Implantation in Both of the Uterine Horns after the Unilateral Intrauterine Insemination in Rabbits

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ABSTRACT. Artificial insemination was carried out by injecting semen into the upper part of the left uterine horn in rabbits with a duplex uterus. Ovulation was then induced by administration of hCG. As a result, implanted fetuses were observed not only in the left uterine horn but also in the right uterine horn. However, when similar insemination was carried out after ligation of the right utero-tubal junction, fertilization did not occur in the right oviduct. From these findings, we conclude that some of the spermatozoa injected into the left uterine horn was discharged into the vagina, entered the right uterine cervical canal, and reached the right oviduct to fertilize the ova.—**KEY WORDS:** implantation, rabbit, unilateral intrauterine insemination.

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It has been reported that in dogs with a bicornuate uterus, the injection of semen into the central portion of the unilateral uterine horn resulted in fertilization not only in the oviduct of the inseminated side but also in the contralateral oviduct [6]. In the present experiment, we injected semen into one of the uterine horns of rabbits with a duplex uterus in order to examine whether implanted fetuses can be observed in the opposite uterine horn. In case fetuses were observed in the opposite uterine horn, the transit route of the sperm from the inseminated uterine horn to the opposite oviduct was also examined. In short, whether the transit route of sperm had been via the peritoneal cavity or via the vaginal cavity was investigated.

Mature female Japanese white rabbits weighing between 2.8 to 3.5 kg and purchased from a commercial breeder (Saitama Experimental Animal Supply Co., Ltd., Saitama, Japan) were used in this study. The rabbits were housed individually in wire netting cages in a conventional environment room. They were given commercial pellets (Labo R Stock; Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) and tap water *ad libitum*. Each animal had been isolated in a separate cage for at least 30 days prior to the experiment. Under general anesthesia with a pentobarbital derivative; Nembutal (Abbott Laboratories, North Chicago, IL, U.S.A.), laparotomy was performed by a midline abdominal incision. Thereafter 0.1 ml of semen was injected into the upper part of the left uterine horn with a glass capillary pipette. After conducting unilateral intrauterine insemination into the left uterine horn, the abdominal muscles and skin were sutured respectively. The semen used for insemination was collected beforehand from proven fertile mature males by means of an artificial vagina method. The day of insemination was designated as Day 0. The inseminated rabbits were laparotomized again under general anesthesia on Day 11 to examine the number of implanted fetuses (Experiment 1). Since fetuses were observed not only in the left but also in the right uterine horn in Experiment 1, we then examined the transit route of the sperm (Experi-

ment 2). In Experiment 2, just prior to the semen injection into the upper part of the left uterine horn, the right utero-tubal junction (UTJ) was ligated with silk thread in order to block sperm ascending transvaginally to the right oviduct from the inseminated left uterine horn. In both Experiments 1 and 2, 75 iu of hCG (Gonotropin; Teikoku Hormone Mfg. Co., Ltd., Tokyo, Japan) was injected intravenously to the rabbits one hour after the semen injection to induce ovulation. In Experiment 2, the rabbits were euthanized thirty hours after the hCG injection with an overdose of Nembutal and both oviducts were excised from the rabbits. The ovulated eggs were collected by flushing the excised oviducts with medium (TCM-199; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 40% inactivated normal rabbit serum.

As shown in Table 1, implanted fetuses on the inseminated side of the uterine horn in three cases and on the opposite side in four cases were observed in Experiment 1. Rabbits Nos. 103 and 104 were delivered of one young each on Day 31. In these two rabbits, no fetuses became implanted on the inseminated left uterine horn on Day 11, so the delivered young must have developed from the fetuses that became implanted on the opposite side of the inseminated uterine horn.

These results indicate that even in rabbits with a duplex uterus, sperm injected into the left uterine horn moved to the right oviduct and participated in fertilization there. Table 2 shows the results of Experiment 2. From the left oviduct, all eggs recovered were found to have cleaved. However, none of the eggs recovered from the right oviduct were fertilized.

In rabbits, some of the sperm ejaculated into the vagina during copulation are released into the abdominal cavity [5]. Hunter [3] considered that the sperm released from one of the tubal fimbrias into the abdominal cavity might enter the corresponding extremity of the other. Actually the transit of sperm across the abdominal cavity and their entrance into the tubal fimbrias to take part in fertilization have been confirmed by intraperitoneal insemination in rabbits [1, 2, 4]. The authors, therefore, consider that there was a possibility that some of the sperm released from the extremity of the left tubal fimbria entered the right tubal fimbria and fertilization took place in the ampulla of the right oviduct. The results of Experiment 1

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Table 1. The number of implanted fetuses and delivered young from unilateral intrauterine insemination in rabbits

Rabbit No.	Conc. of live sperm injected ($\times 10^7$)	Side of semen injection (left)		Contralateral side of semen injection (right)		No. of delivered young
		No. of corpora lutea	No. of implanted fetuses ^{a)}	No. of corpora lutea	No. of implanted fetuses ^{a)}	
101	2.08	5	2	5	1	0
102	7.37	3	1	6	0	0
103	2.04	2	0	6	2	1
104	2.04	5	0	7	2	1
105	5.79	6	3	3	1	0

a) Laparotomy was conducted on the 11th day after insemination to examine the implanted fetuses.

Table 2. Effects of the contralateral UTJ ligation on the fertilization after unilateral intrauterine insemination in rabbits

Rabbit No.	Conc. of live sperm injected ($\times 10^7$)	Side of semen injection (left)			Contralateral side of semen injection (right) ^{a)}		
		No. of ovulation points	No. of eggs recovered	No. of cleaved eggs ^{b)}	No. of ovulation points	No. of eggs recovered	No. of cleaved eggs
201	5.96	7	4	4	5	4	0
202	5.96	3	2	2	5	0	0
203	0.99	4	2	2	4	2	0
204	0.99	4	0	0	2	0	0
205	5.24	3	1	1	6	0	0
206	0.83	8	7	7	6	3	0
207	0.83	6	5	5	3	3	0

a) UTJs of this side were ligated with silk thread immediately before the semen injection into the left uterine horn.

b) Five eggs at the 2-cell and 16 eggs at the 4-cell stage were recovered.

indicates an evidence that the sperm moved from the inseminated left uterine horn to the right oviduct. When the right UTJ was ligated in Experiment 2, however, no fertilized eggs were recovered from the right oviduct. This result means that the ligation at the right UTJ prevented the movement of sperm from the inseminated left uterine horn to the right oviduct.

It is thus concluded that some of the sperm injected into the left uterine horn in rabbits found their way into the vagina, entered the right uterine cervical canal, and migrated to the right oviduct and participated in fertilization there.

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