EFFECTS OF AN INTRA-UTERINE CONTRACEPTIVE DEVICE ON MITOSIS IN THE RAT UTERUS ON DIFFERENT DAYS OF PREGNANCY

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Summary. Changes in the pattern of cell division in the rat uterus with an intra-uterine silk thread suture in one horn were studied during the first 5 days of pregnancy.

There was no appreciable difference between the mitotic counts in the two horns on Days 1 to 4 of pregnancy, except that there was an increase in the luminal epithelial mitotic count on Day 4 of pregnancy in the horn with the device.

There was a marked difference in the luminal mitotic count between the two horns on Days 4 and 5 of pregnancy. While the mean mitotic count was 1.7 in the control horn on Day 5, it was 421 in the horn with the device. The diminution in the mitotic count seen on Day 5 of pregnancy in the control horn was not present in the horn with the device. It is possible that the effect of endogenous progesterone in causing diminished mitosis in the lumen was prevented by the presence of an IUD.

INTRODUCTION

Although the intra-uterine device is today being widely used for purposes of fertility control, its mode of action is not known. Studies carried out on experimental animals reveal that an intra-uterine silk thread suture in one horn of the uterus prevents implantation in that horn (Doyle & Margolis, 1963; Chaudhury, 1964). Chaudhury & Tarak (1965) have unequivocally demonstrated that the IUD in rats does not exert its antifertility effect by causing endocrine changes or by inhibiting sperm migration. In a further study on rats, Sudha, Chakaravarti & Chaudhury (1967) demonstrated that the antifertility effect was not mediated by inflammatory changes induced by the device. Recent studies have given some indication that the anti-implantation effect of an IUD in the rat may be caused by changes induced in the pharmacological properties of the intraluminal fluid of that horn of the uterus where it is placed and that the mechanism of action is a local effect in the uterus itself (Batta & Chaudhury, 1968a, b).

It is also possible that the IUD exerts its anti-implantation effect by altering the pattern of mitosis in the luminal epithelium of the rat uterus. It has already been demonstrated by Finn (1966) that oestrogen at doses higher than $0.1 \mu\text{g}$ renders the uterus insensitive to a decidual stimulus in the mouse and at this dose level, oestrogen also inhibits implantation. It is possible that an IUD in the rat uterus also acts in a similar manner and inhibits implantation by its effect on the patterns of mitosis in the luminal epithelium. Allen, Smith & Gardner (1937) have already demonstrated that oestrogen increased mitosis in the uterine epithelia of ovariectomized mice. Mathur & Chaudhury (1968) have demonstrated that an IUD in one horn of the rat uterus increased the mast cell count in that horn. This could again be reflecting an enhanced oestrogenicity.

In this investigation, the effect of an IUD on the mitotic counts of the luminal epithelium, glandular epithelium and stroma of the rat uterus has been studied on Days 1 to 5 of pregnancy. This has been compared with the counts in the contralateral uterine horn without an IUD, thus enabling one to have controls in the same animal.

MATERIAL AND METHODS

Albino rats of known fertility weighing 150 to 250 g were used. Laparotomies were carried out under ether anaesthesia on cycling females through a low abdominal incision. A silk thread suture was introduced into the right horn of the uterus, making certain that it was inserted through the lumen. After a rest of 10 to 15 days, females at the oestrous phase of the cycle were left overnight for mating with males of proven fertility. Pregnancy was confirmed by the presence of clumps of spermatozoa in the vaginal smear next morning. This was taken to be Day 1 of pregnancy. Colchicine at a dose of $0.1 \text{ mg}/50 \text{ g}$ was injected subcutaneously at 09.30 hours on Days 1, 2, 3, 4 and 5. The animals were killed 7 hr later. The upper, middle and lower portions of both the horns were fixed separately in Carnoy's fixative, which contained absolute alcohol, chloroform and acetic acid in the ratio of 6:3:1. Transverse sections (5μ) were cut and stained in haematoxylin and eosin. Three slides were prepared from each portion of the uterus. Each slide had sections with an interval of 50μ to exclude the possibility of including the same mitotic figures in different sections. Three sections on each slide were studied for the mitotic counts in the luminal epithelium, glandular epithelium and stroma. Luminal counts were taken as a whole for each section. Five glands were examined for counting the glandular mitoses and five fields were selected for counting the figures in the stroma. Thus, forty-five stromal fields for each portion of the uterus were studied under oil immersion at a magnification of $\times 700$. Groups of five animals were taken for each series of experiments. A total of twenty-five rats was used for this study. Since each animal was its own control and one horn of the uterus was compared with the other, the statistical analysis was carried out by the method of paired comparisons (Bailey, 1959).

