A Comparative Study of the Contraceptive Mechanism of the Cu-Intrauterine Device and the Plastic-Intrauterine Device in Rats

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TAKAHASHI, K., HIROSE, Y., HANEDA, A., MIZUKAMI, T., OKAMURA, K. and SUZUKI, M. *A Comparative Study of the Contraceptive Mechanism of the Cu-Intrauterine Device and the Plastic-Intrauterine Device in Rats.* Tohoku J. exp. Med., 1978, 126 (4) 325-333 — After inserting the Cu-IUD and plastic-IUD into the horn of the uterus of rats, vaginal smears were daily observed for twelve estrus cycles, but no abnormal changes were noted. The female rats with the IUD were exposed to impregnation placing together with male rats. Neither the Cu-IUD nor the plastic-IUD affected copulation, and a complete contraceptive result was obtained in these rats. There were no significant differences in any effect examined between Cu-IUD and plastic-IUD groups. In order to investigate the influence of copper on the penetration of sperm, we placed the rats with the IUD under direct observation and then exposed them to natural copulation. By examining various sites of the reproductive organs 30 min after copulation, the hindrance of sperm migration by the Cu-IUD was confirmed. — IUD; experimental study of contraception; rat

With worldwide expansion of population becoming an everincreasing social problem, researches concerning contraception are of great significance, and various contraceptive methods are being investigated on all quarters. Among these are oral contraceptive (pill) and intrauterine devices (IUD), methods which are valued for their superior results and which represent the most widely disseminated birthcontrol techniques to date. With regard to the IUD, besides the heretofore utilized inert IUD, the active IUD has been developed and reported as manifesting a higher level of contraceptive effect. However, as for the mechanism through which the active IUD functions, there are numerous aspects which remain as yet unclear.

The purpose of the present paper is to elucidate the mechanism of the contraceptive action of the Cu-IUD in rats. The experiments were performed with respect to its influence on the estrus cycle and on sperm deposited in the female reproductive tract. On all points a comparative study utilizing the plastic-IUD vis-à-vis the Cu-IUD was concurrently conducted.

MATERIALS AND METHODS

The IUDs employed in our experiments were the Cu-IUD and the plastic-IUD manufactured by Searle Laboratories (Chicago) for use in rats. They are represented in Fig. 1. The Cu-IUD was 200–210 mm in total length, with a copper wire—0.2 mm in thickness and 1 cm in length—shaped into a coil 1 mm in inside diameter, above and below which fixed rings, each 1 mm in width, were attached. The plastic-IUD was of the shape presented in the picture, with a total length of 1 cm and a diameter of 2 mm. All of the IUDs were already sterilized and stored under vacuum-sealed conditions.

Fig. 1. Cu-IUD and plastic-IUD.

Adult female rats of the Wistar strain were used. Vaginal smears were taken every morning to determine the stage of two estrus cycles. Only those rats which exhibited a normal estrus cycle were selected.

The method of insertion of the IUD was as described by Chang et al. (1970). Under ether anesthesia, the uterine horns were exposed through a dorsolateral incision. By means of a scalpel, an incision of 1 mm in the horn of the uterus below its union with the fallopian tube was made and then the IUD was inserted. The position at which each IUD was installed is illustrated in Fig. 2. After fixing the IUD in place with silk thread, we stitched together the opened section and then closed the abdomen. As one of the controls, "sham operations" were performed on some rats, in which, using a scalpel, an incision was simply made in the uterus and then was sewed up again without installing a device.

The first experiment consisted of daily observation of vaginal smears taken every morning at 10:00 a.m. for a period of twelve estrus cycles.

In the second experiment female rats in proestrus were selected and placed together with male rats. The following morning, the rats were checked for the existence of sperm or vaginal plugs. If either was found, we counted that day as the first of pregnancy and thus ascertained the probable day of delivery. Immediately after delivery the rats were sacrificed and the numbers of corpora lutea in both ovaries and implantations in both uteruses were counted.

Fig. 2. Diagram of the rat uterus showing the position of the IUDs. (a), sham operation; (b), Cu-IUD (unilateral); (c), Cu-IUD (bilateral); (d), plastic-IUD (unilateral); (e), plastic-IUD (bilateral); (f), Cu-IUD (uterus junction).

