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Hormonal Changes Associated with Estrogen Induced Luteolysis in the Pregnant Hamster: A Reevaluation of the Luteotropic Complex (42255)

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Abstract. Hamsters were injected sc on Day 1 of pregnancy (sperm positive) with 50 μg estradiol cyclopentylpropionate (ECP) or peanut oil. On Day 5, serum progesterone (P_4) was 10.6 ng/ml in controls vs 3.1 ng/ml after ECP. In the ECP group, serum prolactin (PRL) and follicle stimulating hormone (FSH) did not differ from controls but serum luteinizing hormone (LH) was significantly lower than that of the controls, and usually below the sensitivity of the radioimmunoassay (RIA). After ECP, structural signs of luteolysis (weight and histology) and absence of antral follicles characterized the ovary. Injection of an anti-LH serum on Day 4 halved serum P_4 levels on Day 5 in control animals but caused no further lowering of P_4 in ECP-treated hamsters. Treatment on Days 1–5 with 1.0 IU hCG or 10 μg LH plus ECP on Day 1 restored, by the afternoon of Day 5, serum P_4 to the control range (9–10 ng/ml) and antral follicles were now present. The results indicate that a large dose of ECP causes luteolysis by reducing LH levels and reinforce the concept of a luteotropic complex in the hamster with PRL and FSH constituting the minimal components and LH serving as a synergist.

The maintenance of functional corpora lutea (CL) in the pregnant hamster has been proposed to depend upon a luteotropic complex (LTH complex) with prolactin (PRL) and follicle stimulating hormone (FSH) serving as the minimal components and with luteinizing hormone (LH) modulating the response, with small amounts synergizing and large amounts antagonizing the minimal complex [for references, see (1)]. The first evidence for this concept was provided by experiments in which injection of a long acting estrogen, estradiol cyclopentylpropionate (ECP), led to regression of the corpora lutea of pregnancy by Day 4 (2). The luteolytic action of ECP was reversed by PMS, hCG, and FSH (100–200 μg/day) but not by the same doses of LH or 1 mg PRL (2). The availability of radioimmunoassays (RIAs) now makes it feasible to reexplore this problem by determining serum changes in sex steroids, FSH, and LH after the administration of a single dose of ECP on Day 1 of pregnancy.

Materials and Methods. Female hamsters weighing 80–110 g were maintained on a lighting schedule of 14 hr L: 10 hr D (lights on: 0500 hr). After a minimum of 3 consecutive 4-day cycles, proestrous females were caged overnight with males; the next morning was designated as Day 1 of pregnancy if sperm were present in the vaginal lavage. The basic experimental design consisted of sc injection of hamsters on Day 1 of pregnancy at 0900 hr with either ECP (provided by Upjohn Laboratories, Kalamazoo, Mich.) or peanut oil (as a control). All injections were given in 0.1 ml peanut oil. Four experiments were performed, involving the measurement of several hormones by RIA (see below).

Experiment 1. Hamsters (groups of eight each) were injected on Day 1 with either 50 μg ECP or peanut oil and decapitated at 0900 hr on Days 2–8 and trunk blood was saved for analysis of serum levels of progesterone (P_4), estradiol (E_2), FSH, LH, and PRL. Three ovaries for each group were saved for routine histological preparations and slides were stained with H & E. Average luteal weight was determined by dissecting a minimum of six CL from the ovaries, determining the collective weight and then the average for each animal. Additional groups of animals were ovariectomized on Day 1 of pregnancy and then immediately injected sc with 50 μg ECP and killed on Days 4 and 8 to monitor serum levels of estradiol.

Experiment 2. Hamsters (eight per group) were injected sc on Day 1 with either 25, 50, or 100 μg ECP. Unanesthetized animals were then bled via cardiac puncture on Days 2 and 4 at 0900 hr (0.5 ml blood was collected) and decapitated on Day 6. Serum for all 3 days was assayed for P_4, LH, and FSH.

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Experiment 3. After a single injection of 50 μg ECP or peanut oil on Day 1, animals (eight per group) were bled by cardiac puncture on Day 4 at 0900 hr with sufficient blood withdrawn to measure serum P_4 and FSH. Immediately afterward, the hamsters were injected sc with either a potent equine antibovine LH (anti-LH) which has been characterized in a previous paper (3) or with ergocryptine (ECR), kindly provided by Sandoz, Inc. (East Hanover, N.J.). The ECR was injected in 0.1 ml 70% ethanol. The animals were decapitated the next day at 0900 hr and serum was again saved for RIAs of P_4 and FSH.

