

Studies on Effect of the Intrauterine Device on the Embryonal Development and Transport in the Mouse

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INTRODUCTION

Since the early 1960's a number of investigators have concentrated their efforts to solve the mechanism of the intrauterine device(IUD) which is known as one of the contraceptive measures by blocking implantation and is widely applied by women who wish to control birth. However, no real function of the IUD has been manifested so far. Once Mastroianni and Rosseau (1965) reported that the fertilized eggs of monkey whose uterus was adapted by an IUD passed through the fallopian tubes faster than those of plain one, and they assumed that such a quick arrival of the eggs at the uterus might be a cause of failure to implant to the uterine wall because its structural changes have not been accomodated enough for the embryos. On the other hand, Kelly and Marston(1967) found that the transport of the monkey eggs was normal as seen in the control one, and they stated that the failure of the pregnancy of the monkey would be due to degeneration of the embryos caused by alteration of the uterine milieu or other reasons such as a premature expulsion of the embryos in the IUD uterus. Meanwhile, the investigations on effects of the IUD on the uterine endometrium, activities of the enzymes found in the uterine fluid and changes of the uterine tissues of the various animals have been conducted by many peoples(Brown-Grant; 1969,

Dizzia and Bo; 1969, Chang and Tatum; 1970, Mallikarjunesware; 1972, Chatterjee and Laumas; 1972, Pollak et al.; 1973, and Adadevoh and Dada; 1973).

Doyle and Margolis(1966) observed that the IUD in the mouse uterus slowed slightly down the tubal transport of the embryos, but no any effect on the fertilization or early embryonal development but only on the implantation. They also found that the effect of the IUD is bilateral in the mouse, instead of unilateral in the rat (Craig;1969). In other words, the IUD adapted to one side of the uterine horns gives its effect not only on the same side but also the other horn untreated with the device in the mouse, while in the rat the IUD effect remains its own horn. It also has been reported that the IUD gives the bilateral effect and blocks the fertilization as well in the ewe (Hawk; 1969, Spielman et al.; 1970, Brinsfield et al.;1971). Thus, the effect of the IUD is different by various species of animals, and it is supposed that the different kinds and the length of the foreign bodies might show different way of the effects. Though the IUD is popularly utilized by women nowadays, no body has proved its real function in connection with the contraceptive effect as yet. At this situation, the effect of the IUD on the transport in the fallopian tubes, development of the embryos or implantation to the uterus inserted by the foreign bodies have been investigated in the present studies in order to clarify its function for further

understanding on it.

MATERIALS AND METHODS

In the present studies, C3H/JMS strain of the fertile mice bred randomly in our laboratory were used. The upper portion (one third of the length) of the left horn of the mouse uterus was equipped by a piece of the silk thread (#1) through the uterine wall by means of a surgical needle (#1), and the right horn was remained empty only given with a slight injure for the control horn. At the time of the operation, the uterus was pulled out through slight opening made on the latero-dorsal sides of the trunk.

The experimental groups were divided into two; one for examination of the embryonal transport and the development, and the other for the implantation. On the 14th day after operation, the females were cohabitated with males, and those showing vaginal plug in the next morning were separated from the males for further examination. On day 2, 3 or 4 (from the day of production of the vaginal plug), the mouse was sacrificed by capital dislocation, and the fallopian tubes and the uteri of both sides were cut and washed in saline twice. The fallopian tubes were cut from the uterine horn at the uterotubal junction, and the tubes were cut again into three pieces. The embryos were flushed out from each piece of the tube and from the uterus by means of a capillary pipette, and number of the eggs was scored under a dissecting stereomicroscope. The eggs were fixed with acetic alcohol and stained by 0.5% lacmoid for the examination of the cell stages and the developmental condition. A part of the experimental groups was sacrificed on day 17 to observe the implantation.

