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The Transport of Spermatozoa in Postpartum Estrus of Rats

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Summary

Rats will breed immediately after parturition. However, little is known of the transport of spermatozoa in the female genital tract in postpartum estrus comparing to cyclic estrus. The effects of condition following parturition on the transport of spermatozoa were investigated.

The rats which delivered between 4.00 PM (Day 1) and 10.00 AM (Day 2) were mated with fertile male from 5.00 to 7.00 PM (Day 2). These animals were slaughtered at various intervals after mating, and then the number of spermatozoa was determined in each segment of the female genital tract.

The rate of fertile mating at postpartum estrus was lower than that in cyclic estrus. Large numbers of spermatozoa were observed in the uterine cornua within 15 minutes after ejaculation in cyclic estrus, but 30 minutes after ejaculation in postpartum estrus. Spermatozoa in the ampullae was observed in all animals examined 60 minutes after ejaculation in cyclic estrus, but 240 minutes after ejaculation in postpartum estrus. The first appearance of active spermatozoa in the ampullae was 180 minutes after ejaculation in cyclic estrus, but 300 minutes after mating in postpartum estrus. However, the number of ovulation, of implantation site and of fetus in postpartum rats was normal.

An optimal calving interval is an important matter to the lifetime production in cattle. Thus, the interest in reproductive rate during the postpartum period arise from management and economic conditions. When cows were bred at early estrus after parturition, the conception rate was lower compared to the rate observed at a later estrus. There is a suggestion that this lower fertility appears to be due mainly to the lack of fertilization and that the failure in spermatozoa transport and the infertile state of spermatozoa in the fertilization site are regarded as important factors (1). However, no experimental investigation has been made to examine the possibility that the lower rate of fertilization at early estrus after parturition is due to a specific failure in the transport of spermatozoa.

The present investigation is undertaken to examine the transport and the vitality of spermatozoa in the female genital tract at copulation following

parturition. The rat was used as the experimental animal. Even though rats breed immediately after parturition, little is known of the transport of spermatozoa following parturition. The transport of spermatozoa in the female genital tract in postpartum estrus is compared with that in normal estrus.

Materials and Methods

The animals used were rats from the Wistar strain bred in our laboratory. The animals were provided with food and water ad lib. Vaginal smears were followed regularly and the 4-day cycle was selected for study. Animals were between 200 and 300 g at the initiation of experiments. The female rats were divided into two groups. The female rats at cyclic estrus were mated with a fertile male from 5.00 to 7.00 PM of proestrus, as control (Group 1). The female rats in Group 2 were observed for delivery at 10.00 AM and 4.00 PM beginning on the 20 day of pregnancy. The postpartum rats which delivered between 4.00 PM (Day 1) and 10.00 AM (Day 2) were placed with a fertile male from 5.00 to 7.00 PM (Day 2).

The mating was conducted by one small light. Individual females were observed continuously to determine the precise moment of mating. Fertile mating was confirmed by the observation of coitus and the presence of spermatozoa in the smear. The rats showing spermatozoa in the smear were immediately removed and remained undisturbed until the time of sacrifice. Animals were slaughtered at various intervals after mating, as shown in Table 2. Immediately after the female rat was killed, she was opened ventrally. The genital tracts were flushed with 0.2 ml physiological saline by inserting a needle attached to a hypodermic syringe. Then, the fluid was collected through syringe inserted into the uterine end of the Fallopian tube or into the ampulla and was transferred to a counting vessel. The number of spermatozoa was determined by haemocytometer counts. The spermatozoa was stained with nigrosin. The survival of spermatozoa was determined by their motility.

The conditions of the uterus following parturition were compared with the cyclic estrus rats. The rats were slaughtered at 5.00 PM of proestrus in Group 1, and at 5.00 PM following parturition in Group 2, respectively. The weight, width and length of uterus, and the quantity of uterine fluid were measured. The measurement of width was taken at the implantation site.

Further, the fertility of spermatozoa observed in the vagina was obtained in order to determine the transport and the vitality of spermatozoa at copulation following parturition. Animals were killed and laparotomized on the 18th day after service. The number of corpus luteum, implantation sites and fetus were recorded.

Results and Discussion

The rate of mating and ejaculation.

The rate of mating with a fertile male and of true service confirmed by the presence of spermatozoa in the smear is shown in Table 1. The mating was observed in all animals of Group 1 from 5.00 to 7.00 PM of proestrus, but in only 33 of 55 animals of Group 2. The rate of fertile mating was 41/56, 20/33 in Groups 1, and 2, respectively. Also, there was a difference in sexual behavior and fertile mating between cyclic estrus and postpartum estrus.