Experiment 4. Hamsters were injected on Day 1 of pregnancy with either peanut oil or, in most instances, with 50 μg ECP. Various gonadotropins were then injected sc beginning at Day 1, 0900 hr through Day 5 with hCG and PMS given once daily. Ovine FSH (NIH-S10) and LH (NIH-S18) were given as divided sc doses at 0900 and 1500 hr on Days 1–4 with a single injection at 0900 hr of Day 5. All hormones were administered in 0.1 ml physiological saline. The hamsters were decapitated at 1300 hr on Day 5 and serum was saved for measurement of P_4. In addition, the ovaries were examined with a dissecting microscope and the presence or absence of large antral follicles (>600 μm) was noted.

Radioimmunoassays. Serum estradiol was determined with an antibody kindly provided by Dr. D. Exley (4) and used in an RIA procedure described previously (5). All serum samples were measured in a volume of 300 μl. The lower limit of sensitivity was 2 pg/tube. Serum from chronically hypophysectomized–ovariectomized hamsters contained 12 pg/ml of immunoreactive E_2.

Serum P_4 was measured with an antibody to progesterone provided by Dr. A. Surve (6) and as described previously for this laboratory (3). A volume of 10 μl serum was used. Serum from chronically hypophysectomized–ovariectomized hamsters contained 0.6 ng/ml of immunoreactive P_4.

Rat RIA kits for determination of FSH and PRL were provided by the National Pituitary and Hormone Program, NIADDK and have been previously validated for the hamster (7). Serum LH was determined by two different antibodies. In experiment 1, the ovine: ovine RIA system for LH was used (8) as described for the hamster (9). In the second experiment, LH was measured by the rat:rat RIA as validated for the hamster (7). The specific antibody used was anti-rat LH serum 3. The serum volumes used for measuring FSH, LH, and PRL were, respectively, 100, 100, and 50 μl. Reference preparations for standards were rat LH-RP-1 (0.03 NIH-LH-S1), FSH-RP-1 (2.11 NIH-FSH-S1), and PRL-RP-1 (11 IU/mg).

For all experiments, Students t test at $P < 0.05$ was used to establish statistical significance.

Results. Following the administration of 50 μg ECP on Day 1 of pregnancy, luteal weight was essentially unchanged through Day 7 whereas in the control group the CL began to increase in size on Day 6 (Fig. 1). Ovarian histology revealed that the first signs of luteolysis after estrogen treatment occurred on Day 4 in the form of incipient infiltration of polymorphonuclear leukocytes. Throughout the 8-day period, healthy antral follicles were never present in the ECP-treated animals.

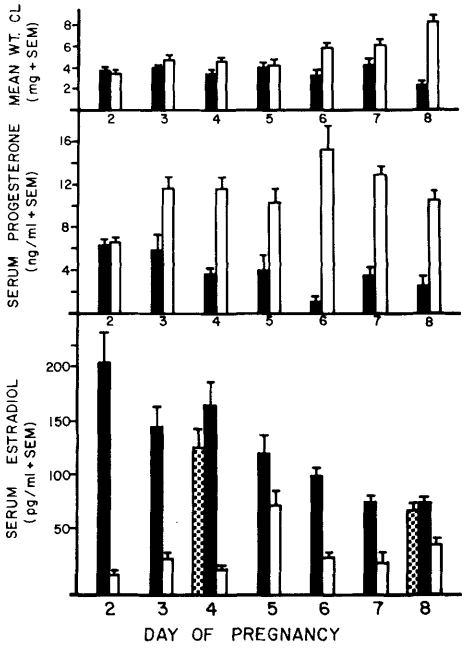

FIG. 1. Effects of a single sc injection of 50 μg ECP (black bar) or peanut oil (clear bar) on Day 1 of pregnancy on luteal weight and serum levels of progesterone and estradiol. An additional group of animals were ovariectomized on Day 1, injected with 50 μg ECP, and serum estradiol was determined on Days 4 and 8 (stippled bar).

Serum P_4 levels began to diverge on Day 3 and, in general, ran $\frac{1}{3}$ to $\frac{1}{4}$th lower in the ECP-treated groups (Fig. 1); except for inexplicably low levels on Day 6, serum P_4 was always in the detectable range (>1 ng/ml).

