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Effect of a Single Dose of Pretrauma Estrogen on the Decidual Response in the Spayed Progesterone Treated Rats

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Summary

The relationships between the effectiveness of a single injection of pretrauma estrogen and the uterine response to the decidual stimulus were investigated in ovariectomized rats treated with various kinds of estrogen (estrone or 17β-estradiol) at varied times and or with various combination of progesterone and estrogen. Progesterone treatment was kept constant for all groups of animals during the period between ovariectomy and autopsy. Uterine weight was measured 96 hours after trauma and compared.

When the 1 μg of esterone was given for four days (from the day before traumatization to the day before autopsy), there was maximum induction of decidualization and the magnitude of uterine response was the same as in normal pseudopregnancy rats.

When a single dose of estrogen was given at 12 to 24 hours before traumatization, successful decidualization can be induced in the ovariectomized rats which had received 4 mg of progesterone daily. The maximum response was obtained when the spayed animals received a single dose of estrone at 12 hours before traumatization.

It appears therefore that the estrogen surge hypothesis of Shelesnyak and his collaborators can account for those conditions in which maximum sensitivity of the uterine horn to deciduoma-inducing stimuli reached a peak at 12–24 hours after estrone treatment.

Uterine responsibility to deciduoma-inducing stimulation in the spayed rats varied with various combinations of estrone and progesterone or with duration of dosing and with the use of hormone. Uterine sensitivity and velocity of decidual reaction did not alter with the kind of estrogen or administration route of the hormone (subcutaneous or intravenous injection).

The fact is well known that the synergistic action of estrogen and progesterone is needed for the respone of uterine endometrium in the process of decidual cell reaction and that implantation of blastocyst was induced by the synergic action of both hormones.

It is also demonstrated that the priming effect of estrogen is very important to the induction of deciduoma formation. Shelesnyak and his collaborators (1, 2) gave evidence for the existence of an estrogen surge about 24 hours before the time of maximal sensitivity to decidoma inducing stimuli when performed just before or at 12 noon on day 3 of pregnancy or pseudopregnancy. The effect of a single dose of estrone on the delayed rat blastocysts have been made by Yasukawa & Meyer (3) and Takeuchi et al. (4)

However, the pattern of estrogen secretion during the period between fertilization and implantation or between induction of pseudopregnancy and application of mechanical, electrical and chemical stimuli to the uterus is not clear. There is also no information on the relationship between the time of estrogen treatment or the kind of estrogen and uterine sensitivity.

The present experiments were designed to obtain information on the relation or effectiveness of a single dose of oestrogen at various times before traumatization, using ovariectomized rats which had received progesterone treatment. Also, one experiment was attempted to discern whether or not the hormone treatment allows the formation of decidual mass of the same weight as those observed in normal pseudopregnancy as the intact control.

Materials and Methods

Experimental animals: Adult virgin female rats of the Wistar strain, weighing from 150 to 240 gram, derived from the colony maintained at our laboratory, were used throughout the experiments.

In the first experiment, intact animals exhibiting regular 4-day cycles served as controls (Group 1). They were made pseudopregnancy by stimulating the cervix with a bipolar electrode on the day of proestrus and estrus. (5).

On the fourth day of pseudopregnancy, a midventral laparatomy was performed under ether anaesthesia, and the right uterine horn was traumatized. A blade-tipped needle was inserted into the uterine lumen from the end of the uterine bifurcation to the top of the uterine horn and then withdrawn so as to scratch the intire length of the antimesometrial endometrium.

In group 2–6, the animals were ovariectomzied by the drosal route followed by injections of progesterone for one week before the beginning of the estrogen treatment. The schedules of hormone treatment and traumatization for experiment 1 are shown in Fig. 1.

Progesterone and estrogen were given subcutaneously in sesame oil. Daily, all the rats were given 4 mg of progesterone from the day of ovariectomy until the day before autopsy. 1μg of estrone treatment was performed according to the experimental design given in Fig. 1.

In a second experiment, all the spayed rats in each of these three main groups were further divided into four or five subgroups.