TABLE 1. *The Rate of Mating and of True Service in Each Group.*

Group	Number of animals	Rate of mating	Rate of detecting spermatozoa
1	56	56/56	41/56
2	55	33/55	20/33

It has been shown that sexual behavior in female rats occurs in the early afternoon of proestrus and is fully observed by 7.30 PM on the day of proestrus (2, 3). This investigation suggests also that sexual behavior occurs earlier during the day of proestrus and that high mating response rate occurs by 5.00 to 7.00 PM.

Postpartum ovulation in the rat was observed by Blandau and Soderwall (4), Hoffman and Schwartz (5). They (5) have reported that all animals which delivered between 4.00 PM (Day 1) and 8.00 AM (Day 2) had not ovulated by 4.00 PM (Day 2) but had ovulated by 10.00 AM (Day 3). These results indicate that assuming a latent period of about 12 hours between LH release and ovulation, LH release can begin as early as 10.00 AM or as late as 6.00 PM and that critical time for LH release in the postpartum estrus is long compared to the 4 day cyclic estrus. These data demonstrate that the coitus occurs in close proximity to the critical period for ovulatory release of LH. It seems that the period of LH release necessary for sexual behavior is different in the delivery period and that the mating in postpartum estrus must fall within a more extended time interval than cyclic estrus. Thus, the animal which has not been observed mating in postpartum estrus is late in LH release and there may not have occured a rise in secretion of ovarian hormone to produce estrus between 5.00 and 7.00 PM.

The transport of spermatozoa in the female genital tracts.

The number of spermatozoa ejaculated into the right and left cornua in female rats killed at various intervals after mating is summarized in Table 2. Large numbers of spermatozoa were observed throughout the uterine cornua of the

control animals examined 15 minutes after ejaculation, but not of the postpartum animals. In postpartum rats, the spermatozoa migrated into the uterine examined 30 minutes after mating. The average number of spermatozoa ejaculated into right and left cornua in control animals killed 30 minutes after mating was

TABLE 2. *The Number of Spermatozoa Observed in Each Cornu of Each Group at Various Intervals After Mating.*

Time, in minutes, between ejaculation and killing of rats	Group	Number of animals	Number of spermatozoa reached in the uterine segment ($\times 10^4$)	
			Right	Left
15′	1	3	3933(0 — 6200)	4733(800— 9000)
30′	1	5	19080(2400— 80000)	4680(200—11000)
	2	5	880(0 — 2000)	2440(200—74000)
60′	1	7	11915(900— 35700)	9386(0 —27200)
	2	6	10775(200— 60000)	11990(200— 4000)
120′	1	6	6100(600— 20400)	567(0 — 1400)
	2	6	41640(2200—160000)	8920(0 —40000)
180′	1	5	12780(400— 27200)	5450(0 —11900)
	2	6	3033(1200— 8000)	4167(1200— 3200)
240′	2	6	2166(400— 4800)	1033(200— 2000)
300′	2	8	1063(200— 3400)	806(200— 2200)

190,800,000 and 46,800,000 respectively, and in postpartum animals was 8,800,000 and 24,400,000 respectively. In a number of animals, the concentration of spermatozoa was notably less in one than in the other cornu. The number of spermatozoa for the two sides, from animal to animal and from time to time vary so widely that a significant difference in the number of spermatozoa ejaculated into each cornu between cyclic estrus and postpartum estrus has not been observed.

TABLE 3. *The Number of Spermatozoa Observed in Each Ampulla of Each Group at Various Intervals After Mating.*

Time, in minutes, between ejaculation and killing of rats	Group	Number of animals	Rate of animal observed spermatoza	Number of spermatozoa in the ampullae	
				Right	Left
15′	1	3	0/3	0	0
30′	1	5	1/5	0.6(0— 3)	0
	2	5	0/5	0	0
60′	1	7	7/7	1.3(0— 3)	2.6(0—14)
	2	6	2/6	1.3(0— 5)	0.1(0— 1)
120′	1	6	6/6	3.4(0— 8)	1.7(0— 3)
	2	6	3/6	1.2(0— 3)	0.6(0— 3)
180′	1	5	5/5	23.4(5—60)	30.7(8—60)
	2	6	3/6	2.5(0—10)	2.5(0—10)
240′	2	6	6/6	10.1(0—40)	2.7(0— 6)
300′	2	8	8/8	3.5(6— 7)	0.6(0— 2)

It is known that at the time of estrus the cervix is highly contracted, that the cornua become distended with fluid and that the ejaculation takes place in such a manner that the semen is transported en masse through the cervix, directly into the favorable uterine environment (6, 7). However, the uterine fluid was scarce in the postpartum estrus, as shown in Table 5. Thus, the transport of spermatozoa into the uterus seemed to be late.

