

Precocious Sensitization of the Uterus in Pseudopregnant Rats¹ (34869)

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In pregnant rats progesterone secretion increases by Day 5 of pregnancy (1, 2) and an estrogen surge is observed on Day 4 (3). The elevated secretion of the ovarian hormones by Day 5 of pregnancy makes the uterus sensitive to blastocysts, and implantation takes place during the night of Day 5. After Day 6 the uterus loses its sensitivity to accept blastocysts (4). This temporal aspect of the uterine sensitivity is also observed in pseudopregnant rats (5), reflecting a similar pattern of ovarian hormone secretion (2, 6). Sensitization and subsequent desensitization of the uterus is also observed in ovariectomized rats treated with various sequences of progesterone and estrogen (7, 8). It has been shown that the level of progesterone secretion during the first 2 days of pseudopregnancy or pregnancy is relatively low, and that daily progesterone treatment started 2 days prior to concomitant estrogen injection can initiate implantation (blueing reaction) in the pregnant rats ovariectomized on Day 2 (9). It was of interest to examine whether progesterone and estrogen administered exogenously could influence the uterus and produce the same sensitization-desensitization pattern earlier than the naturally occurring sequential changes in the uterus. In this paper we report such a precocious sensitization of the uterus by exogenous hormones in pseudopregnant rats.

Materials and Methods. Sprague-Dawley strain rats (200–250 g body weight) were kept in a temperature-controlled room with the light schedule of 14 hr light and 10 hr darkness. The estrous cycle was traced by daily vaginal smears taken each morning. All the rats used in this study were made pseudo-

pregnant by mechanical stimulation of the uterine cervix with a glass rod on the day of estrus. The day of cervical stimulation was designated as Day 1 of pseudopregnancy and the preceding day (the day of proestrus) was accordingly designated as Day 0. Steroids were dissolved in sesame oil and administered subcutaneously. Daily dose of progesterone was 5 mg in 0.3 ml oil. The dose of estradiol was 0.2 μ g in 0.1 ml per day. Traumatization of the uterine horn was performed by scratching the antimesometrial side of the endometrium along the entire uterine horn with a curved needle. Traumatization was applied only on the left horn; the right horn serves as control.

A total of 64 pseudopregnant rats was divided into 11 groups, each group containing 5–6 rats. These groups were conveniently divided into two experimental series (Series A and B), according to the treatments which are described below and summarized in Table I.

Series A consisted of 6 groups of rats designated A-1 to A-6.

A-1 and 2. No hormones were given and the uterine horn was traumatized on Day 3 of pseudopregnancy (A-1) or on Day 5 (A-2).

A-3, 4, and 5. Progesterone was given daily from Day 0 to Day 8 and the uterine horn was traumatized on Day 2 (A-3), Day 3 (A-4), or Day 4 (A-5), and estradiol was given on the day of traumatization.

A-6. Progesterone was given daily from Day 1 to Day 8 and the uterine horn was traumatized on Day 3 and estradiol was given on this day.

Series B consisted of five groups of rats and all the rats of this series received daily injection of progesterone from Day 0 to Day 8.

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TABLE I. Effect of Ovarian Hormones on Deciduoma Formation after Uterine Traumatization on Day 2, 3, 4, or 5 of Pseudopregnancy in the Rat.

Group no.	Period of progesterone injection (5 mg/day)	Day ^a of estradiol injection (0.2 µg)	Day of uterine traumatization	Day of autopsy	ND/N ^b	Weight of uterine horn (mg)	
						Control	Traumatized
A-1	no injections		3	9	0/6	132.3 ± 25.4 ^c	173.5 ± 38.7
A-2	no injections		5	9	6/6	126.1 ± 22.7	1242.2 ± 353.3
A-3	Day 0-8	2	2	9	0/6	136.3 ± 5.5	143.7 ± 11.2
A-4	Day 0-8	3	3	9	6/6	148.9 ± 16.6	560.7 ± 493.3
A-5	Day 0-8	4	4	9	6/6	167.4 ± 43.6	1125.2 ± 594.9
A-6	Day 1-8	3	3	9	3/5	129.8 ± 19.2	553.1 ± 357.1
B-1	Day 0-8	3	5	9	2/6	159.8 ± 10.1	330.7 ± 297.9
B-2	Day 0-8	4	5	9	3/6	126.3 ± 25.7	316.6 ± 201.9
B-3	Day 0-8	3 & 4	5	9	0/6	147.2 ± 10.5	210.7 ± 23.8
B-4	Day 0-8	2 & 3	3	9	1/5	162.4 ± 40.0	177.4 ± 67.2
B-5	Day 0-8	1, 2 & 3	3	9	0/6	187.4 ± 27.3	209.8 ± 25.9

^a Day 0 = day of proestrus; one day before the cervical stimulation.

^b ND/N = number of rats with deciduoma/number of rats in group.

^c Mean ± SD.

B-1, 2, and 3. Estradiol was given on Day 3 (B-1), Day 4 (B-2), or Days 3 and 4 (B-3), and the uterine horn was traumatized on Day 5.

