THE INFLUENCE OF THE CONTROL OF PROLONGED PREGNANCY IN THE LACTATING PREGNANCY RAT ON SUBSEQUENT LACTATION AND REPRODUCTION

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By

Saburo TAKEUCHI, Shichiro SUGAWARA and Genzaburo MIURA

Laboratory of Animal Reproduction, Faculty of Agriculture, Tohoku University, Sendai, Japan.

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The relationship between lactation and reproduction in cattle is an important problem in the field of animal reproduction. These investigations almost require work with animal populations that are managed for the specific purpose of analyzing the records dealing with reproduction. Because, it is hard to research experimentally with cattle, it is possible that the information gained from research on small animals may serve as a guide for studies with cattle and another large animals. With this in mind, some research has been devoted to the relationship between lactation and reproduction in rats. An earlier report (8) gave evidence that influences of litter size and the nursing period on the recurrence of post-weaning estrus and subsequent fertility in rats.

Early works found that the length of the lactating pregnancy is correlated with the number of suckling young in rodents (5, 9). However, the influence of length of prolonged gestation in the lactating rat on the growth of suckling young and the subsequent reproductive functions has never been investigated. The present study was undertaken to obtain additional information on the relationship between lactation and reproduction, i.e. the effects of control of delayed implantation of lactating rats on the lactation, the functions of gonads and the fertility were determined.

Materials and Methods

All rats used in these experiments were virgin females of the Wister strain, weighing from 200 to 250 g. Vaginal smears were taken daily for at least three consecutive cycles. The rats were divided into four groups, each consisting of thirteen to twenty five animals which have 4-day cycles. The details of the experimental plan are shown in Table 1. For the sake of uniformity in suckling stimuli, the number of the litter was adjusted to eight or twelve immediately after parturition.

Thus, the delivered young were allow to remain with the mother for 21 days following parturition. In Group 3 and 4, each rat was placed in a separate cage with a sexually mature male on the day before parturition was expected. Fertile mating was confirmed in the morning at 10 A.M. by the presence of spermatozoa in vaginal smears. The day of discovery of sperm in the vagina is day 1 of pregnancy. Males were removed from the cages as soon as spermatozoa were found. If no sperm were found in the vagina, the rat was discarded. In Group 2 and 4, estrogen was administrated in order to shorten the gestation period of pregnancy rats. Estrone was administrated throughout as subcutaneous injection in 0.2 ml sesame oil daily. Daily doses of estrone (0.5 or 1.0μg) was administrated in each treated group from day 5 through day 13 of pregnancy. The number of young suckled was checked daily. In addition, body weights of both the mothers and their litters were taken. Particular attention was directed to the appearance of placental sign and the occurence of parturition. Animals were killed and laparotomized on post-weaning vaginal estrus (Group 1 and 2). Beginning on the 22nd day of pregnancy, animals were observed for delivery at 10 AM and the post-partum animals were examined for ovulation at 4 PM on 2 days following delivery (Group 3, 4). The ovaries were examined for the number of corpus luteum and ovulation point. The number of ovum in the oviducts was also observed. The procedures and methods used in each group are illustrated graphically in outline form by Figure 1.

Results and Discussion

Control of gestation period

The effect of the estrogen injection on the appearance period of placental sign and the length of gestation is summarized in Table 1. The length of gestation for normal pregnancy of rats in our laboratory ranged from 22 to 24 days with an average of 23.0±0.38 days (M±S.D.). The duration of pregnancy (Group 3) was prolonged as numerous investigations. The studies of Weichert (9) indicate that the prolongation of gestation in lactating pregnant rats correlates with the number of suckling young. In the present experiment, the length of lactating pregnancy is also in relation to the number of sucklings. If estrogen was administrated from day 5 through day 13 of pregnancy, the implantation of blastocyst began earlier and the length of pregnancy was shortened. This shortening of the gestation period bears no definite relationship to the doses of estrogen and the number of sucklings. Thus, the gestation period in Group 4 was nearly the same as normal pregnancy.

Effects of control of implantation on lactation

The growth rate of a suckling litter serves to measure the lactational performance in rats. The increase of body weight of a suckling litter is given in Table 2.

Table 1. Appearance of placental sign and gestation period in postpartum pregnancy lactating rats.

Group		No. of suckling youngs	Mating in postpartum	Injection of estrogen (estrone, μg)	No. of rats	Placental sign period (day following pregnancy)	Gestation period (day)
I	1-1	8	−	−	17		
	1-2	12	−	−	11		
II	2-1	8	−	+(0.5)	8		
	2-2	8	−	+(1.0)	6		
	2-3	12	−	+(1.0)	14		
III	3-1	8	+	−	5	16.0±1.90*	25.4±1.62*
	3-2	12	+	−	8	21.0±2.48	30.1±2.37
IV	4-1	8	+	+(0.5)	5	14.4±1.00	23.8±0.20
	4-2	8	+	+(1.0)	8	14.6±1.87	24.5±0.30
	4-3	12	+	+(1.0)	8	13.7±1.28	24.5±0.87

* M±S.D.

