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On the Ovulation Induced in Pseudopregnant Rats

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Summary

It has been reported that the ovary of the pregnant rat undergoes rhythmic follicular maturation. However, there is no knowledge as to whether the follicular maturation is rhythmic in pseudopregnant rats. The present investigation was designed to analyse the follicular development leading to ovulation, the release of ovulating hormone and the fertility of pseudopregnant rats treated with hormones.

An injection of 20 IU of HCG resulted in the incidence of ovulation in all animals at the various times of pseudopregnancy. The number of egg yield for rat was lower on day 4, 5 and 11 of pseudopregnancy. The injection of estrogen did not lead to ovulation in all animals during pseudopregnancy. The number of egg yield for rat was lowest on day 2 and 3 of pseudopregnancy. In the present experiment, the rhythmical pattern of follicular maturation during pseudopregnancy was not observed. The formation of deciduoma during pseudopregnancy affected the number of ovulations.

In the pseudopregnant, Nembutal prevented the ovulatory activation of the hypophysis when administreted at 28–30 hours after estrogen injection.

Mating during pseudopregnancy was observed with injection of estrogen, but not of HCG. It was found that the ovulation is not correlated with the mating and that fertility was reduced.

It is well known that ovulation is inhibited during pregnancy and pseudopregnancy in the rat, but that the development of follicles takes place and ovulation is induced by electro-chemical stimulation of the preoptic area of the brain, by injection of gonadotropine and estrogen. It has frequently been claimed that the ovary of the pregnant rat undergoes rhythmic follicular maturation, representing a persistence of the pattern of the estrus cycle (1–3). The reason for the cyclicity of follicle ripening is now known. The question is posed as to whether the cyclicity of follicular maturation occurs during pseudopregnancy.

It has previously been reported that the potency of gonadotropins in rat pituitary during pregnancy and pseudopregnancy increases and shows in part a rythmic alteration, but the pattern of the alteration during pregnancy is not the same as that during pseudopregnancy (4–7). The difference between pregnancy and pseudopregnancy is in part likely responsible for the implantation of the ova. There is no knowledge as to whether the follicular maturation in association with

ovulation induced by the administration of gonadotropin or estrogen is rhythmic in pseudopregnant rats. The present investigation was therefore designed to analyse the follicular development leading to ovulation, and then attention was focussed on the difference between pseudopregnancy and pregnancy to determine whether the follicular maturation could be correlated with the onset of decidual cell reaction. Further, the time of ovulating hormone release and the fertility of pseudopregnant rats treated with estrogen were observed.

Materials and Methods

The animals used in all experiments were adult virgin female rats of the Wistar strain maintained in this laboratory, with an average body weight of 200 g. The animals were provided with food and water ad lib.. All animals were checked by daily vaginal smear for the presence of at least two consecutive regular cycles of 4 days duration immediately before use. Daily smearing was continued during pseudopregnancy until the animals were killed.

Pseudopregnancy was induced by electrical stimulation of the uterine cervix on the day of proestrus and estrus as described previously (8). The estrus period on which the vaginal smear showed cornification was taken as 0 day of pseudopregnancy.

Series 1. Ovarian sensitivity to HCG and estrogen — The HCG preparation used was Puberogen from Tomoda Seiyaku Co. Ltd. and the dose in 0.15 ml. The NaCl solution was injected s.c. at about 10.00 AM on each day of pseudopregnancy. The estrogen used was estradiol and the dose in 0.5 ml of oil was injected s.c. at about 10.00 A.M. on each day of pseudopregnancy. The rats were killed at 24 and 48 hours after treatment of HCG, estrogen to examine the presence of tubal ova under a dissecting microscope and the number of eggs was counted.

Series 2. The effect of deciduoma formation on ovarian sensitivity to HCG. — Rhythmic follicular maturation was observed in the pregnant rats. When this was not observed in pseudopregnancy, the difference between pregnancy and pseudopregnancy must be considered. The difference between pregnancy and pseudopregnancy may be due to the implantation of ova and the decidual reaction of the uterus. The experiment was carried out in order to test whether the decidual cell reaction results in decreased ovulation of pseudopregnant rats treated with HCG. At 10.00 A.M. on the 4th day of pseudopregnancy, a midventral laparotomy was performed under ether anaesthesia and both uterine horns were traumatized. These animals were injected with HCG on the 5th or 7th day of pseudopregnancy and killed 24 hours post-injection to examine the ovulation rate and the number of eggs.