As anticipated, serum E_2 was elevated in the ECP-treated hamsters, ranging from a high of 200 pg/ml on Day 2 to 85 pg/ml on Day 8. The fact that serum E_2 was virtually superimposable between the intact and ovariectomized ECP-treated hamsters on Days 4 and 8 indicates that the circulating values reflect the exogenous hormone.

Despite the elevated levels of E_2 in the ECP group, serum PRL was unaffected and this was also true for FSH (Fig. 2). Beginning on Day 2, however, serum LH was consistently undetectable in the ovine:ovine RIA (Fig. 2).

In the next experiment, the effects of 3 doses of ECP were compared with control values. For the controls, serum P_4 showed a steady increase over the 6-day period whereas there was no dose–response relationship evident for

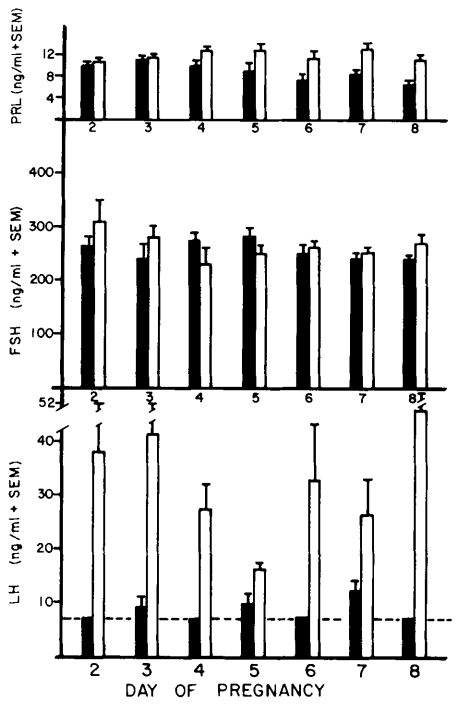

FIG. 2. Effects of 50 μg ECP (black bar) or peanut oil (clear bar) on serum gonadotropin levels. For LH, the dotted line represents the detectable limit of the RIA. These values are from the animals shown in Fig. 1.

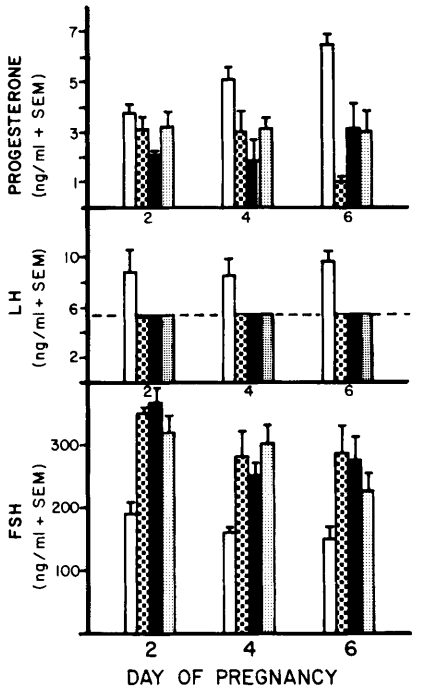

FIG. 3. Effects of peanut oil (clear bar) or 25, 50, or 100 μg ECP (respectively, the next 3 bars) administered on Day 1 on subsequent hormone levels. For LH, the dotted line represents the detectable limit of the RIA.

25 to 100 μg ECP, with values usually in the range of 1 to 3 ng/ml (Fig. 3). Serum FSH and LH (measured by rat RIA kits) remained relatively unchanged in the control groups on Days 2–6 but for the ECP-treated hamsters, serum LH was below the sensitivity of the assay. In contrast to the results of Experiment 1, serum FSH was significantly elevated for all three ECP-treated groups compared to the controls (Fig. 3). Whether this was due to the method of blood collection or merely chance, can not be ascertained.

Administration of anti-LH to control hamsters on Day 4 of pregnancy resulted 24 hr later in a sharp drop in serum P_4 whereas the levels were unaffected in the ECP-treated hamsters (Table I). Similarly, ergocryptine which inhibits PRL secretion (10), was effective in the controls but after ECP treatment neither 100 μg nor 1 mg ergocryptine further reduced serum P_4 (Table I).

