Effects on Lactation of Progesterone Injections Administered Before and After Parturition in the Rat (37056)

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(Introduced by R. P. Reece)

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Cowie and Folley (1) have hypothesized that the onset of lactation is inhibited by high titers of circulating progesterone, such as those that characterize gestation. At or near parturition, when blood levels of progesterone decline, the block to lactation is removed, and milk secretion ensues. In a test of this view, Herrenkohl (2) injected rats with varying doses of progesterone from Days 18 through 23 of gestation and found that 2 mg doses twice daily were highly effective in inhibiting postparturient lactation. However, when only postparturient rats were given similar doses of progesterone, lactation was only moderately suppressed (3).

A suppression of lactation may be due to an inhibition of milk secretion or milk release, or to a reduction in the motivation to suckle young (1, 4–9). Progesterone injections during late pregnancy presumably inhibited lactation by a primary failure of milk release (2). Postpartal injections, however, also suppressed nursing behavior (3). To better identify the period when progesterone inhibits only lactation, the effects on lactation of progesterone injections administered during late pregnancy were compared with progesterone injections continued after birth.

Materials and Methods. Virgin Sprague-Dawley rats, 60–70 days old and weighing 175–225 g, were bred with males and injected with progesterone or oil either from Days 18 through 23 of gestation only, or from Days 18 through 22 of gestation and then for the first 5 days postpartum. Experimental females were injected subcutaneously with 2 mg doses of progesterone in 0.1 ml of sesame oil twice daily. Control rats received only the oil. From Day 18 of gestation on, the animals were housed and fed according to laboratory procedures (2). 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. In addition, the mammary glands were scored as positive for the presence of milk if: (a) from the day of birth on, milk was observed in the stomachs of the young; or (b) in cases of prolonged gestation, milk appeared evident in biopsy mammary samples from the inguinal region every other day from day 23 of gestation until the day of birth.

The animals were also observed in standard nursing tests for 15 min each day in which the duration of crouching behavior was scored. The nursing test began when 3 foster pups the same age as the litter were introduced into the cage each morning, 0.5 hr after the female's own litter had been removed. For the next 15 min, the duration of nursing behavior was continuously scored.

By removing the litter and replacing it during the nursing test with young that had been removed overnight from their mothers, a strong suckling stimulus was insured. The

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TABLE I. Lactational Performance and Nursing Behavior of Rats Injected with Progesterone Before and After Parturition.

Period of treatment	Treatment (mg)	No. rats	Postpartum day 1				Postpartum day 5		Mammary gland condition (% of rats with secretion)	Nursing tests		
			Day of birth		No. live pups ($\bar{X} \pm SE$)	Mean wt/ pup (g) ($\bar{X} \pm SE$)	No. live pups ($\bar{X} \pm SE$)	Mean wt/ pup (g) ($\bar{X} \pm SE$)		Crouching behavior		Milk ejection (% of tests)
			Median	Range						% of rats	Duration (min) ($\bar{X} \pm SE$)	
Prepartum days 18–23	2 p[a]	7	26	24–27	1.3 ± 0.2	7.9 ± 3.7	2.0 ± 0.1	7.6 ± 1.5	86	100	4.8 ± 0.6	26
	Sesame oil	10	23	0	9.2 ± 0.0	6.7 ± 1.0	5.8 ± 0.1	10.3 ± 1.6	100	100	7.0 ± 2.2	94
			Fisher test[c]		$t = 42.66$[c]	$t = 0.34$	$t = 28.67$[c]	$t = 1.31$			$t = 1.02$	X^2 test[d]
Prepartum days 18–22	2 p[a]	9	24	24–25	2.1 ± 0.2	8.8 ± 3.2	5.5 ± 0.3	9.9 ± 4.0	56	44	3.8 ± 0.4	70
Postpartum days 1–5	Sesame oil	10	23	0	10.6 ± 1.0	6.9 ± 2.9	5.6 ± 0.0	14.2 ± 1.3	100	100	5.5 ± 1.0	96
			Fisher test[c]		$t = 8.79$[c]	$t = 0.47$	$t = 0.35$	$t = 1.08$		Fisher test[b]	$t = 1.67$	X^2 test[c]

[a] Progesterone.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .001$.

nursing tests thus served both as an index of motivation to nurse as well as for milk ejection. Milk ejection was scored as positive when active suckling by foster pups led to milk release as determined at the end of each test by their stomachs being distended with milk.

Results. Administration of 2 mg doses of progesterone twice daily from Days 18 through 23 extended the median day of birth to Day 26, thereby increasing gestation by an average of 3 days above the control group (Table I). This difference in median day of birth was significant by the Fisher's exact test applied to the combined median score. Similar doses of progesterone from Days 18 through 22 also significantly extended gestation, but only to Day 24.

Regardless of the period of treatment, rats injected with progesterone had significantly fewer live pups at birth than did control rats. However, the differences in mean weight per pup were not significant.