Fig. 3. The dotted lines show where each horn was divided into three pieces before sectioning.

In the third experiment rats which were already inserted with the IUD and were ligated at the right uterus, as shown in Fig. 3, were used. Male and female rats were raised separately under light control consisting of reversed cycles of light and dark. That is, the rearing room was kept completely dark from 9:00 a.m. through 6:00 p.m. and illuminated from 6:00 p.m. through 9:00 a.m. with electric lights for ten days. After reversing the estrus cycle in this manner, the male and female rats were placed together in a single pair in each cage from 9:00 a.m. to 11:00 a.m., while keeping the rearing room in the darkened condition, and were placed under direct observation. Females which then experienced copulation were removed immediately. Thirty minutes after copulation, their abdomen was opened and the uterus was exposed. Then, as indicated in Fig. 3, with utmost care we cut and divided up the tissue. Each part was flushed with 1 ml of physiological saline and, after centrifugation, the number of acquired sperm was counted by use of a Thoma blood cell count plate.

RESULTS

Effect on the estrus cycle

The influence of the Cu-IUD and plastic-IUD on the estrus cycle is indicated in Fig. 4. Although for a period of two to three estrus cycles following installation of the devices some rats indicated disturbance of the estrus cycle, thought to be derived from the trauma of operation, after this initial postoperative period a normal

Fig. 4. Effect on estrus cycle in rats with sham operation and IUDs.

estrus cycle was restored in nearly all animals. No evidence of abnormal estrus cycle which might be caused by the Cu-IUD or the plastic-IUD was noted.

Effect on mating

When female rats were placed in cohabitation with male rats, it was found that the rats which had an operation for the installation of IUD took a somewhat longer period until copulation than did the rats which did not have an operation (Table 1). Furthermore, the Cu-IUD group required 1.2 ± 0.2 proestrus cycles until copulation, while the plastic-IUD group required 1.7 ± 0.2 proestrus cycles. But there was no significant difference between the two groups.

Effect on anti-fertilization

The numbers of corpora lutea were as follows (in the left and the right ovary, respectively): 7.3 ± 0.6 and 7.3 ± 0.8 for the non-treated group, 6.0 ± 0.6 and 6.8 ± 0.8 for the sham operation group, 6.5 ± 0.8 and 5.3 ± 0.6 for the Cu-IUD group, and 5.6 ± 0.4 and 5.6 ± 0.5 for the plastic-IUD group. There was no significant statistical difference between the non-treated and treated groups. Either, the operation and insertion of IUDs appeared to exert no effect on ovulation.

The numbers of implantations were as follows (in the left and the right uterus, respectively): 5.3 ± 0.5 and 5.3 ± 0.7 for the non-treated group, 4.6 ± 1.5 and 5.2 ± 1.9 for the sham operation group, 5.3 ± 0.6 for the non-inserted side of the Cu-IUD group, and 5.0 ± 0.6 for the non-inserted side of the plastic-IUD group. There was no significant difference between the non-treated group and the non-inserted side of the IUD group.

No implantation was observed on the inserted side of the Cu-IUD or the plastic-IUD group, and thus the perfect contraceptive effect was evidenced. Fifteen