Series 3. Release of ovulating hormone after estrogen treatment — The time relationship between administration of estrogen and secretion of luteinizing hormone in pseudopregnant rats has been investigated (9, 11, 12). In these

investigations, the morphological changes in the ovaries were taken as the criteria of LH secretion. We have also attempted to determine the temporal relationships between the estrogen treatment and the secretion of ovulating hormone by observing when the ovulation is suppressed by an appropriately timed injection of Nembutal. On the 7th day of pseudopregnant the rat was injected with Nembutal (4 mg/100 g) at different times after estrogen treatment and killed to examine the ovulation. Then, the release of hypophysial ovulating hormone after estrogen treatment was demonstrated.

Series 4. The reproductive function of pseudopregnant rats injected with HCG or estrogen — The fertility in day 7 of pseudopregnant rats treated with HCG or estrogen was observed in order to determine whether the eggs ovulated with treatment of these hormones have normal fertilization and embryonic survival. The pseudopregnant rats were placed in the cage of a fertile male overnight on the day, next day after injection of HCG, estrogen, respectively. The vaginal smear was observed in the morning of the following day to confirm coitus. The animals were killed and laparotomy on the 18th day after service. The number of corpus luteum, of implantation sites and of fetuses were recorded.

Results

The induction of ovulation by HCG treatment — This experiment was carried out to estimate the dose of HCG that brings about ovulation in all animals at various times during pseudopregnancy. The injection of 5 and 10 iu HCG did not result in ovulation in all animals, as shown in Table 1. The ovulation rate on days 4 and 5 of pseudopregnancy was lower in rats injected with 10 iu HCG. The ovulation rate and the number of egg for rats killed 24 hrs. after 20 iu HCG treatment at different stages of pseudopregnancy are given in Table 2. The ovulation was observed at each stage of pseudopregnant rats injected with 20 iu HCG. This is comparable to the control injected at diestrus, which did result in normal ovulation. The number of eggs per rat on days 4 and 5 of pseudopregnancy was significantly lower ($P<0.01$) than the control. There was again a significant reduction of egg yield per rat on day 11 of pseudopregnancy. However, a rhythmical pattern of ovulation number during pseudopregnancy was not expected in these experiments.

The induction of ovulation by estrogen treatment — The injection of estrogen at various stages of pseudopregnancy did not lead to ovulation in all animals, as shown in Table 3. This is comparable to the control injected at diestrus, which did result in normal ovulation.

The rate of ovulation and the number of egg yield per rat on days 2 and 3 of pseudopregnancy was significantly lower ($P<0.01$) than the control. Thereafter a significant variation of egg yield was not observed. Also, this experiment did not indicate a rhythmical pattern of egg yield. The absence of follicles competent to

TABLE 1. *Administration of 5, 10 and 20 iu HCG during Pseudopregnancy*

Dose of HCG	Day of pseudopregnancy	Rate of ovulation	No. of ova for ovulated rat
5 iu	3	0/4	0
	4	0/5	0
	7	0/5	0
	11	0/2	0
10 iu	3	8/15	4.4±4.0*
	4	1/9	3.0
	5	1/8	4.0
	6	2/6	2.0±0.0
	7	4/8	1.8±1.5
	11	3/8	6.0±4.6
20 iu	3	6/6	10.0±1.8
	4	8/8	6.4±0.9
	7	6/6	9.7±4.1
	11	6/6	7.3±1.9

*M±S.D.

TABLE 2. *Administration of 20 iu HCG during Pseudopregnancy*

Day of pseudopregnancy	Rate of ovulation	No. of ova for ovulated rat
2	5/5	9.4±3.6
3	6/6	10.0±1.8
4	8/8	6.4±0.9
5	5/5	7.2±3.3
6	5/5	8.2±2.2
7	6/6	9.7±4.1
8	5/5	10.6±2.6
10	5/5	9.6±2.1
11	6/6	7.3±1.9
12	7/7	9.9±3.2
Diestrus	7/7	12.5±1.3

TABLE 3. *Administration of 10 μg Estradiol during Pseudopregnancy*

Day of pseudopregnancy	Rate of ovulation	No. of ova for ovulated rat
2	2/8	2.5±2.1
3	3/8	5.0±1.7
4	4/10	6.8±3.8
5	5/8	7.6±4.0
6	3/6	8.7±4.5
7	8/10	10.3±5.2
8	6/10	8.8±2.3
9	4/8	9.8±6.0
Diestrus	6/6	10.3±2.0

respond to estrogen treatment during pseudopregnancy could explain the partial failure to induce the ovulation.