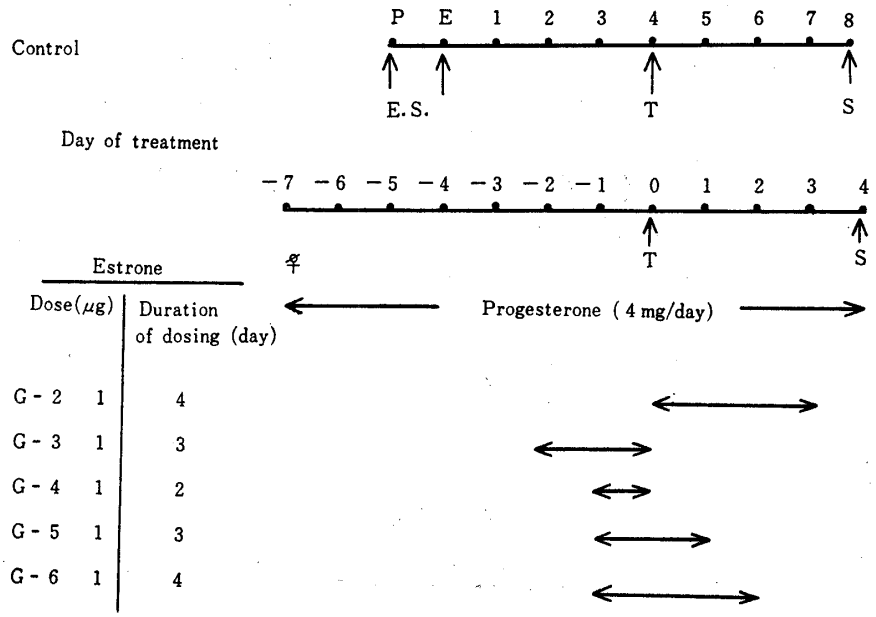

FIG. 1. Experimental design for the first experiment.
p : proestrus, E : estrus, E.S. : electrical stimulus, T : traumatization,
S : sacrifice, ♀ : ovariectomy

In main groups 1, 2 and 3, daily 2, 4 and 8 mg of progesterone were given form the day of ovariectomy until the day before autopsy. The single pre-trauma injections of estrogen were given at 0, 6, 12, 24 and 48 hours before traumatization as shown indicated in Fig. 2.

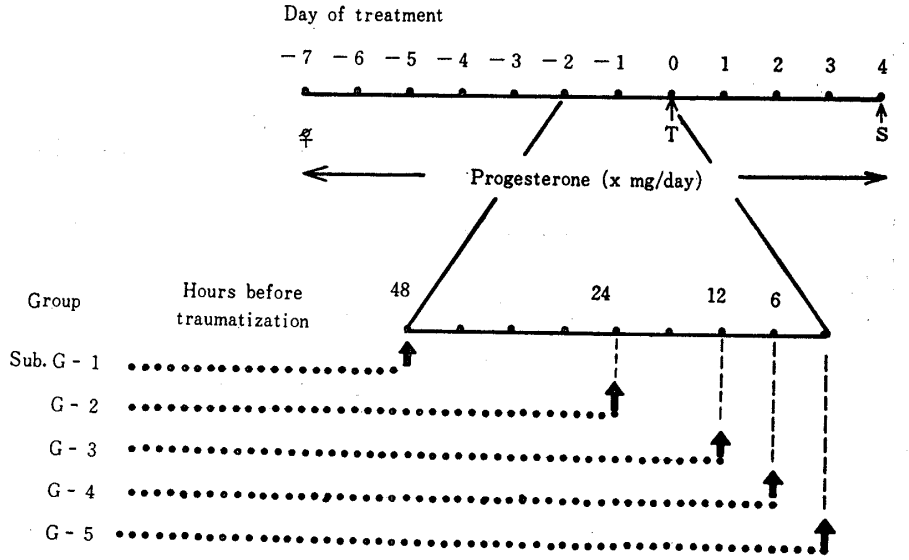

FIG. 2. Schedule for the single pre-trauma injection of estrone in the second experiment.
♀ : ovariectomy, T : traumatization, S : sacrifice
* The animals in each sub-group 5 were not treated exception for main group 2.

In a third experiment, in order to ascertain whether or not the magnitude and the velocity of the decidual reaction differs according to the kind of estrogen and the route of administration, the animals received subcutaneously or intravenously a single injection of 1 μg estrone or 0.1 μg of 17β estradiol at various time before traumatization. Estrone (1 μg) and 17-β-estradiol (0.1μg) dissolved in 70 percent ethyl alcohol were administered through the inguinal vein.