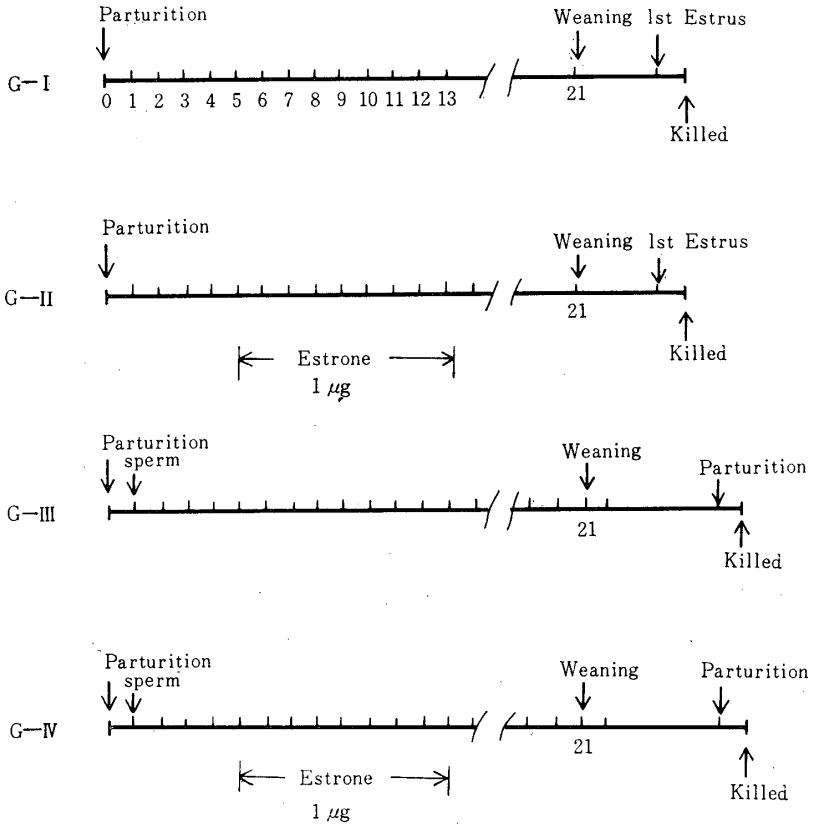

Fig. 1. Schedule of experimental procedures.

The difference between Group 1 and Group 2 during 14 days is significant ($P<0.05$). The injection of estrogen seems to increase milk secretion. The evidence to date shows that in the rat estrogens inhibit milk secretion (2). In the present

experiment, low doses of estrogen injection may stimulate milk secretion, but its mode of action is unknown. The difference between Group 1 and Group 3 is insignificant as is the difference between Group 2 and Group 4. It seems that the yield of milk would not be affected by pregnancy. The needs of the fetus for nutrition take priority over the needs of the dam. However, this effects on fetal growth increase in latter two-thirds of gestation. On the other hand, the milk secretion of rats increases until about the fourteenth day of postpartum. Accordingly, it would seem probable that the milk secretion would not be affected by gestation.

Table 2. Increases of body weight in suckling youngs 14 days and 21 days after parturition.

Group		No. of mother	14 days after parturition (g)	21 days after parturition (g)
I	1-1	17	154.0±23.73*	313.9±49.80*
	1-2	7	200.0±32.04	387.0±52.15
II	2-1	8	165.3±20.42	319.9±33.10
	2-2	6	139.8±21.66	291.1±45.47
	2-3	11	224.0±20.86	425.7±91.57
III	3-1	5	146.0±15.36	263.6±37.13
	3-2	16	197.0±34.35	377.3±71.96
IV	4-1	2	165.0±52.50	282.0±96.00
	4-2	6	143.9±66.78	320.0±24.50
	4-3	6	218.6±27.78	427.6±55.88

* M±S.D.

Effects of control of implantation on the subsequent reproductive functions

The number of corpus luteum, ovulation point, ovum in oviducts, litter size and normal fetus are given in Table 3. The litter size at first parturition of virgin rats in our laboratory ranged from 4 to 15 with an average of 9.4±2.68. The litter size in lactating pregnancy rats was statistically different between suckling young 8 and 12 ($P<0.01$). The litter size of lactating pregnancy rats was higher in estrogen injected rats (Group 4) than non treated (Group 3) ($P<0.01$). The litter size of lactating pregnancy rats is correlated with the number of suckling young and the length of delayed implantation. The increase of the number of sucklings leads to an increase of milk secretion and the duration until the occurrence of implantation. The increase of milk secretion and duration of delayed implantation appears to have an influence on the mortality of the embryo. The fetal viability (percentage of normal fetus against corpus luteum) is also considerably higher in estrogen injected rats (Group 4) than in the non treated (Group 3). The shortening of the delayed implantation period by an estrogen injection may maintain the viability of pre-implantation ovum. The number of ovulation points is

Table 3. Number of corpus luteum, ovulation point, ovum in oviduct, litter and percentage of fetus production (no. of living youngs/no. of corpus luteum).