Ovulation by HCG treatment in pseudopregnant rats induced a deciduoma

reaction — The effect of deciduoma formation on the ovulation induced with HCG treatment was investigated on the 5th and 7th day of pseudopregnancy. The results obtained are shown in Table 4. The injection of 10 iu HCG on the 7th day of pseudopregnancy did result in ovulation in 50 percent of the control, but not in animals having a deciduoma formation. The ovulation rate and the number of egg yield on the 7th day of pseudopregnant rats injected with 20 iu HCG did not indicate a significant effect of deciduoma formation. However, the ovulation rate at day 5 of pseudopregnancy was reduced by the formation of deciduoma. The difference of ovulation induced with HCG treatment between pregnancy and pseudopregnancy was in part suggested from the decidual reaction of uterus. Then, it is possible that the egg yield on days 4, 5, 7 and 11 decreased and that the rythmical pattern of follicular maturation was somewhat expected in pregnancy.

TABLE 4. *Effects of Uterine Traumatization on the Ovulation induced with HCG Injection at days 5 and 7 of Pseudopregnancy*

Dose of HCG	Stage	Rate of ovulation	No. of ova for ovulated rat
10 iu	Day 7	0/6	0
20 iu	5	5/8	7.4±2.4
	7	6/6	7.8±3.9

The release of ovulating hormone after estrogen treatment. — In order to observe the releasing time of the ovulating hormone of pseudopregnant rat after injection of estrogen, the temporal relationship between estrogen treatment and Nembutal injection was determined; When is the ovulation suppressed ? It was found that the blockage of ovulation reached 100 per cent when Nembutal given 28 and 30 hours after estrogen administration.

The reproductive function of pseudopregnant rats injected with HCG or estrogen. — The mating confirmed by the presence of spermatozoa in the vaginal smear was observed in 4 of 7 animals injected with estrogen, but not in the animals injected with HCG. Then the relationship between the occurrence of mating and the ovulation in pseudopregnant rats injected with estrogen was observed and these results shown in Table 5. Usually ovulation in both mated and unmated rats occurs and there is no evidence that ovulation is correlated to the

TABLE 5. *Effects of Nembutal at Different Times after Estradiol Injection on the Ovulation*

Interval from injection of estradiol to Nembutal	Rate of ovulation	No. of ova for ovulated rat
26 hrs.	3/4	7.3±3.1
28	0/4	0
30	0/6	0
32	3/5	5.3±1.5

mating. There is no evidence to show that the number of ovulations is reduced on day 7 of pseudopregnancy, but the rate of implantation and fetal survival was reduced.

TABLE 6. *Relationship between the Occurrence of Mating and Ovulation*

Group	No. of animals	No. of ovulated animals
mated	6	4
unmated	3	2

TABLE 7. *Reproductive Function of Pseudopregnant Rat Injected with HCG and Estrogen*

Treatment	Rate of mating	Rate of pregnancy	No. of corpus luteum	No. of implantation site	No. of fetus
HCG	0/5	—	—	—	-
Estradiol	4/7	1/7	15	10	7

Discussion

The incidence of ovulation by 20 iu HCG injection was obtained in all animals at each stage of pseudopregnancy in the present experiment. Greenwald has reported that he was unable to induce ovulation even with doses of HCG as high as 40 iu on days 3, 7, 11, and 15 of pregnancy (4). On the other hand, Brown-Grant has found the injection of 15 iu HCG did not result in ovulation in any rats on days 2, 5, and 8 of pseudopregnancy (1). In those studies, the animals were injected at 3.00 P.M. and killed 18 to 20 hrs later. In the present study, the animals were treated at 10.00 A.M. and killed 24 hrs later. The reason for the differences between the two studies is now known; the differences of strain and time of injection may be involved. The rate of ovulation induced with 10 iu HCG treatment was lower on days 4 and 5 of pseudopregnancy. The ovaries appeared to be relatively insensitive to the ovulatory action of HCG in early pseudopregnancy, probably as a results of retarded maturation or atresia of follicles.