RESULTS AND DISCUSSION

In the present studies, the developmental stages

of the embryos were divided into the six groups such as, 1-cell, 2-4-cell, 8-16-cell, morula, blastocyst and the abnormal embryos.

Table 1 showed number of the eggs in different stages recovered in different portion of the tubes. As the table showed, the majority of the eggs have cleaved to the 2-4-cell stage in the second day of the pregnancy, and they were found in the middle portion of the tube. Some of the eggs in the control already reached to the posterior portion of the tube while was found none of these in the IUD bearing side. This implies that the slowing down of the transport begins to appear at this stage of the embryos on Day 2. There was unexpectedly high frequency of the abnormal eggs such as fragmentation or the cytolysis during cleavage in the control side. 79% of the embryos proceeded to 8-16-cell stage in the third day of the pregnancy, and one fourth of the embryos was found in the uterus in the control side while 69% of those were at 8-16-cell stage, and only 8.2% of those arrived at the uterus in the IUD side. Abnormal development of the embryos increased in the IUD side twice as much as in the control and most of them still remained in the fallopian tube. On day 4, 74.8% of the embryos in the control and 61.1% of those in the IUD side developed to morula. 60.4% of the morula were found in the uterus of the control, while only 40.0% in the IUD horn. The difference of proportions between two is apparently significant statistically. More number of morulae in the fallopian tubes and less in the uterus in the IUD bearing horn than in the control showed delayed transport of the embryos in the IUD side. In the IUD horn was seen the increased number of the abnormal embryos most of which were still remained in the fallopian tube. The incidence of the abnormal embryos was different significantly between the control and the IUD horns. Conseruently,

Table 1. Effect of the intrauterine device on development and transport of the mouse embryos

Day 2

Treatment in uterine horn		1*		2~4		Abnormal		Total
		Ftm**	Ftp	Ftm	Ftp	Ftm	Ftp	
none	No. of eggs %	10 (5.4)	0	118(64.1)	12 (6.5)	37(20.1)	7 (3.8)	184
IUD	No. of eggs %	11 (7.0)	0	119(75.8)	0	27(17.2)	0	157

Test of significance: 2~4 Ftm; $X^2=5.42$, $p<0.03$

Day 3

Treatment in uterine horn		Ft 1		2~4		8~16		Abnormal		Total
		Ft	Ut	Ft	Ut	Ft	Ut	Ft	Ut	
none	No. of eggs %	8(6.3)	0	0	0	70(54.7)	31(24.2)	14(10.9)	5(3.9)	128
IUD	No. of eggs %	7(5.7)	0	2(1.6)	1(0.8)	74(60.7)	10 (8.2)	27(22.1)	1(0.8)	122

Test of significance: 8~16 Ut; $X^2=11.70$, $p<0.01$

Abnormal Ft; $X^2=5.71$, $p<0.02$

Day 4

Treatment in uterine horn		Morula		Blastocyst		Abnormal		Total
		Ft	Ut	Ft	Ut	Ft	Ut	
none	No. of eggs %	20(14.4)	84(60.4)	3 (2.1)	25(18.0)	2 (1.4)	5 (3.6)	139
IUD	No. of eggs %	19(21.1)	36(40.0)	4 (4.4)	17(18.9)	11(12.2)	3 (3.3)	90

Test of significance: Morula Ut; $X^2=9.17$, $p<0.01$

Abnormal Ft; $X^2=11.88$, $p<0.01$

* Cell stage of the embryo

** Place where the embryos were recovered. Ftm: Mid-portion of the fallopian tube, Ftp: Posterior portion of the tube, Ft: Fallopian tube, Ut: Uterus

the present studies showed gradual slow down of the embryonal transport and increase of the production of the abnormal embryos in the IUD facilitated uterine horn as the day passed. Total number of the embryos recovered on day 4 also decreased in the IUD side compared with that in the control one. It was expected that almost same number of the ovulation and the fertilization would be occurred in the left or right side of the reproductive organs. However, in the present studies, 139 embryos from the right horn; control side and only 90 from the left horn; IUD side were counted. Smaller number of the embryos in the IUD side might be due to more expulsion of the embryos during development than in the control because of the local