Group		No. of suckling youngs	No. of mothers	Corpus luteum	Ovulation point	Ovum in oviduct	Litter size	Fetus production (%)
I	1-1	8	17	10.5±2.66*	13.1±5.60*	9.2±4.93*		
	1-2	12	11	13.4±1.85	13.0±1.85	12.1±2.08		
II	2-1	8	8	9.6±2.64	14.1±3.52	12.6±3.71		
	2-2	8	6	9.8±4.56	14.3±2.48	11.5±3.68		
	2-3	12	14	12.4±1.97	10.8±2.33	7.9±4.59		
III	3-1	8	5	15.0±1.00	10.2±3.20	10.5±1.80	9.0±3.4*	56.6
	3-2	12	17	13.4±1.32	10.4±2.12	9.5±2.78	7.5±2.39	56.5
IV	4-1	8	5	14.6±1.43	13.2±2.14	13.6±1.30	7.6±1.85	52.0
	4-2	8	8	11.9±2.03	4.5±5.95	3.0±5.20	8.7±3.10	73.6
	4-3	12	8	16.0±1.94	1.85±4.70	1.85±4.70	11.1±2.63	77.3

* M±S.D.

considerably higher in Group 1 and Group 2 and there is no statistical difference between these two groups. It is also recognized that the injection of estrogen has no significant effect on the ovulation rate. Statistical analysis showed the following differences: Group 1 v. 3: $P<0.01$; Group 2 v. 4: $P<0.01$, Group 3 v. 4: $P<0.05$. The lowest of ovulation point is obtained in Group 4. In the lactating pregnancy rats, the pregnancy seems to affect the ovulation at postpartum estrus. The ovulation point in ovaries were observed in all animals in Group 1, 2, 3, but not in Group 4. The ratio of ovulated animals to experimental animals was 8/13, 1/8 in subgroup 4–1,2 and 4–3, respectively. The interval from weaning to parturition was 7.4±3.09 days, 3.2±2.39 days in Group 3, and Group 4 respectively and the difference between these two groups is significant (P 0.01). The complication of the suckling and pregnancy in almost the same period seems to affect the ovulation in Group 4.

It was indicated that the follicular development proceeds to a point just short of the preovulatory swelling and does not rupture during pregnancy (3, 7), but the ovulation occurs soon after parturition and the occurrence of ovulation has relations with the time of delivery (1). Hoffmann and Schwartz (4) have shown that post-partum animals which were delivered between 4 PM the first day and 8 AM the second day had ovulated by 10 AM on, the third day.

It has been reported that the suckling stimuli cause a reduction of pituitary LH release, as indicated by inhibition of follicular growth and ovulation (6, 7). The authors have shown that the interval from weaning to the first vaginal estrus was 2, 8 (2–4) days following 21 days of suckling with 12 sucklings (8).

In Group 4, the ovulation was examined on 5 days, 2 days post-weaning, and

post-partum respectively the ovulation of almost all the animals was expected. However, the ovulation was not observed in all animals in Group 4 as mentioned above. The complication of suckling and pregnancy may also cause the change in the time of the critical period for LH release, and certain factors seem to affect the axis of hypothalamus-hypophyse-gonads, until it is posible for the maternal system to provide the proper enviroument for reproduction. Accordingly the ovulation in Group 4 seems to begin later.

The interval from weaning to the first vaginal estrus cornification is given in Table 4. The recurrence of vaginal estrus is also correlated with the number of suckling young as previously reported (5). The injection of estrogen has no significant effect on the recurrence of the vaginal estrus after weaning.

Table 4. Average intervals from parturitions to the first vaginal estrus (day)

Group		No. of mothers	Parturition to estrus
I	1-1	17	22.3±2.02*
	1-2	9	24.9±1.20
II	2-1	8	22.4±2.12
	2-2	6	23.9±2.55
	2-3	14	24.7±1.90

* M±S.D.

Summary

The effects of control of delayed implantation of lactating rats on the milk secretion and the reproductive functions were investigated. The control of delayed implantation is made by the injection of estrogen from day 5 through day 13 of pregnancy.

The milk secretion in lactating pregnant rats would not be affected by control of delayed implantation. The litter size and the fetal viablity of lactating pregnant rats increase by the shortening of delayed implantation. The ovulation of lactating pregnant rats seems to be delayed by shortening of delayed implantation.

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