TABLE 1. *Antifertility effects of Cu-IUD and plastic-IUD in rats*

Group	Rat No.	Number of proestrus cycles until mating	Number of corpora lutea		Number of implantations	
			Left	Right	Left	Right
No treatment control	1	1	7	6	5	5
	2	1	8	7	5	4
	3	1	8	4	7	3
	4	1	4	11	4	9
	5	1	9	8	7	5
	6	1	8	6	6	5
	7	1	7	9	3	6
	Mean±S.E.	1±0	7.3±0.6	7.3±0.8	5.3±0.5	5.3±0.7
Sham operation control (bilateral)	1	1	6	9	6	8
	2	2	4	9	2	5
	3	1	5	5	4	6
	4	1	8	5	6	2
	5	4	9	6	5	5
	Mean±S.E.	1.6±0.5	6.0±0.6	6.8±0.8	4.6±1.5	5.2±1.9
Cu-IUD (unilateral)	1	1	8	6	0	6
	2	2	8	5	0	5
	3	1	6	7	0	7
	4	1	8	3	0	3
	5	1	3	4	0	4
	6	1	6	7	0	7
	Mean±S.E.	1.2±0.2	6.5±0.8	5.3±0.6	0±0	5.3±0.6
Plastic-IUD (unilateral)	1	1	6	5	0	5
	2	1	6	7	0	7
	3	2	4	4	0	4
	4	1	6	5	0	5
	5	2	6	9	0	9
	6	2	6	6	0	4
	7	2	4	4	0	2
	8	2	8	6	0	5
	9	2	4	4	0	4
	Mean±S.E.	1.7±0.2	5.6±0.4	5.6±0.5	0±0	5.0±0.6

female rats of the IUD-treated group in Fig. 2-c, e, f were exposed to copulation but none became impregnated.

Effect on penetration of sperm into the reproductive organs

Table 2 indicates by percentages the number of sperm found in each region of the reproductive organs, and thus a comparison of sperm penetration is made. In the non-treated group, the sperm penetration was confirmed 49.2% in (A), the lower one-third of the uterus; 13.3% in (B), the middle one-third of the uterus; 17.4% in (C), the upper one-third of the uterus; and 0.1% in the uterine tube. In the Cu-IUD group, it was 64.3% in (A), 19.9% in (B), 15.7% in (C) and a small number of sperm in the uterine tube. A reduction of number of sperm could be seen in the positions through the IUD-installed region. In particular, in one example sperm were completely absent from (B) through the uterine tube. Furthermore, as shown in Table 2, "4*" represents an example which, 5 min after initial copulation, experienced copulation a second time. This example showed

TABLE 2. *Effect of Cu-IUD and plastic-IUD on the sperm penetration*

Group	Rat No.	(A) Lower 1/3 of uterus	
		Number of sperm	%
		($\times 10^6$)	
No treatment	1	2.91	38.2
	2	2.76	58.6
	3	4.93	47.7
	4	5.43	51.1
	Mean $\pm$ S.E.	4.01 $\pm$ 0.7	49.2
Cu-IUD	1	3.95×10^3	100
	2	2.75×10^5	62.6
	3	4.35×10^6	62.3
	4*	2.51×10^5	42.7
	Mean $\pm$ S.E.	3.39 $\pm$ 0.4 $\times 10^6$	64.3
Plastic-IUD		($\times 10^6$)	
	1	1.11	47.4
	2	3.25	38.2
	Mean $\pm$ S.E.	2.18 $\pm$ 0.9	42.8

* See text.

percentage tendencies resembling those of the plastic-IUD group. As for the plastic-IUD group, while it showed the same results as the non-treated group in the region of the uterus, with a slightly higher percentage at (C), in the region of the ovaries it showed the same trend as that of the Cu-IUD group.

DISCUSSION

Copper represents a metallic ion which is associated with the metabolic activity of tissue in living things. With regard to reproduction, the authors reported that when copper salt is intravenously injected into domestic rabbits, it acts on the hypothalamus and ovulation is induced (Suzuki et al. 1972a, b). Furthermore, in terms of the toxic action of copper, it was reported that its action hinders the formation of sperm and has an effect on sperm activity (Saito et al. 1967).

Taking note of these actions, Zipper et al. (1968, 1969a) reported that when metallic substances such as copper and zinc were inserted into the uterus of domestic rabbits, a superior contraceptive effect was achieved. In rats and hamsters the antifertility effect of copper was confirmed in the experiments of various metals by Chang et al. (1970). In human, Zipper et al. (1969b) showed that the addition of copper to a plastic "T"-shaped IUD greatly enhanced the contraceptive effect. Besides metallic substances, Doyle and Clewe (1968) published an active IUD to which synthetic progestin had been added, and today this is being investigated in a form such as the Progesterone T (Zador et al. 1976).