In the final experiment, the efficacy of various gonadotropins in reversing the effects of ECP was evaluated (Table II). Serum P_4 was

TABLE I. EFFECTS OF DIFFERENT TREATMENTS ON DAYS 1 AND 4 ON SERUM PROGESTERONE

Group	Treatment	Hormone levels on day	Serum progesterone (ng/ml ± SEM)
1	Day 1: peanut oil	4	9.9 ± 1.6 (8)
	4: 100 μl anti-LH	5	4.4 ± 0.9 (8)*
2	Day 1: 50 μg ECP	4	2.3 ± 0.7 (8)
	4: 100 μl anti-LH	5	1.7 ± 0.4 (8)
3	Day 1: peanut oil	4	8.3 ± 0.5 (8)
	4: 100 μg ergocryptine	5	5.7 ± 0.8 (8)*
4	Day 1: 50 μg ECP	4	3.8 ± 1.2 (8)
	4: 100 μg ergocryptine	5	2.8 ± 0.7 (8)
5	Day 1: 50 μg ECP	4	3.0 ± 0.8 (8)
	4: 1 mg ergocryptine	5	2.2 ± 0.6 (8)

* $P < 0.01$.

restored to control levels by daily injection of hCG or 10 μg LH which also enabled antral follicles to mature (the latter parameter was not evaluated in the 2 or 5 μg LH groups).

Discussion. The major factor responsible for the slow decline in luteal function in the ECP-treated hamster is the absence of detectable levels of serum LH (Figs. 1–3) in the face of maintenance of PRL and FSH. The latter hormones have been termed the minimal LTH complex (1) and indeed were capable of sustaining serum P_4 levels at 2–4 ng/ml in the present study. After estrogen treatment, the sharp dichotomy between maintained FSH and virtually nonexistent LH raises the spectre of possibly separate releasing factor(s) for the two hormones. The possibility exists that undetectable amounts of immunoreactive LH may still have bioactivity but this seems to be negated by the inability of a very potent anti-LH to further decrease serum P_4 in ECP-treated animals (Table I). Since PRL is necessary for the morphologic persistence of CL in the hamster (1) I had anticipated that ergocryptine would also act as a luteolytic compound. This was true for the control animals where serum P_4 was halved in 24 hr but not for the ECP-treated groups (Table I). The most likely explanation is that a larger time span may be needed as the endpoint for complete cessation of progesterone secretion. This seems to be the case when ergocryptine is given to interrupt pregnancy in the hamster (10).

The final experiment dealt with the obvious effects of LH replacement as the missing component of the LTH complex and showed that

TABLE II. EFFECTS OF EXOGENOUS GONADOTROPINS ON SERUM PROGESTERONE AND FOLLICULAR GROWTH IN ECP-TREATED HAMSTERS

Treatment (Days 1–5)		Serum progesterone (ng/ml ± SEM) on Day 5 at 1300 hr	No. animals with antral follicles
Peanut oil + saline		10.6 ± 0.7 (8)*	8/8
50 μg ECP	+ saline	3.1 ± 0.6 (8)	0/8
	+ 1 IU hCG	8.1 ± 1.2 (8)*	8/8
	+ 1 IU PMS	4.4 ± 0.9 (8)	8/8
	+ 200 μg FSH	4.7 ± 0.5 (8)	0/8
	+ 1 μg LH	5.0 ± 0.6 (8)	0/8
	+ 2 μg LH	5.0 ± 1.2 (8)	—
	+ 5 μg LH	3.9 ± 0.9 (8)	—
	+ 10 μg LH	7.7 ± 1.2 (8)*	8.8

* $P < 0.01$ compared to ECP + saline value.

serum P_4 was brought up to normal levels by 10 μg LH or 1.0 IU hCG (Table II). In the present experiment, the ability of 10 μg LH to restore normal serum levels of progesterone after ECP treatment (Table II) contrasts with the initial results reported in 1965 (2). Two likely explanations for the different results are that in the early study, luteal histology was used as the endpoint but more importantly the levels of LH were probably excessive: 50 to 200 μg (2). Thus, the amount of LH injected was luteolytic not luteotropic. Previous studies have shown that LH alone or combined with PRL is ineffective in maintaining P_4 secretion and pregnancy in hypophysectomized hamsters (1). Daily injection of either LH or hCG also restored follicular development (Table II). This lends further credence to the findings in the first two experiments which indicated undetectable levels of serum LH after ECP. I have previously reported that 200 μg daily of FSH to hypophysectomized hamsters fails to mature antral follicles whereas the same amounts (and same batch) do restore antral follicles to hypophysectomized rats or mice [references in (11)]. The hamster evidently requires tonically higher levels of LH for optimal development of antral follicles.

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