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Effects of Progesterone Injections Administered During Late Pregnancy on Lactation and Nursing Behavior in the Rat[1] (37950)

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Ovarian hormones have long been known to facilitate lactation (1). However, under certain conditions, as when administered to lactating rats, they markedly suppress it. Among the reasons advanced have been a disturbance of secretory events in the mammary glands (e.g., biochemical changes in the milk, no milk secretion at all due to involution of the mammary glands, etc.), the failure of milk release, or poor nursing behavior (2–7).

Recent reports have particularly implicated progesterone in the inhibition of lactation (8–10). Injections of progesterone into late pregnant rats, for example, markedly depressed litter growth. Under these conditions, of 2-mg progesterone injections twice daily from Days 18–23 of gestation, nursing behavior remained normal (8). Inhibition of nursing behavior only occurred when progesterone injections were resumed after birth, after having been administered prepartally from Days 18 through 22 (10). However, lactation remained undisturbed.

Thus the inhibition of lactation, apart from nursing behavior, occurred when progesterone was injected prepartally from Days 18–23, and not from Days 18–22. The question arises as to whether only one day, Day 23, may be critical for the inhibition of lactation. We tested this view by comparing the effects on lactation and nursing behavior of progesterone injected into late pregnant rats on one of two days late in gestation, on either Day 18 or Day 23.

Materials and Methods. Virgin Sprague–Dawley rats, 60–70 days old and weighing 175–225 g, were bred with males and injected subcutaneously with 2 mg of progesterone in 0.1 ml of sesame oil twice daily on either Day 18 or Day 23 of gestation. Control rats received only the oil. From Day 18 of gestation on, the animals were housed and fed according to laboratory procedures (8–10). They were observed daily for the onset of birth, and on that day, litters were reduced or brought up to 6 pups/litter. Litters were then maintained daily at 6 pups for 5 days thereafter. Throughout this time, the mortality of the litters was scored daily as were the weight gains of the surviving young.

The animals were also observed in standard nursing tests for 15 min each day in which the latency and duration of crouching behavior (assuming a lactation-like posture over young) was scored. For further descriptions of the tests, see Herrenkohl and Rosenberg (11). The nursing test began when 3 foster pups the same age as the litter were introduced into the case each morning, 0.5 hr after the female's own litter had been removed. Thus, despite variability in the litter condition, nursing behavior was always scored in the presence of healthy viable young.

Results. Table I summarizes the lactational performance and nursing behavior of rats injected with progesterone on Day 18 or 23 of gestation. It shows first of all that 2-mg doses of progesterone twice daily on

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TABLE I. Lactational Performance and Nursing Behavior of Rats Injected with Progesterone on Days 18 or 23 of Gestation.

	Treatment during gestation			
	Day 18 2 mg Progesterone (n = 10)	Day 18 Sesame oil (n = 11)	Day 23 2 mg Progesterone (n = 11)	Day 23 Sesame oil (n = 10)
Postpartum Day 1				
Day of Birth (median)	23	23	25***[a]	23
Ratio of dead to live pups (No.)	1/117	4/122	49/41***	3/119
Wt change in litter between Days 1 and 2 (g) $\bar{X} \pm$ SE	3.2 ± 1.2	2.2 ± 0.9	1.7 ± 1.9	4.5 ± 0.8
Postpartum Day 5				
Ratio of dead to live pups (No.)	0/60	1/65	0/66	0/58
Wt change in litter between Days 4 and 5 (g) $\bar{X} \pm$ SE	9.3 ± 0.9	9.8 ± 1.0	5.3 ± 1.6**	10.4 ± 0.6
Nursing Tests				
Crouching Behavior				
% of Rats	100	100	100	100
Latency (sec/10)	20.3 ± 2.0*	13.3 ± 2.4	20.8 ± 4.2	22.7 ± 6.4
Duration (sec/10)	29.9 ± 2.5	30.7 ± 4.1	39.7 ± 3.8*	27.6 ± 3.3

[a] By Fisher's test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

prepartal Day 23 significantly extended the median day of birth about 2 days beyond that of the control group, to Day 25. It also reveals a significant difference in mortality: over half the pups in the Day 23 progesterone group were dead, compared with only a few deaths in the oil group. By Day 2, progesterone pups gained only about ¼ the weight of control young. This difference, however, was not significant.

Labor was markedly prolonged only in those females injected with progesterone on Day 23. Under this treatment, females appeared to remain in protracted labor for several days, often in a state of piloerection and with periodic hemorrhage from the vaginal canal. Occasionally, a fetus, dead or alive, would pass through the canal. However, in most cases, fetuses were observed partially lodged in the uterus or dangling from the vaginal canal. Females often ate the carcasses of dead pups, which explained in part the reduction in the total number of pups dead or alive in the Day 23 progesterone group (n = 90) compared with the other groups ($n \geqq 118$). Cannibalism of live pups was rare.

By postpartal Day 5, almost all the pups survived, regardless of treatment. However, litters in the Day 23 progesterone group weighed significantly less than control young, and between Days 4 and 5, they gained only about ½ as much weight.

Despite the presence of progesterone on Day 18 or 23, all the females crouched over pups in the behavior tests. However, treatment on Day 18 significantly extended the time for the onset of crouching by about 50%. As Table I shows, the Day 18 progesterone group differed significantly from the control one with regard to the latency of crouching. In contrast, progesterone treatment on Day 23 markedly extended crouching duration. The amount of time spent crouching by the Day 23 progesterone group differed significantly from that in the control group by over 30%.

Discussion. Two-milligram doses of pro-

gesterone twice daily on Day 23 of gestation markedly depressed litter growth, as shown by the low weight gains in pups, particularly by the 4th and 5th day. Moreover, they reduced the number of live pups, most likely as a consequence of disruptions at birth (see *Results*). Like doses of progesterone on Day 18 had no such effects.

Survival of young in the rat, as in most mammalian species, depends upon at least two sets of maternal events. One set, primarily a physiological one, involves adequate synthesis and ejection of milk from the mammary glands. The other, primarily a behavioral one, involves appropriate postural adjustments on the part of the mother so that the young reach her teats and suck.

Ovarian hormones, particularly progesterone, have long been implicated in nursing behavior. Under certain conditions, progesterone has been known to inhibit nursing behavior (21, 22). Moltz, Levin, and Leon (21) injected progesterone into late-pregnant caesarian-sectioned rats and found that they rejected foster young. Roth and Lisk (22) implanted progesterone into the hypothalamus of late-pregnant primiparous rats and reported that placements in the mammillary region moderately reduced retrieval of young but left lactation undisturbed.

Thus depressed litter growth in the present report could be due to a disturbance in secretory activity in the mammary glands, failure of milk release, or poor nursing behavior (1–8, 10, 25, 26). Judging from the latency and increased duration of crouching behavior in the Day 23 progesterone group, nursing behavior appeared at least normal, if not above normal. This rules out poor nursing behavior and implicates instead a failure in milk secretion and/or milk release (8, 10).

Summary. Subcutaneous doses of 2-mg progesterone in 0.1 ml sesame oil twice daily on Day 23 of gestation significantly depressed litter growth, particularly on postpartal Days 4–5, and markedly reduced the number of live pups. Like doses of progesterone on Day 18 had no such effects. Because the latency and duration of crouching behavior remained at least normal, the depressed litter growth in the Day 23 group could not be due to poor nursing behavior. It implicates lactation alone.

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