

Effects of Multiple Small Doses of Progesterone on Estrogen and Gonadotropin Secretion in the Cyclic Hamster (41330)

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Abstract. The sc injection of 10 μg of progesterone at 0900, 1200, and 1500 h of Day 3 of the hamster estrous cycle (Day 1 = morning of ovulation) delayed ovulation by at least one day. Following progesterone treatment, the temporal changes in hormone levels on Day 3 were determined. Serum estradiol declined significantly at 1300 hr (1 hr after the second injection of progesterone) and was depressed until at least 2200 hr of Day 3. Serum estradiol returned to normal levels by 0900 hr, Day 4. Serum levels of progesterone ranging from 1.2 to 2.8 ng/ml were associated with the decline in estradiol; these levels of progesterone are within the physiological range of 5 ng/ml produced by the corpora lutea on Days 1 and 2 of the cycle. The rapid initial fall in estradiol produced by exogenous progesterone was not paralleled at 1300 hr by similar declines in FSH, LH, or PRL. However, at subsequent time intervals on Day 3, serum LH was 3–4 ng/ml less in the progesterone-treated hamsters. Therefore, the use of multiple small doses of progesterone to suppress estrogen secretion does not resolve the question of whether progesterone can directly affect follicular function without exerting central inhibitory effects on LH release.

A single subcutaneous injection of 2.5 mg progesterone on Day 1 of the cycle (morning of ovulation) delays ovulation by 2 days in the hamster (1) whereas 50 μg injected on Day 3 delays ovulation by 1 day in all hamsters and even 25 μg of progesterone is effective in 75% of the treated animals (2). After 50 μg of progesterone, serum estradiol falls significantly within 3 hr paralleled by a decline in serum LH, but not FSH (2). Similarly, in the 4-day cyclic rat, a minimal injection of 800 μg of progesterone is required to delay ovulation by one day and this is associated with rapid decreases in serum estradiol and LH (3). Thus, in the cyclic hamster and rat, the effective doses of progesterone which delayed ovulation evidently lowered estrogen by inhibitory effects on the hypothalamic–pituitary axis and not by direct action on the antral follicles.

The purpose of the present experiments was to establish the minimal effective dose of multiple injections of progesterone needed to delay ovulation by one day in cyclic hamsters and to determine the effect of this treatment on serum levels of estradiol, progesterone, gonadotropins (FSH and LH), and prolactin.

Materials and Methods. Golden hamsters (*Mesocricetus auratus*) weighing 80–120 g were used, after at least three consecutive 4-day estrous cycles were monitored by checking every 4 days for the conspicuous postovulatory vaginal discharge (Day 1). Day 1 corresponds to the morning of ovulation; Day 4 corresponds to proestrus.

In the first experiment, hamsters were given three sc injections on Day 3 at 0900, 1200, and 1500 hr and killed on Day 5 between 0900 and 1000 hr. The ovaries were then inspected for newly formed corpora lutea and the oviducts flushed to recover ova, still embedded in granulosa cells. The injections on Day 3 consisted of either progesterone or the vehicle peanut oil in a volume of 0.1 ml. This experiment showed that 10 μg of progesterone \times 3 injections blocked ovulation in all hamsters and this regimen was used in the second experiment.

To determine the effects of exogenous progesterone on hormone levels, hamsters were injected sc beginning on Day 3 at 0900 hr with either 10 μg of progesterone or 0.1 ml of peanut oil. At times indicated in Table II, groups of six hamsters each were de-

capitated and trunk blood saved for RIA of steroid and peptide hormones by previously described methods (4,5). The antiserum for the progesterone RIA was produced in rabbits against progesterone-11 α -bovine serum albumin (6) and for the estradiol RIA the antiserum was produced in rabbits against 17 β -estradiol-6-(*O*-carboxymethyl)oxine-bovine serum albumin (7).

Rat RIA kits for determinations of FSH and PRL were provided by NIAMDD. Antibodies used were anti-rat FSH serum 6 and anti-rat PRL serum 3. The reference preparations used as standards were rat FSH-RP-1 (2.1 \times NIH-FSH-SI) and PRL-RP-1 (11 IU/mg). Serum LH was determined by the ovine-ovine RIA system of Niswender *et al.* (8). Anti-ovine LH serum (GDN-15) and an ovine LH for iodination (LER-1056C2) were kindly provided by Drs. Gordon D. Niswender and Leo E. Reichert, respectively. Serum LH is expressed in terms of NIAMDD rat LH-RP-1 (0.03 \times NIH-LH-SI).