For the gross exmaination of the deciduoamta, all the animals in each experiment were autopised an four days after traumatization. The uterine horns were separated and weighed immediately after removal from the mesentery.

The grade of decidual response was estimated by the increase in the uterine weight of the traumatized horn composed with those of the nontreated another horn.

Results

Uterine Response according to the Time and Duration of Estrone Treatment

An uterine weights in the spayed animals, which had been treated with estrone administered subcutaneously at various times before traumatization and for various durations are summarized in Table 1. In the control group (G-1), the right uterine was traumatized at 10.00–10.30 A.M. on the fourth day of pseudopregnancy, and the grade of uterine response was (R-L=R'+S.D.) 1080.1+ 152.3 mg. Decidualization (uterine response) was minimum or absent in the animals in which 1 μg of estrone had been administered for three days, (starting 48 hours before traumatization to that day) and the value of the traumatized uterine was 74.1±36.0 mg.

TABLE 1. *The Uterine Response to Various Durations and Times of Estrone Injection in Spayed Rats Treated with 4 mg of Progesterone daily*

Group	No. of animals	Weight of uterine horn (M±S.D.)		Grade of response (R−L=R'±S.D.) mg
		Left (mg)	Right (mg)	
G-1 (Control)	7	140.2±26.7	1219.0±121.9	1080.1±125.3
G 2	7	114.9±22.1	382.1±106.1	267.1±214.2
G-3	6	112.5±20.4	170.0± 32.7	74.1± 36.0
G-4	4	63.2±12.4	445.5±148.7	382.2±143.4
G-5	6	88,3± 8.8	663.0±158.1	562.1±181.7
G-6	5	138.0±20.6	1349.0±224.1	1211.6±211.9

In groups 4, 5 and 6 in which the estrone treatment was started 24 hours before traumatization the decidual response increased in proportion to the duration of the estrone treatment.

The average uterine weight in the groups 4, 5 and 6 were 382.2, 562.1 and 1211.6 mg, respectively. In group 6 treated with 1μg of estrone on the fourth day, the grade of uterine response was maximum and the value of the uterine weight

was about the same as those obtained in the intact control animals.

Uterine Response to a Single Pre-trauma Injection of Estrone

In main groups 1, 2 and 4, the average weight of the traumatized uterus in the animals receiving progesterone alone daily after spaying was 122.9, 215.7 and 137.3 mg, respectively (refer Table 2 and Fig. 3).

Maximum decidual response was obtained from the animals treated with 4 mg of progesterone. There was a signfiicant difference in the average weight of uterus between groups 1 and 3, but no difference statistically between groups 1 and 2.

In each main group, the spayed animals treated with a combination of estrone and progesterone 12 to 24 hours before traumatization had maximum weight of traumatized uterine horn. The maximum average weight of the uterine horn was obtained from the animals receiving a combination treatment of progesterone and estrone (a ratio 1:4000), but the value of the uterine weight was about half of the normal pseudopregnancy or the spayed animals treated with 1 μg of estrone for four day from the day before traumatization.

TABLE 2. *The Uterine Response to Single Injection of Estrone at Various Times before Traumatization in Spayed Rats Treated with Various Doses of Progesterone*

Group		No. of animal	Dose of progesterone (mg/day)	Estrone treatment		Weight of uterine hora (M±S.D.)		Grade of response (R−L=R′ ±S.D.) (mg)
Main	Sub.			Hours before traumatization	Dose (μg)	Left (L) (mg)	Right (R) (mg)	
1	1	5	2	48	1	105.7±74.1	294.7± 63.3	189.0± 58.5
	2	7	2	24	1	85.5±18.4	417.3±178.0	330.4±179.2
	3	7	2	12	1	94.8±16.9	486.5± 48.9	391.8± 35.3
	4	6	2	6	1	72.6± 7.6	101.3± 15.3	33.4± 13.1
	5	7	2	—	0	80.9±22.2	203.8± 88.0	122.9± 8.7
2	1	6	4	48	1	97.5±11.0	166.8± 36.4	70.3± 51.7
	2	6	4	24	1	112.9±30.1	541.6± 91.3	429.0± 72.2
	3	9	4	12	1	116.3±21.3	658.2± 62.3	541.8± 57.5
	4	9	4	6	1	93.0±22.1	230.0± 52.2	151.4± 33.2
	5	6	4	0	1	111.5±34.7	227.1± 67.3	107.8± 53.1
	6	15	4	—	0	115.4±22.7	318.6±147.2	215.7±136.9
3	1	5	8	48	1	108.0±12.3	193.0± 26.4	85.0± 20.3
	2	7	8	24	1	80.0± 9.6	500.4± 37.2	413.6± 28.3
	3	6	8	12	1	87.1±10.8	454.1± 54.3	367.0± 48.1
	4	7	8	6	1	117.1±14.9	475.1±122.6	358.0±118.6
	5	6	8	—	0	82.3± 9.9	216.7± 13.0	137.3± 15.9

