INTRA-UTERINE DEVICE AND EMBRYONIC SURVIVAL IN THE RAT

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The mode of action of intra-uterine devices (IUDs) has been postulated by several investigators to involve a pharmacologically active substance capable of inhibiting the uterine decidual response or of actively cytolysing the pre-implantation blastocysts (Marston & Chang, 1965; Marston & Kelly, 1969; Doyle & Margolis, 1963; Batta & Chaudhury, 1968a, b; Parr, Schaedler & Hirsch, 1967). An investigation of the effect of uterine secretions on ovarian function and embryonic survival in the rat is reported here.

Rats (180 to 250 g; Holtzman Co.) were maintained as previously described (Anderson, Melampy & Chen, 1967). Daily vaginal smears were taken. Day 1 was characterized by the appearance of spermatozoa in mated animals. In the first series of experiments (Table 1), laparotomies were carried out under ether anaesthesia, using clean but not sterile technique, at all stages of the oestrous cycle. A silk thread (no. 5-0) was inserted midway in the right horn of the uterus according to the method of Parr *et al.* (1967). In the same animals, a passage was made with a scalpel (blade no. 11) between the uterine horns by incising the left side of the uterus, 2 mm anterior to the cervical os, through which a pair of iris scissors was inserted (Group A, Table 1). By cutting and puncturing the thin intercornual septum several times, a permanent supra-cervical passage was effected. This uterine incision was not sutured. In seven other animals (Group B, Table 1), this uterine passage was made, but no IUD was inserted in the right horn. Thirteen rats on which no operation was performed acted as controls (Group C, Table 1). All rats were mated, beginning 1 week after surgery. They were killed on Day 13 and the number of living and resorbing embryos was recorded. The patency of the interuterine passage was tested by injecting 1 ml saline into one horn. Only those animals in which saline passed readily into the opposite horn were included in the experimental group.

At autopsy, there were no embryos in the IUD-bearing (right) horn. However, the opposite horn in rats with a passage between the horns exhibited a highly significant ($P < 0.01$) reduction in the number of embryos (average 0.7; Table 1). The control animals contained a normal number of live embryos (average 6.6). Thus, an IUD in one uterine horn produced a drastic bilateral effect by reducing embryonic survival rates, presumably by action of some substance diffusing from the IUD horn to affect the other horn. Batta &

Chaudhury (1968b), Wood & Kirby (1968) and Marston & Kelly (1969) have also performed similar experiments, though with a larger and more traumatic uterine anastomosis, and have reached a similar conclusion. Wood & Kirby (1968) found that this factor would not diffuse through myometrial tissue but required luminal continuity. In the results presented here, however, there was a reduction ($P < 0.05$) in embryonic survival rates in rats which received sham-operations (Group B, Table 1) as compared with those on which no operation was performed (Group C). Other workers have reported similar findings (Batta & Chaudhury, 1968b; Marston & Kelly, 1969).

To determine if the horn with the IUD contains an active principle which reduced embryonic survival, the horn having the IUD was flushed and the

TABLE 1
BILATERAL EFFECT OF AN IUD ON EMBRYONIC SURVIVAL WHEN A PASSAGE EXISTS BETWEEN
UTERINE HORNS IN THE RAT

Group	Treatment	No. of rats	Living embryos in horn opposite IUD		Embryonic survival in contralateral (left) horn†	
			No.	Mean $\pm$ S.E.	Mean %	Mean arcsin and S.E.
A	IUD in right horn; passage between horns	8	6	0.7 $\pm$ 0.5	11.5	11 $\pm$ 4.6*
B	No IUD in either horn; passage between horns	7	33	4.7 $\pm$ 0.9	60.0	49 $\pm$ 10.0**
C	Unoperated controls	13	86	6.6 $\pm$ 0.6	83.5	68 $\pm$ 4.1

* Significant at 0.01 level by analysis of variance.

** Significant at 0.05 level by analysis of variance.

† Embryonic survival rate was calculated as: No. living embryos/no. corpora lutea $\times$ 100.

fluid collected from that horn was injected into the opposite, intact horn of the same rat on Day 3 or 4. For these experiments, 115 rats were randomly allotted to six groups (Table 2). An IUD was inserted into the right uterine horn of rats in Group 1, and no operation was performed on the animals in Groups 2 to 6. After three oestrous cycles, all rats were mated. On Day 3 or 4, rats in Groups 1 to 5, anaesthetized with ether, were subjected to laparotomy using aseptic techniques. In the rats in Group 1, the middle portion of the right horn, which contained the IUD, was occluded with small bulldog clamps, and 0.08 ml medium TC 199 (Difco) was injected. The same amount of fluid was injected into the lumen of the right horn, which had no IUD (Group 2). After flushing the medium six to eight times, 0.05 ml of it, now containing uterine fluid and debris, was withdrawn with a 23-gauge needle. This fluid was then injected through a 26-gauge needle into the opposite (left) horn at the tubo-uterine junction of the same animal (Groups 1 and 2). Rats in Group 3 received 0.05 ml TC 199 medium in the left horn. Uterine fluid (0.05 ml) was aspirated from oestrous females and injected into the left horn of mated rats at Day 3 or 4 (Group 4). The fresh uterine fluid was injected within 5 min of collection and its contents were not analysed. A 26-gauge hypodermic needle was inserted into

the uterine lumen of the left horn of the mated rats in Group 5 at laparotomy but no fluid or medium was injected. All operations were carried out between 12.00 and 17.00 hours on either Day 3 or 4. The rats in Group 6 received no operative treatment.