None of the differences in egg yield induced with HCG were statistically significant except on days 4, 5 and 11 of pseudopregnancy, where the number of eggs was significantly lower. Greenwald has reported a reduction in mean number of follicles at days 3 and 10 of pregnancy, apparently resulting from atresia of follicle (4). It is possible that the occurrence of atresia or the variation in speed of follicular maturation results in the presence of follicles at different stages of maturation in the ovaries. At that time the follicles are insensitive to the ovulatory action of HCG on days 4, 5, and 11 of pseudopregnancy.

It has been found that the ovary of pregnant rats undergoes rhythmic follicular maturation (1–3). However, the cyclicity of follicular ripening is

lacking in the present study, indicating an endocrinological difference between pregnancy and pseudopregnancy. It is thought that a change in the follicular population which might correlate with nidation is found in pregnancy. Then, the experiment was designed to test whether deciduoma formation results in decreased ovulation in pseudopregnant animals. The effects of deciduoma formation on the ovulation rate and the egg yield were observed on days 5 and 7 of pseudopregnancy. In these rats, implantation commences late on day 5 of pregnancy. It is then possible that the reduced follicular maturation might correlate with implantation. Thereafter, cyclic maturation was not obtained. This result may be in accord with the Greenwals' experiment (4), which indicated that the size distribution of the follicles, remained relatively constant from day 4 through day 14 of pregnancy.

The effects of estrogen injection on ovulation at different stages of pseudopregnancy were determined. The incidence of ovulation was lower and the number of egg was fewer than in the results obtained with 20 iu HCG treatment. The absence of follicles competent to respond to estrogen explains the partial failure to reproduce the results in HCG treatment. The work of Everett (10) clearly demonstrated that ovulation following estrogen administration is due to the release of gonadotropin from the animals own pituitary. Van Rees et al have reported that the amounts of HCG necessary to induce ovulation during pseudopregnancy varied markedly (14). Then, it is possible that the stimulation by estrogen might result in a release of gonadotropin from the pituitary gland, in amounts insufficient to induce ovulation in early pseudopregnancy. These experiments may indicate a difference in sensitivity of the hypothalamus-hypophyseal system to estrogen between normal cyclic and pseudopregnancy rats.

It has been generally accepted that an injection of estrogen is followed within 48 hours by ovulation in the pregnant rat (11, 12). However, the time of ovulatory hormone release in pseudopregnant rats injected with estrogen was not carefully investigated.

The stimulating effect of estrogen on ovulation was uniformaly blocked by Nembutal given 28 to 30 hours after estrogen administration. These results may support the view that activating impulses to the anterior pituitary in pseudopregnancy are sharply limited by Nembutal, just as in certain critical hours during proestrus. The criterion taken for LH secretion was cholesterolization, which could be expected to be positive within 42 hours and from 22–32 hours after estrogen instances of partial blockage occured in pregnant and pseudopregnant rats (9). Then, the ovulation period obtained in the present experiment did not coincide in time with the positive cholestrolzation in the corpora lutea. This discrepancy is probably due to a quantitative difference in the character of the activation process between ovulation and the cholesterolization of the corpora lutea.

Mating was not observed in animals injected with HCG. On the other hand, the injection of estrogen resulted in mating 4 out of 7 pseudopregnant rat. The results of Brown-Grant indicated that 10 μg estradiol benzoate given at metoestrus induced mating in 10 out of 11 rats when placed with males at diestrus and four of eight rats mated when treated with 2.5 μg estradiol benzoate (13). This investigation suggests also that the treatment of HCG or estrogen may not cause a rise in the secretion of ovarian hormone to induce sexual behavior in pseudopregnant rats. When estrogen was administrated at metestrus, rats that did not mate did not ovulat. However, ovulation was induced in both mated and unmated rat in the present experiment. It seems that the ovulation induced by the estrogen injection during pseudopregnancy is not correlated to mating. A reduction of fertility was observed in these animals. It is probable that the ova and the condition of the genitals tract was not the same as in the nontreated animals.

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