In main group 3, the maximum response of decidualization occurred in the animals receiving estrone treatment 24 hours before traumatic stimulus. In each main group, however, there was no difference statistically between sub-groups 2 and 3.

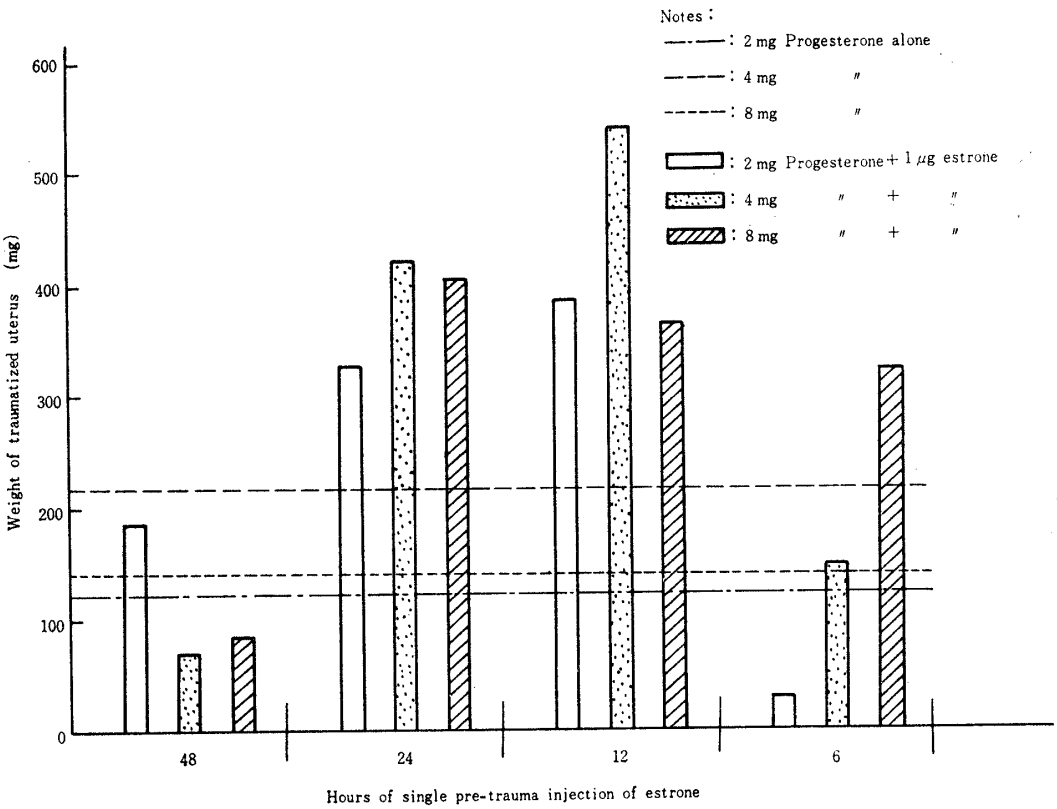

FIG. 3. Uterine response to a single pre-trauma injection of 1 μg estrone

TABLE 3. *The Uterine Response to Single Pre-trauma Treatment of 17β-Estradiol in Spayed Rats Treated with 4 mg of Progesterone daily*

Group	No. of animals	Estradiol treatment		Weight of uterine horn (M±S.D.)		Grade of response (R−L=R′ ±S.D.) mg
		Hours before traumatization	Dose μg	Left mg	Right mg	
1	9	24	0.1	81.1+13.4	390.3+121.9	310.3+121.6
2	8	12	0.1	78.4+10.2	574.0+158.7	495.6+163.1
3	10	6	0.1	105.1+19.5	137.3+ 19.3	32.2+ 11.3

When a single pre-trauma injection of esterone was given 48 and 6 hours before traumatization no increase in the weight of the traumatized uterine horn was noted. Especially, the average weight of the uterine horn in the animals which received a single pre-trauma treatment of esterone at 48 hours before decidual stimulus was reduced to about half to 1/3 of the uterine in the animals treated with progesterone alone.

