pregnancy (5), and indirectly by the fact that one injection of ergot alkaloids (inhibitors of pituitary but not placental mammotropin) interrupts pregnancy if given before but not after the eighth day (13), it seemed reasonable to expect a greater influence of RCM as pregnancy progressed toward Day 12 when that hormones' highest activity has been detected (4, 5, 14). Furthermore, it is difficult to understand why a daily dose of 100 μg of ICSH in itself should be more "luteotropic in rats hypophysectomized on Day 8 than in those with pituitary ablation on an earlier day of gestation. In our earlier experiments (6, 8, 9) neither ICSH nor an FSH-ICSH combination maintained pregnancy in rats hypophysectomized from Days 1 to 6. That these hormones are important for the ovarian secretion of estrogen was readily demonstrated in the same experiments in which only hypophyseal or placental mammotropin provided the luteotropic activity.

The question of how the completely abnormal hyperluteinized ovaries induced by the high 100 μg daily dose of ICSH (with slight FSH contamination) may respond differently from the normal ovary of pregnancy in some but not all cases to the only luteotropin being secreted in $\overline{H}$ rats on the eighth day of gestation (RCM), remains to be answered by further experimentation that should include steroid titrations. The abnormality of the ovaries [see photomicrographs in Ref. (12)] subjected to 100 μg ICSH in gelatin daily for 5 days should be appreciated in attempts to understand some of the cases of pregnancy-maintenance. There also arises the question of whether the secretion of known amounts of estrogen from such ovaries effects the release of possibly increased amounts of chorionic luteotropin (as is true of hypophyseal MH-LTH).

The late Professor Alloiteau (15) summarized most succinctly the views supported by our own research as follows: "Le corps jaune ne serait maintenu réactivable que si l'intervalle entre la privation en LTH hypophysaire et l'apport de LTH placentaire est court," and "le placenta sécrète, dès sa formation, une substance lutéotrophe dont la quantité augmenterait peu à peu." This investigator had the support of our previous findings which showed that in rats hypophysectomized on Day 6 of gestation, pregnancy could be maintained by giving them a daily equivalent of five 12-day placentas (fetal), or one of these plus 0.5–1.0 μg of estrone. Our unpublished findings that approximately 0.5 μg of estrone equivalent may be extracted as the momentary content of twelve 12-day placentas explains the nonnecessity of adding estrone if a sufficient number of placental equivalents are injected.

That an anti-LH serum may terminate pregnancy during its first half (16, 12) when the RCM titer is increasing need not only be explained on the basis of its counteracting the required estrogen level; but quite plausibly by the fact that it is developed against the hormone upon which the formation, but not the progestogen-secretion of the luteal cell depends. Once formed the luteal cell persists but cannot be said to function to any significant degree in the absence of hypophyseal or chorionic LTH. And if it is deprived of either of the LTH's for 48–72 hr these "tropic" hormones become "lytic" (17, 18); and induce degenerative changes in the corpora lutea not unlike those illustrated as the result of anti-LH serum (16, 12). This suggests that an antibody to LH may alter the luteal cell, or its surface membrane in such a way as to prevent the hypophyseal or chorionic luteotropins from acting during a critical interval on the appropriate enzyme systems that lead to progesterone synthesis and not to luteolysis.

Summary. A high dose of ICSH (100 μg in

gelatin daily for 5 or 6 days) in Long-Evans rats hypophysectomized ($\overline{H}$) on Days 7, 8 or 9 of pregnancy induced hyperluteinization in the ovaries. The usual number of 10–12 corpora lutea of pregnancy were found and also about twice that number of theca-luteinized corpora. This latter finding, and the presence of stimulated ovarian follicles showed that the ICSH had a minor but significant contamination with FSH in a ratio that could be expected to induce estogen secretion.

All 4 rats $\overline{H}$ on Day 7 and injected for 6 days with ICSH showed resorbing sites, and no living fetuses on Day 13. Of 14 rats $\overline{H}$ on Day 8 and injected for 5 days with ICSH, nine showed living fetuses (av 11) on Day 13. Of 5 rats $\overline{H}$ on Day 9 and injected for 5 days with ICSH, all showed living fetuses (av 10) on Day 14.

Since rat chorionic mammoluteotropin (RCM) secretion begins on Day 7; and increases to a peak on Day 12, it would seem that its luteotropic activity was responsible for adequate amounts of progesterone in cases of successful maintenance of pregnancy. Undoubtedly the ICSH plus FSH combination induced the necessary small amount of estrogen to be secreted; and this in turn may have induced the placenta to secrete increased levels of RCM.

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