Data were analyzed following analysis of variance by using Duncan's multiple range test (9) and differences were judged significant if $P < 0.05$.

Results. The dosage regimen which inhibited ovulation by one day in all animals was the series of three injections of 10 μ g progesterone (Table I). The ovaries on Day 5 all contained large antral follicles which would have presumably ovulated by the next day (2).

The effects of 10 μ g progesterone or 0.1 ml peanut oil (the control group) on steroid and peptide hormone levels are shown in Table II. A significant fall in serum estradiol occurred at 1300 hr—one hour after

the second injection of 10 μ g progesterone—and thereafter the serum levels of E₂ were depressed until at least 2200 hr of Day 3. The drop in estradiol was associated with circulating levels of progesterone of 1.2 to 2.8 ng/ml.

The initial fall in serum estradiol after progesterone treatment at 1300 hr was not paralleled at that time by similar declines in FSH, LH, or PRL (Table II). After progesterone treatment there was a significant increase in serum FSH at 1600 hr of Day 3, a phenomenon which has previously been observed (2). Serum PRL only differed at 1900 hr, Day 3 between the groups.

The situation for serum LH after progesterone treatment was more complicated. Analysis of variance revealed a significant difference in LH ($F = 9.45$; $P < 0.01$) between the progesterone- and vehicle-treated groups but not for within groups with time ($F = 2.09$; not significant). By the Duncan's test, the serum LH values at 1600 hr, Day 3 were just approaching significance ($P > 0.05$) but by Student's *t* test a highly significant difference ($P < 0.02$) existed between the controls and progesterone-treated hamsters. The highest serum LH levels were found in the control group at 1900 hr, presumably correlated with the peak levels of estradiol. At 1900 hr, Day 3 serum LH was significantly lower ($P < 0.05$) by the multiple range test in the progesterone-injected hamsters. The overall LH value from 1000 until 2200 hr Day 3 for the progesterone versus peanut oil-treated animals was 12.8 ± 0.6 ng/ml ($n = 28$) and 18.4 ± 1.0 ($n = 29$), respectively. The mean value for the control group is inflated by the LH increase observed at 1900

TABLE I. EFFECT OF DIFFERENT DOSES OF PROGESTERONE (P) INJECTED SC ON DAY 3 ON SUBSEQUENT OVULATION

Treatment on Day 3:			No. ovulating Day 5/total	Mean No. ovulations \pm SEM
0900 hr	1200 hr	1500 hr		
1 μ g P	1 μ g P	1 μ g P	4/4	15.0 \pm 0.4 (4)
5 μ g P	5 μ g P	5 μ g P	7/8	12.4 \pm 0.9 (7)
10 μ g P	P.O. ^a	P.O.	8/8	12.4 \pm 0.8 (8)
10 μ g P	10 μ g P	P.O.	3/8	15.3 \pm 1.3 (3)
10 μ g P	10 μ g P	10 μ g P	0/8	0

^a Peanut oil (0.1 ml).