Effect of a Single Dose of Pre-trauma 17β-estradiol on the Decidual Reaction in Spayed Rats

As shown in Table 3, the maximum response of the traumatized uterine horn

TABLE 4. *The Weight of the Traumatized Uterine Horn in Spayed Rats Treted with a Single Pre-trauma Estrogen intravenously.*

Group	No. of animal	Estrogen treatment			Weight of uterine horn (M±S.D.)		Grade of response (R−L=R′ ±S.D.) mg
		Hours before traumatization	Kind of hormone	Dose μg	Left (L) (mg)	Right (R) (mg)	
1	11	24	E-rone[1)]	1	98.7±30.9	372.1± 79.3	274.3± 96.7
2	7	24	E-diol[2)]	0.1	84.6±12.5	324.6± 96.0	240.0±106.0
3	5	12	E-rone	1	105.8±21.7	372.5± 87.1	266.8± 68.3
4	9	12	E-diol	0.1	96.2±17.9	452.1±147.4	355.9±148.0
5	9	6	E-rone	1	86.8±28.0	292.1± 88.8	198.3±98.5
6	7	6	E-diol	0.1	86.6±16.7	312.5±100.6	226.0±91.4

E-rne: Estrone ; E-diol : Estradiol

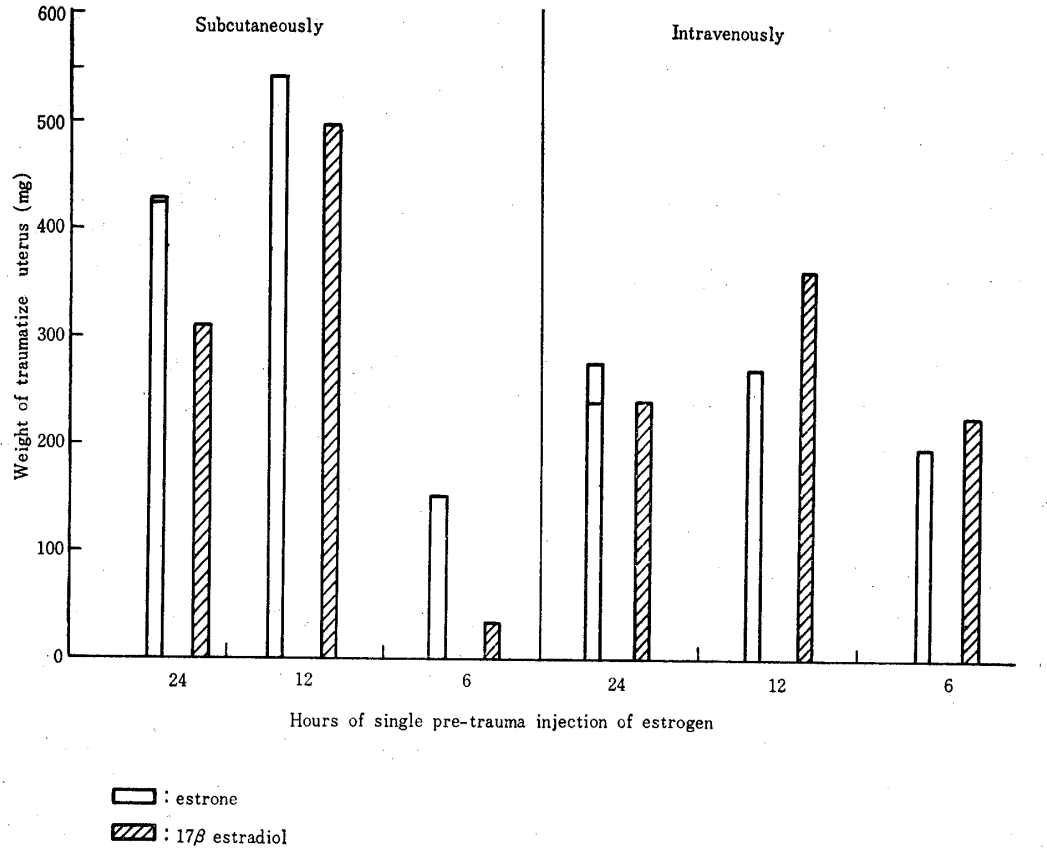

FIG 4. Effect of the route of administration of estrogen on the decidual reaction in the spayed rats treated with 4 mg of progesterone daily.

was obtained from the spayed animals in which 0.1 μg of 17β-estradiol was administered subcutaneously 12 hours before the traumatic stimulus.

The difference in the grade of uterine response was shown between groups 1 and 2. Also, the weight of the traumatized uterine horn, in which a single dose of

estrogen was injected subcutaneously 12 hours before traumatization, did not differ from the estrone treated animals and the estradiol-treated ones (refer Table 2, 3 and 4).

Effect of the Route of Estrogen Administration on the Decidual Response

The results are given in Table 4 and Fig. 4. The decidualization and the velocity of decidual response were not affected by the kind of estrogen and the route of the hormone administration. The weight of the traumatized uterus in the rats which had received a subcutaneous injection of estrogen was heavier than in those administered intravenously, though there was no statistical difference in the uterine weight among the groups.

Discussion

Since Loeb ovserved that the uterine responsibility to the decidual stimulus was dependent on the presense of functional corpora lutea, the hormonal requirements for decidualization have been investigated by many workers. It was cleared that the essential hormonal requirements for decidualization is limited to an appreciate ratio of progesterone and estrogen (Rothchild et al; Krebhiel; Mayer & Meunier: Youchim & De Feo.; Harper). Yochim & De Feo (9) have reported that daily treatment of of 1 μg of estrone and 2 mg of progesterone is necesary to obtain an optimal decidualization reaction in ovariectomized pseudopregnant rats.