effect of the IUD which produces cellular debris, polymorphonuclear leucocytes or uterine infection some of which may act as a toxic factor to the embryos. It has been known that the embryos on the way from morula to blastocyst stage are in the most sensitive to the alteration of the environment (Chang and Tatum; 1970, Webb; 1973 for rat, and Bartke; 1968, 1970 and Marton and Kelly; 1969 for mouse) and such alteration would affect the morulae. Although it has been expected more number of the blastocysts than of the morula on day 4 if the animals are untreated with devices in the uterus, more than half of the total number of the embryos still remained at the morula stage in both uteri one of which was facilitated with the device. This

means that the effect of one side of the uterine horn bearing IUD would extend to the other side which is remained untreated, so the inhibition of the development appeared in the plain horn as well as in the IUD one. The results are consistent with those of Doyle and Margolis (1966) and Bartke(1968) who found that the unilateral IUD gave effect bilaterally in the mouse. That is, the slow transport and inhibition of the embryonal development were observed in the plain gonoduct like as in the IUD facilitated one. Viewing on the fact of more production of the abnormal embryos in the IUD horn, it might be understood that the IUD possibly blocked the smooth journey of the embryos down from the fallopian tube to the uterine lumen and gave fatal damage to them inside the tube. Same observation as of us has been done by Craig(1969) who found delay of the embryos in the tube, decreased number of the blastocysts in the uterus and increased number of the abnormal eggs in the IUD bearing uterine horn of the rat. Doyle and Margolis (1966) found that all embryos at blastocyst had already reached to the uterus in the control side while all remained only in the fallopian tube in the IUD equipped horn in day 4 mouse. Marston and Kelly(1969) stated that delay of the mouse embryos in the tube is caused mainly by the mechanical obstruction because the IUD was inserted in the upper portion of the uterus at the very near of the utero-tubal junction, by accumulation of the cellular debris or

by the uterine infection which possibly gives damage on the embryos.

On Table 2, average number of the normal and abnormal embryos per uterine horn on Day 2 through 4 are shown. In untreated horn number of the normal embryos were nearly homogeneous between different days (6.3 per horn), instead of gradual decrease of the number of the normal one in older days in the IUD bearing side (from 6.5 in day 2 to 3.8 per horn in Day 4). Meanwhile, the production of the abnormal embryos in the control side markedly decreased from 2.2 to 0.4 per horn on Day 2 to Day 4. However, in the IUD side the production of the abnormal embryos was not decreased as greatly as in the control one in the older days. Dizzia and Bo(1969), Brown-Grant (1969), Chatterjee and Laumas(1972) and Adadevoh and Dada(1973) also found almost similar results with rat to ours. Such a marked decrease of the number of the normal embryos in the IUD side might be due to the loss of the embryos caused by the insufficient environmental conditions such as production of the cell debris, or uterine infections as described by a number of investigators.

Fifteen mice, one side of whose uterine horn was facilitated with a silk thread, were survived for 17 days of the pregnancy in order to observe whether the embryos succeed to implant. Two of 15 uterine horns untreated showed the sites of the implantation leaving the trace of the absorption, but no sign suggesting implantation

Table 2. Average number of embryos per uterine horn in different days of pregnancy