TABLE II. EFFECTS OF PROGESTERONE OR PEANUT OIL INJECTED ON DAY 3 OF THE HAMSTER CYCLE ON SERUM LEVELS OF HORMONES^a

Time and treatment	Estradiol (pg/ml ± SEM)	Progesterone (ng/ml ± SEM)	FSH (ng/ml ± SEM)	LH (ng/ml ± SEM)	PRL (ng/ml ± SEM)
1000 hr P ₁₀	66 ± 7 ^b	0.9 ± 0.4(5)	174 ± 5	14.6 ± 1.9	3.3 ± 0.3
D3 P.O.	75 ± 7	0.2 ± 0.09	111 ± 5	19.7 ± 2.6	3.3 ± 0.3
1300 hr P ₁₀	31 ± 5*	2.8 ± 0.5*	173 ± 7	12.9 ± 1.0	2.9 ± 0.3
D3 P.O.	61 ± 9	0.5 ± 0.3	158 ± 8	15.2 ± 1.7(5)	2.3 ± 0.3
1600 hr P ₁₀	33 ± 4*	2.8 ± 0.5*	203 ± 13*	11.6 ± 0.9	3.4 ± 0.7
D3 P.O.	69 ± 14	0.8 ± 0.2	138 ± 7	17.6 ± 1.8	3.9 ± 0.5
1900 hr P ₁₀	39 ± 7*	2.4 ± 0.5*	171 ± 8	13.2 ± .6(5)*	5.0 ± 0.8*
D3 P.O.	98 ± 12	1.1 ± 0.4	167 ± 6	23.5 ± 1.8	2.9 ± 0.3
2200 hr P ₁₀	40 ± 8*	1.2 ± 0.7(5)	184 ± 15	11.9 ± 1.6	4.1 ± 0.5
D3 P.O.	95 ± 12	0.1 ± 0.1	167 ± 7	15.8 ± 1.2	3.3 ± 0.5
0900 hr P ₁₀	84 ± 9	0.1 ± 0.09	142 ± 2	21.4 ± 1.1	4.1 ± 0.7
D4 P.O.	66 ± 12(5)	0.9 ± 0.06	197 ± 16	18.5 ± 1.2(5)	4.4 ± 0.5

^a 10 μg progesterone (P₁₀) or peanut oil (P.O.) injected sc at 0900, 1200, and 1500 hr.

^b n = 6 for all groups unless otherwise noted.

* Significantly different (P < 0.05) from control (P.O.) value.

hr, Day 3. If the mean value for each group is therefore calculated from 1000 through 1600 hr, the averages for the progesterone versus peanut oil groups were 13.1 ± 0.80 ng/ml (n = 17) and 17.6 ± 1.2 ng/ml (n = 17), respectively.

Discussion. The salient finding of this study is that the minimal effective regimen of progesterone which delayed ovulation by one day (Table I), led to a significant decline in serum estradiol initially at 1300 hr, Day 3 without concomitant decreases in LH, FSH, and PRL (Table II). However, insofar as LH is concerned, the situation became cloudy thereafter with several of the progesterone-treated groups showing borderline (1600 hr) or significant (1900 hr) differences from the control group.

In a previous study, a single injection of 50 μg progesterone in the cyclic hamster produced circulating levels of 8–10 ng/ml of progesterone and in most instances, serum LH was below the sensitivity of the RIA (2). Similarly, in the cyclic rat injection of 800 μg of progesterone on diestrus II resulted in serum levels of 40–60 ng/ml of progesterone and within 1 hr concomitant significant declines in serum estradiol and gonadotropins (3). Moreover, this was paralleled by a drastic decrease in the *in vitro* production rate of progesterone, testosterone, and estradiol by the antral follicles (3). In the control hamsters on Day 3, serum progesterone fell below the limits of sensitivity of the RIA < 1 ng/ml (Table II). The exogenous progesterone raised serum progesterone to 2.8 ng/ml which is below the level of 4–6 ng/ml found on Days 1 and 2 of the hamster cycle when functional corpora lutea are present (4). Hence, the effective dose of progesterone used in this study fell within the physiological levels of the hormone present during the estrous cycle unlike the possible pharmacological amounts employed in our previous investigations of the hamster (2) and rat (3).

Although antral follicles have already differentiated by the afternoon of Day 2 of the hamster cycle, the levels of serum estradiol are very low until the functional demise of the corpora lutea at 0400 hr of Day 3 (10). Using the ovine-ovine RIA for LH, we have previously reported that after

serum progesterone levels decline on Day 3 in the hamster cycle there is an approximate doubling in serum levels of LH (11) which coincides with the striking increase in circulating levels of estrogen. Thus, progesterone may exert a double-barreled effect on the regulation of estrogen secretion by acting to suppress LH release from the pituitary and by acting directly on the follicles. The use of low doses of progesterone—after the corpora lutea are no longer functional—may be a way of dissociating the hypothalamic–pituitary actions of the hormone from its direct ovarian effects. However, in the present study, after the initial decline in serum estradiol which was not associated with evident changes in the hypothalamic–pituitary axis, there was a fall of 3–4 ng/ml of LH in the progesterone-treated hamsters. How much of the subsequent suppression in serum estrogen, therefore, represents direct and/or indirect effects of progesterone is still unresolved. Obviously, considerably more research is required to determine whether progesterone at different stages of the cycle can directly inhibit follicular function.

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