The number of spermatozoa recovered from the ampullae of females killed at the various intervals after coitus is summarized in Table 3. It was also observed that a great variation existed from animal to animal in the number of spermatozoa in the ampulla. The number of spermatozoa in the ampulla was very small in contrast to that in the uterus. In female rats killed 15 minutes after mating, spermatozoa was not found in the washing of the ampulla. At 30 minutes after mating, spermatozoa were found in the ampulla of the control animals in only one out of five oviducts examined. The spermatozoa had migrated into the ampulla in all control animals examined 60 minutes (or later) following ejaculation. In the postpartum group, all animals displayed spermatozoa in the ampulla 240 minutes (or longer) after mating. The number of spermatozoa in the ampulla of control

TABLE 4. *The Number of Motile Spermatozoa in Ampullae at Several Intervals after Mating.*

Time, in minutes, between ejaculation and killing of rats	Group	Rate of animal observed spermatozoa	Number of spermatozoa in ampullae	
			Right	Left
120′	1	0/6		
	2	0/6		
180′	1	4/5	2	1
			0	1
			3	0
			1	2
	2	0/6		
240′	2	0/6		
300′	2	3/8	1	0
			3	0
			2	0

animals increased during the later intervals until a maximum number was obtained from a single tube in several animals killed 180 minutes after mating. In the postpartum group, however, the number of spermatozoa in the ampulla did not increase to the extent one would have expected. The first appearance of the active spermatozoa in the ampullae was obtained 180 minutes after mating in the control group, but 300 minutes after mating in the postpartum group, as shown in Table 4. In each time interval examined after mating, it was noted that the number of spermatozoa in the ampullae was less in the postpartum group when compared with the control group.

It has been indicated that spermatozoa is found in the ampullae in 100 percent of the animals examined 1 hour after ejaculation (8), that relatively few spermatozoa arrive at the site of fertilization in the rats and the number of spermatozoa obtained here is also few (9). These results are similar to the observations obtained in this investigation. However, the transport of spermatozoa into the ampulla was not so rapid in control animals obtained by Blandau and Money (8). It is generally recognized that the spermatozoa rate ascent to the site of fertilization appears to be influenced by the time of mating in the rat (10,11). The female rats examined here were mated from 5.00 to 7.00 PM and the mating in the early evening might interfer with the normal ascent of the spermatozoa to the ampulla.

In the postpartum animals the number of spermatozoa observed in the ampulla is considerably fewer than that observed in the control animals. This fewer spermatoza in the postpartum group may be due to the few spermatozoa migrating

TABLE 5. *The Weight, Dimension and Fluid of the Uterus (Mean±S.D.)*

Group	Number of animals	Body weight (g)	Weight of uterus (g)	Width of each cornu (cm)		Length of each cornu (cm)		Quantity of uterine fluid (ml)	
				Right	Left	Right	Left	Right	Left
2	11	225.8 ± 39.6	2.19 ± 0.42	0.78 ± 0.17	0.86 ± 0.19	4.9 ± 1.17	5.2 ± 1.23	—	—
1	10	231.2 ± 24.8	0.55 ± 0.13	0.38 ± 0.06	0.40 ± 0.04	2.7 ± 0.34	2.7 ± 0.42	0.18 ± 0.05	0.18 ± 0.04

TABLE 6. *The Fertility of Postpartum Rats (Mean±S.D.).*

Number of rat	Number of corpus luteum	Number of implantation site	Number of fetus
16	14.9±1.75	11.8±3.02	10.9±3.05

from the uterus to the oviduct or to a late transport of spermatozoa into the female genital tracts. The results of the uterine measurement are shown in Table 5. The uterine horn at postpartum estrus had a large diameter and consequently a larger endometrial surface area. The large endometrial surface over which the spermatozoa scatter may bring about a marked reduction in the number of spermatozoa reaching the oviducts. It has been reported that the transport mechanism of spermatozoa is dependent upon the muscular activity of the uterine and oviductal tissue, and that both oviductal activity and gametic migration rates are influenced by hormonal factors. The uterine and oviductal environment in postpartum estrus may have an adverse effect on the responsiveness to the ovarian hormones and consequent transport of the spermatozoa in the genital tract.

The fertility in a single mating at early evening was observed to ascertain the transport of active spermatozoa in postpartum animals. The results of reproductive performance are shown in Table 6. Corpus luteum were used as indices of ovulation. There is no evidence to show that the number of ovulation, of implantation sites and of fetus was reduced. Therefore, it seems that the restriction on the passage of spermatozoa through the female genital tract is relaxed later, that the uterine environment of postpartum estrus has not an adverse effect on the fertility of spermatozoa, and that the active spermatozoa do arrive at the site of fertilization in postpartum rats.

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