The results obtained in the present experiments indicated that 1 μg of estrone injected for four days (from the day before traumatic stimulus to the day beofre autopsy) allows a successful induction of decudaulization in ovariectomized, progesterone-treated rats. Shelesnyak et al (2) discovered that animals ovariectomized at 12:00 hours of the day 3 of pseudopregnancy failed to respond to the decidual inducing stimulus, and that if ovariectomy was performed 12 hours later, the uterus responded normally. They concluded that the estrogen surge occurs at 12:00 hours of day 3 of pseudopregnancy or pregnancy and that the source of the estrogen is ovary.

It was also reported by Cartoni & Bignami (10) that the administration of a single dose of estrogen 18 hours before traumatization allows the development of large decidualization. But, in our experiments a single administration of estrogen 12 hours before decidual stimulus obtained the maximal response of the uterus. This descrepancy in the time (the hours from the administration of the hormone to the initiation of decidual response) seems to be due to the different kinds of the hormone used e.g. estrone and estradiol benzoate.

In our experiment, we also tried to examine whether or not the uterine response to decidual stimulus or the velocity of decidual reaction is affected by the kind of estrogen and the route of hormone administration.

It was very clear that the velocity and magnitude of the decidual reaction have not relation to the kind of estrogen or the route of hormone administration (subcutaneous or intravenous). Rather, decidualization was dependent on the time after administration. When a uterine horn was treated with a traumatic stimulus from 12 hours to 24 hours after a single dose of estrogen the dcidualization had reached maximum response.

Therefore, it may be concluded that a period of about 12 hours (after estrogen surge or secretion) is the essential factor for the occurrence of decidual reaction in the uterus in the progestational stage.

The relationship between the delayed rat blastocyst and the single dose of estrogen have been investigated by Yasukawa & Meyer and Takeuchi et al and demonstrated that the delayed blastocyst begins to grow as the increase in their area. Accompaning are morphological changes within 12–24 hours after a single administration of estrone and that the implantation occurred within the subsequent 24–30 hours.

It is very interesting that the response of the uterine horn and blastocyst to a single dose of estrogen occurred similary within the 12 hours after hormone administration.

Acknowledgements

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