Day	Number of mouse	Treatment in uterine horn	Number of normal embryos	Number of abnormal embryos	Total
2	20	none	7.0	2.2	9.2
		IUD	6.5	1.3	7.8
3	20	none	5.4	1.0	6.4
		IUD	4.7	1.4	6.1
4	20	none	6.6	0.4	7.0
		IUD	3.8	0.7	4.5

was detected from the other 15 opposite side of the uterus adapted by IUD. The present results are consistent with the findings of Bartke (1968) and Doyle and Margolis(1966) who observed few percent of the implantation sites in the control horn in the mouse. On the contrary, Craig(1969) reported that rat succeeded to develop embryos in the control horn without showing any damage even though the rat was equipped by an IUD on the other side of the uterus in which no any implantation was occurred. This implies that the rat apparently gives unilateral effect on the embryonal development while mouse does bilateral. A group of people(Dizzia and Bo; 1969, Brown-Grant; 1969, Chatterjee and Laumas; 1972 and Adadevoh and Dada; 1973) explained that the waste of the fertility in the IUD equipped animals is due to the fact that the uterus bearing IUD fails to form decidual reaction on or around the portion of the IUD site because of increasing in production of the estrogen stimulated enzyme activity which suppresses secretion of progesterone whose enough quantity is necessary to maintain the fetal development.

In conclusion of the present studies, it became apparent that the transport of the eggs in the gonoduct facilitated an IUD is delayed slightly. The effect of the IUD set in one sided of the uterine horns was extended to the opposite horn which was remained untreated. In other words, delay of the embryonal development, decrease number of the total embryos and disturbance of the implantation were observed in both uterine horns.

The reasons of the delay of the egg transport and development, of production of more number of the abnormal embryos, and of disturbance of the implantation in the mouse whose uterus is inserted by an IUD are remained uncertain, and more extensive studies on these subjects should be conducted for understanding the real function

of the IUD which is utilized among human beings widely.

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—국문초록—

생쥐의 자궁내 裝置가 胚兒發生 및 移動速度에 미치는 影響에 관한 研究

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생쥐(C3H/JMS)의 한쪽 자궁에 縫合系를 삽입한 후 初期胚兒의 發生過程, 移動速度 그리고 着床의 상태를 관찰하였다.

임신한지 2일째 되는 날에 자궁에 장치를 받지 않은 쪽에서는 初期胚의 일부가 이미 輸卵精의 뒷부분에 도달하고 있었으나 장치를 받은 쪽에서는 아직도 輸卵精 中部에 머물러 있었다. 3일째가 되면 對照가 되는 바른쪽 자궁에서는 8~16세포기에 드는 胚兒가 79%에 이르며 24%는 이미 자궁에까지 도달하고 있으나 장치를 한 쪽에서는 8~16세포기의 胚兒가 69%가 되며 겨우 8.2%만이 자궁에 이르고 있다. 4일째가 되면 對照子宮에서는 전체 胚兒의 75%가 그리고 장치 자궁에서는 61%가 桑實胚를 이루며 前者에서 60%, 後者에서는 40%가 자궁내에 진입하게 된다. 正常的인 생쥐에서는 임신 4일이 되면 거의가 胚囊期에 이르는 것으로 알려져 있으나 한쪽 자궁에 실을 장치한 생쥐에서는 對照子宮이거나 장치 자궁이거나 모두 胚囊의 形成이 늦어지고 있다.

한 자궁당 발견되는 正常胚의 平均値는 對照區에서 6.3정도로 임신 2일에서 4일 사이에 큰 차이가 없으나 處理區에서는 2일에서 4일 사이에 6.5에서 3.8로 감소한다. 異常胚는 對照區에서 2일째에 2.2였던 것이 0.4로 크게 줄지만 處理區에서는 對照區에서 처럼 급격한 감소를 볼 수 없다.

임신한지 17일 되는 날 對照區에서 15개의 자궁 가운데 단지 2개의 자궁만이 着床흔적을 보였지만 處理區에서는 전혀 着床되었던 흔적을 볼 수 없었다.

결국 생쥐에서는 한 쪽 자궁에 異物質을 삽입했을 때 장치를 받은 자궁뿐 아니라 다른 한 쪽 자궁에서도 着床이 일어나지 않을 뿐 아니라 異常卵子의 發生을 증가시키며 胚兒의 卵割速度가 늦어지고 또 胚兒의 移動이 늦어진다는 것을 알게 되었다.