Under both progesterone treatments, females appeared to remain in protracted labor for several days, in a state of piloerection and with periodic hemorrhage from the vaginal canal. Occasionally a fetus, dead or alive, would pass successfully through the canal. However in most cases, fetuses were observed to be partially lodged in the uterus or dangling from the vaginal canal. The females would then eat the carcasses of dead pups, with cannibalism of the live ones being rare.

By postpartum Day 5 however, rats injected with progesterone from Days 18 through 23 suffered a high mortality of young. On the average, 4 out of 6 pups in the standard litter had to be replaced each day because each morning only about 2 pups remained alive. The surviving pups, however, did not gain significant weight. In contrast, there was no significant reduction in the number of live pups reared by rats injected with progesterone from Days 18 through 22 and treated again from Days 1 through 5. Control and experimental females alike reared 5–6 pups. Moreover, there were no significant differences in litter weight.

The nursing tests revealed that progesterone injections during late pregnancy did not affect milk secretion at or near parturition, Judging from the presence of milk in the biopsy samples of the mammary glands or by the fact that pups had milk. Almost all (86% or 6/7) of the rats injected with progesterone through Day 23 had milk as did all of the control rats. Although only 56% (5/9) of the rats injected with progesterone through Day 22 and then for the first 5 days postpartum had milk compared with 100% of their respective control group, this difference was not significant (Fisher's exact test, $p > .05$).

All of the rats injected with either progesterone or oil through Day 23 crouched over the young. However, only 44% (4/9) of the rats injected with progesterone through Day 22 and then again after birth crouched over young as compared with 100% of the control rats, a difference that was significant at the .05 level. There were no significant differences in the duration of crouching behavior.

In only 26% (9/35) of the milk ejection tests did the rats injected with progesterone through Day 23 release milk, compared with 94% (47/50) of the tests with control rats. This difference was highly significant ($X^2 = 42.71$, $df = 1$, $p < 0.001$). The difference between progesterone and oil rats injected both before and after parturition was also significant ($X^2 = 9.54$, $df = 1$, $p < 0.01$).

Discussion. Two milligram doses of progesterone twice daily from Days 18 to 23 of gestation had a greater inhibitory effect on lactation than did injections given on Days 18 through 22, as shown particularly by the high mortality among young during the first 5 days postpartum. By contrast, females injected with progesterone through Day 22 rarely lost young during the same 5 days. Moreover, progesterone injections through Day 23 caused a greater prolongation of gestation than did injections through Day 22.

The primary action of progesterone injections through Day 23 appeared to be on lactation and not nursing behavior. All of the experimental females, for example, assumed a lactation posture over young. Because the suppression of lactation was achieved by exogenously introducing high levels of proges-

terone during late pregnancy, at a time when endogenous levels decline (10, 11), the present findings support the view of Cowie and Folley (1), and Meites and Turner (5) among others, that the onset of lactation during gestation is inhibited by high titers of progesterone.

However the primary effect on lactation appeared not to be on milk secretion, as the authors above suggest (1, 5), but rather on milk release. Despite the presence of milk in the mammae of all but one of the animals injected through Day 23 (as revealed by biopsy), they could not release milk in most of the nursing tests. Moreover, progesterone is known from other reports (12, 13) not to inhibit lactogenesis but rather to enhance it.

In addition, progesterone injections in rabbits (14) and rats (15) during late pregnancy markedly prolong gestation and disrupt labor. This finding, supported in the present report, further implicates an oxytocin deficiency (16) and an inhibitory action of progesterone on processes of milk release.

When progesterone was readministered for the first 5 days postpartum after prepartal injections from Days 18 through 22, only nursing behavior was suppressed. Most of the females so treated did not crouch over young in the nursing tests, for example, although obviously they nursed pups at other times, judging from the low mortality among the young. This result confirms previous results that progesterone injections *after* parturition do not markedly suppress lactation (3, 4, 6, 9). Furthermore it supports the view that under certain conditions progesterone may reduce maternal behavior (17).

At least three factors, singly or in combination, have been used in the past (3) to explain differences in results between pre- and postpartal progesterone injections. These have included the facts that (a) a normal birth had already ensued before progesterone was introduced and (b) young were already present. The agreement between past results (3) and present ones in which birth was not normal and young died (particularly in the Day 18 through 23 condition) rules out these factors.

One possibility remaining is that although hormones are essential for the initiation and maintenance of lactation, the degree or kind of control they exert may diminish with time since parturition. Later in lactation, other factors may assume primacy in the regulation of lactation, such as behavioral adaptations in the mother or changing stimulus characteristics of the young. Precedent for this view has already been set by Grosvenor's observations (18) that immediately after birth suckling is the essential stimulus for release of prolactin from the adenohypophysis whereas later in lactation exteroceptive cues from the pups can also stimulate prolactin release.

Summary. Lactation is not suppressed in rats by 2 mg doses of progesterone subcutaneously administered in 0.1 ml sesame oil twice daily from Days 18 through 22 of gestation. Even though progesterone is resumed for the first 5 days postpartum, almost all the young survive. By contrast, 2 mg doses of progesterone administered through Day 23 of gestation markedly disrupt lactation. Almost all of the pups die at or after birth even though nursing behavior is normal and no additional progesterone has been introduced.

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