To continue, as regards further work on the Cu-IUD, much is being conducted of late and reports are coming out concerning the basic and clinical aspects of IUD types such as the Copper T (Zipper et al. 1969b), the Copper 7 (Newton et al.

into the female genital tract of rats at 30 min after copulation

(B) Middle 1/3 of uterus		(C) Upper 1/3 of uterus		Uterine tube	
Number of sperm	%	Number of sperm	%	Number of sperm	%
($\times 10^6$)		($\times 10^6$)			
3.03	39.8	1.67	21.9	10^3	0.01
1.34	28.4	0.60	12.7	10^4	0.2
2.85	28.1	2.34	22.6	10^4	0.1
3.90	36.7	1.30	12.2	10^3	0.01
2.78 ± 0.5	33.3	1.48 ± 0.3	17.4	$5.5 \pm 2.3 \times 10^3$	0.1
($\times 10^6$)		($\times 10^6$)			
0	0	0	0	0	
1.54	35.0	0.10	0.02	0	
1.50	21.7	1.05	15.0	5	
1.17	19.9	2.20	37.4	16	
1.05 ± 0.3	19.9	0.83 ± 0.4	15.7		
($\times 10^6$)		($\times 10^6$)			
0.56	23.9	0.67	28.6	50	
2.47	29.0	2.79	32.8	10	
1.52 ± 0.8	26.5	1.73 ± 0.9	30.7		

1972), the Anderson-Ansell "Latex Leaf" (Sadovsky et al. 1975) and so on.

Clinically speaking, reports on the Copper T were the earliest to appear, although at first it was the Copper 7 which received general usage. At present it is maintained that, in terms of contraceptive efficacy and of possible ill effects such as expulsion, bleeding and pain related to the various types of IUD, IUDs more safe and long-lasting than Copper 7 are the Copper T, especially TCu-380A and TCu-220C (Tatum 1977).

As yet, the mechanism of Cu-IUD action remains to be unclear to our knowledge. With regard to the influence of the IUD on the estrus cycle in rats, we have only the report of Doyle and Margolis (1964), who utilized a silk-IUD and noted an apparent effect on the cycle. In the first of our experiments, no alterations were observed in the estrus cycle.

In the second of our experiments, the following results were obtained: In regard to the number of days until copulation, the installation of Cu-IUD resulted in no changes when compared with the non-treated animals; neither the Cu-IUD nor the plastic-IUD exerted any effects on ovulation; in respect to contraceptive effect from the standpoint of implantations, both the Cu-IUD and the plastic-IUD evidenced 100% contraceptive efficacy; the contraceptive effect remained the same no matter where in the uterus the IUD was placed. Other reports of a similar type were published concerning rats, hamsters (Chang et al. 1970) and rabbits (Zipper et al. 1969; Nutting and Mueller 1975a, b).

In keeping with discussions on IUD action to date, our first and second experiments confirm that the action of the IUD is a localized one, but does not affect the body as a whole. However, there is still a question as to what, in fact, this action really consists of, and as to what kind of role is fulfilled by copper. Our third experiment, which involved observation of sperm activity and migration in the

reproductive organs, was under taken in order to elucidate this problem.

Table 2 indicates that a reduction in number is evident with regard to sperm which penetrate beyond the Cu-IUD site. The number is especially small in the ovaries. In these data it could be assumed that a clue to understanding the contraceptive action of the Cu-IUD lies hidden.

Hefnawi et al. (1975) reported that sperm penetration and motility were greatly impaired in Cu-device users. They discussed in relation to the local action of copper on the cervical milieu effecting spermatozoal migration. A number of reports concerning sperm (Hefnawi et al. 1975; Ullmann and Hammerstein 1972; Kesserü and Camacho-Ortega 1972; Oster 1971, 1972) are being conducted from the human spermatozoal standpoint. However, perhaps because there are so many difficulties relating to storage of sperm, etc. in experiments dealing with small animals like rats, we don't see many reports along this line.

With the exception of one example in which the number of sperm in the ovaries was 0, we did note the presence of sperm there, although in greatly reduced numbers. Whether or not these remaining sperm possessed a fertilizing capacity becomes the next clue toward ascertaining the action of the Cu-IUD.

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