B-4 and 5. Estradiol was given on Days 2 and 3 (B-4) or on Days 1, 2, and 3 (B-5) and the uterine horn was traumatized on Day 3.

On Day 9 of pseudopregnancy all the animals of both series of experiments were sacrificed and each uterine horn was examined for deciduoma, and the weight was recorded. Presence or absence of deciduoma and increase in the weight of the horn due to decidual growth were taken as criteria of uterine sensitivity. Those uterine horns which showed a little swelling were fixed in Bouin's solution for further histological examination.

Results are summarized in Table I. In pseudopregnant, nontreated animals traumatization of the uterus on Day 3 did not produce deciduoma (A-1), whereas traumatization on Day 5 resulted in production of deciduomata in all of the animals (A-2). Treatment with progesterone from Day 0-Day 8 of pseudopregnancy and a single injection of estradiol on the day of traumatization changed the uterine sensitivity. Although traumatization on Day 2 did not produce

deciduoma (A-3), that on Day 3 (A-4) and Day 4 (A-5) produced deciduomata in all the animals. The magnitude of response was greater on Day 4 than on Day 3 ($p < .01$). However, when daily progesterone treatment was started on Day 1, traumatization on Day 3 resulted in deciduoma in only 60% (3/5) of the animals.

In series B estradiol was given earlier than uterine traumatization. When estradiol was given on Day 3 (B-1) or 4 (B-2) and the uterus was traumatized on Day 5, deciduomata were formed in only 33% (2/6) or 50% (3/6) of the animals. Treatment with estradiol for two successive days (Days 3 and 4) before the uterine traumatization completely abolished the decidual formation (B-3). Treatment with estradiol on Days 2 and 3 and the uterine traumatization on Day 3 resulted in decidual formation only in one of five rats (B-4), and estradiol injection from Day 1, through Day 3 inhibited completely the formation of deciduoma (B-5).

Discussion. It was confirmed that under normal circumstances the uterus is not sensitive to traumatization and decidual response on Day 3 of pseudopregnancy but becomes sensitive on Day 5 (5). The present data demonstrate that uterine sensitivity can

be advanced to Day 3 by increasing progesterone level for 3 days prior to estrogen injection and trauma on Day 3. The longer the progesterone treatment, the more response was obtained. The increase in the uterine sensitivity appears to be due not only to a longer progesterone treatment but rather due to the time of the uterine traumatization, that is, the closer this was to the maximal response time (Day 5), the greater was the response. Two days pretreatment with progesterone did not produce deciduomata when the uteri were traumatized on Day 2 (A-3) but deciduomata were produced when the uteri were traumatized on Day 3 (A-6). In this connection Psychoyos (8) showed in ovariectomized rats that blastocysts transferred into the uteri on the third day of progesterone treatment implanted when estrogen was injected on the day of transfer. Since the recipient rats used in his experiment had been ovariectomized for about 1 month before progesterone treatment, endogenous ovarian hormones are considered to be completely depleted before use. As the rats used in our experiment were pseudopregnant, our finding of a poor deciduomal response on Day 3 appears to be due to a hormone imbalance during early pseudopregnancy.

These experiments also show that estrogen treatment 1 or 2 days prior to traumatization (on Day 5) reduced the uterine sensitivity in progesterone-treated pseudopregnant rats (B-1, 2, and 3). Prevention of the precocious sensitization was obtained when estrogen treatment was given 1 or 2 days prior to traumatization on Day 3. It is interesting to notice that estrogen injection on Day 4 of

pseudopregnancy reduced the uterine response on Day 5. Since estrogen surge is normally observed on Day 4 of pregnancy or pseudopregnancy (3, 6), estrogen level higher than normal on this day must have altered the uterine sensitivity on the following day.

Summary. Precocious sensitization of the uterus to respond to trauma with deciduoma formation was obtained in pseudopregnant rats by prior daily treatment with progesterone combined with estrogen injection and traumatization on Day 3. The sensitivity of the uterus was reduced on Day 5. Estrogen treatment prior to traumatization prevented the precocious sensitization.

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1. Eto, T., Masuda, H., Suzuki, Y., and Hosi, T., *Jap. J. Animal Reprod.* **8**, 34 (1962).
2. Hashimoto, I., Henricks, D. M., Anderson, L. L., and Melampy, R. M., *Endocrinology* **82**, 333 (1968).
3. Yoshinaga, K., Hawkins, R. A., and Stocker, J. F., *Endocrinology* **85**, 103 (1969).
4. Dickman, Z., and Noyes, R. W., *J. Reprod. Fert.* **1**, 197, (1960).
5. DeFeo, V. J., *Endocrinology* **72**, 305 (1963).
6. Shaikh, A. A., and Abraham, G. E., *Biol. Reprod.* **1**, 378 (1969).
7. Rothchild, I., and Meyer, R. K., *Physiol. Zool.* **15**, 216 (1942).
8. Psychoyos, A., *C. R. Acad. Sci.* **257**, 1153 (1963).
9. Psychoyos, A., "Egg Implantation," Ciba Found. Study Group No. 23, p. 4. Churchill, London (1966).

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