There was a reduction in embryonic survival by Day 13 in rats which received sham-operations on Day 4 (Group 5) as compared with those on which no operation was performed (Group 6; $P < 0.05$). Also, there was a significant difference ($P < 0.05$) in embryonic survival between animals after sham injection (Group 5) and after injection of fluid (Groups 2 and 3) on Day 4. Thus,

TABLE 2

EFFECT OF INTRA-UTERINE INJECTIONS OF UTERINE FLUIDS AND PHYSIOLOGICAL MEDIA ON EMBRYONIC SURVIVAL IN THE RAT

Group and treatment*	Day of injection	No. of rats	Living embryos in injected (left) horn		Embryonic survival in injected horn	
			Total no.	Mean $\pm$ S.E.	Mean %	Mean arcsin and S.E.
1. TC medium 199† flushings from horn with IUD	3	12	16	1.3 $\pm$ 0.7	23	15 $\pm$ 7.2
	4	10	0	0.0	0	
2. TC medium 199† flushings from intact horn	3	13	27	2.1 $\pm$ 0.7	41	34 $\pm$ 9.0
	4	10	4	0.4 $\pm$ 0.4	7	13 $\pm$ 11.7
3. TC medium 199†	3	10	19	1.9 $\pm$ 0.8	33	29 $\pm$ 10.7
	4	9	8	0.9 $\pm$ 0.3	10	13 $\pm$ 4.4
4. Uterine fluid from intact horn of oestrous females	3	9	21	2.7 $\pm$ 0.7	37	33 $\pm$ 8.8
	4	8	4	0.5 $\pm$ 0.3	8	11 $\pm$ 4.0
5. Laparotomy and insertion of hypodermic needle into uterine lumen	3	8	40	5.0 $\pm$ 1.0	78	67 $\pm$ 10.8
	4	11	21	2.0 $\pm$ 0.6	49	38 $\pm$ 9.4
6. Unoperated controls	—	15	86	5.9 $\pm$ 0.6	80	67 $\pm$ 3.6

* Total volume of fluid injected was 0.05 ml in each animal.

† Difco Laboratories, Detroit, Michigan, U.S.A.

injection of this quantity of fluid into the uterine lumen had an additional detrimental effect upon embryonic survival.

When the uterine fluid or TC 199 medium (Groups 1 to 4) was injected into the uterine lumen on Day 3, embryonic survival was reduced as compared with that in the rats of Group 5 ($P < 0.05$) or Group 6 ($P < 0.05$), but embryonic loss was not as extensive as that found at Day 4. Embryonic survival rates of rats given uterine fluid or TC 199 medium on Day 3 were similar in Groups 1 to 4 ($P > 0.05$). Thus, uterine fluid (Group 4), TC 199 medium (Group 3), uterine fluid from an intact horn (Group 2), and uterine fluid from a horn containing an IUD (Group 1) reduced embryo survival by a similar amount. These results are not in agreement with Batta & Chaudhury (1968a). There is a suggestion that the fluids obtained from the horn with the IUD (Group 1) on Day 3 contained anti-fertility activity (23% embryonic survival) as compared with the injection of fluid from horns not having an IUD (Group 2; 41% embryonic survival), though these differences were not statistically significant.

It appears, from the results of the first experiment (Table 1) and those of other workers, that an anti-fertility factor(s) originates in the IUD-bearing uterus and also that this substance diffuses from one uterine horn to another. Results from the second experiment (Table 2), however, involving an attempt to recover this factor from an IUD-bearing horn and to inject it into a normal horn, failed to give clear evidence of such a factor. Surgical manipulation may have masked the anti-fertility effect of the substances obtained from IUD-bearing uteri. McLaren (1968) has reported that 0.05 ml saline injected into the uterus of mice on Day 4 prevented blastocysts from surviving. Batta & Chaudhury (1968a) transferred uterine fluid from IUD-containing horns of oestrous rats into pregnant horns on Days 2 and 4, and they observed a sharp reduction in embryonic survival. However, transfer of uterine fluid from horns having no IUD had little effect on embryonic survival at these times. The discrepancy between the results reported here and those of Batta & Chaudhury (1968a) may be explained by differences in experimental techniques. In addition, autopsies were carried out on the rats of Batta & Chaudhury (1968a) on Day 10, a stage at which it is sometimes difficult to distinguish between viable and non-viable embryos, whereas the animals in experiments reported here were examined on Day 13, when substantial numbers of resorbing sites were found.

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