RESULTS

The mean number of mitotic figures seen in both uterine horns on different

days of pregnancy is seen in Table 1. Each value is the mean of the results obtained in groups of five rats. There was no statistically significant difference between the mean mitotic counts in the upper, middle and lower portions of each horn.

TEXT-FIG. 1. Mean mitotic figures in the luminal epithelium (O), glandular epithelium (x) and stroma (□) of the control rat uterus from Days 1 to 5 of pregnancy.

TEXT-FIG. 2. Mean mitotic figures in the luminal epithelium (O), glandular epithelium (x) and stroma (□) of the rat uterus with an IUD from Days 1 to 5 of pregnancy.

Text-figure 1 shows the changes occurring in the mitotic counts of the luminal epithelium of the control horn on different days of pregnancy and also the

TABLE 1
MEAN MITOSIS IN LUMINAL EPITHELIUM, GLANDULAR EPITHELIUM AND STROMA IN DIFFERENT GROUPS OF PREGNANT RATS WITH AN IUD ON DAYS 1 TO 5 OF PREGNANCY

Tissue	Day 1			Day 2			Day 3			Day 4			Day 5		
	Control	IUD	P	Control	IUD	P	Control	IUD	P	Control	IUD	P	Control	IUD	P
Luminal epithelium	22.1 ± 21.8	46.6 ± 72.1	> 0.05	391.2 ± 159.9	301.6 ± 135.2	> 0.05	345.6 ± 148.5	405.4 ± 237.4	> 0.5	160.0 ± 164.4	397.4 ± 134.5	< 0.01	1.7 ± 2.5	421.0 ± 149.8	< 0.001
Glandular epithelium	12.7 ± 13.4	27.3 ± 42.8	> 0.5	133.6 ± 58.1	166.7 ± 59.1	> 0.05	80.4 ± 73.3	90.8 ± 36.5	> 0.5	9.0 ± 10.9	11.4 ± 10.7	> 0.05	1.2 ± 1.7	4.4 ± 4.2	> 0.05
Stroma	31.5 ± 13.9	47.6 ± 94.4	> 0.05	28.4 ± 27.0	31.4 ± 16.1	> 0.05	36.8 ± 39.8	100.9 ± 73.1	< 0.05	370.8 ± 121.7	431.5 ± 128.6	> 0.05	167.0 ± 77.1	315.4 ± 63.1	< 0.01

Each value represents the mean ± S.D. for five animals.

PLATE 1

FIG. 1. Microphotograph of a section of the uterine horn with an IUD on Day 5 of pregnancy. $\times 400$.

FIG. 2. Microphotograph of a section of the uterine horn without an IUD on Day 5 of pregnancy. $\times 400$.

changes in the glandular epithelium and stroma where the mitotic counts in the upper, middle and lower portions have been taken.

Text-figure 2 shows the results obtained for similar parameters in the horn with the IUD.

Text-figure 3 shows the comparison between the mean mitotic counts obtained in the luminal epithelium of both uterine horns on Days 1, 2, 3, 4 and 5 of pregnancy. The increased mitotic count on Day 5 of pregnancy in the horn with the device can be clearly seen.

Plate 1, Fig. 1 shows that the luminal epithelium of the uterine horn with the device contained numerous mitotic figures. Plate 1, Fig. 2 shows that, on the same day of pregnancy, mitosis was very much diminished in the contralateral horn.

TEXT-FIG. 3. Mean mitotic figures in the luminal epithelium of the control horn (—) and horn with the IUD (- - -) on Days 1 to 5 of pregnancy.

DISCUSSION

Changes in the luminal epithelium

Changes in the mitotic activity of the control horn were studied on different days of pregnancy. The mitotic counts on Day 1 of pregnancy ($22.1 \pm \text{S.D. } 21.8$) increased considerably on Days 2 and 3 to $391.2 \pm \text{S.D. } 159.9$ and $345.6 \pm \text{S.D. } 148.5$, respectively. These counts decreased gradually to $160.0 \pm \text{S.D. } 164.4$ on Day 4 and $1.7 \pm \text{S.D. } 2.5$ on Day 5 of pregnancy. The horn with an IUD showed similar changes in increased mitotic activity from 46.6 ± 72.1 on Day 1 to $301.6 \pm \text{S.D. } 135.2$ on Day 2 of pregnancy and this was maintained.

Changes in the glandular epithelium

There was a significant rise in the glandular epithelial mitotic count of the

control horn on Day 2 ($133.6 \pm \text{S.D. } 58.1$) and Day 3 ($80.4 \pm \text{S.D. } 73.3$) of pregnancy when compared to the figures of $12.7 \pm \text{S.D. } 13.4$ on Day 1 of pregnancy. The values decreased to $9.0 \pm \text{S.D. } 10.9$ and $1.2 \pm \text{S.D. } 1.7$ on Days 4 and 5 of pregnancy. A similar increase on Days 2 and 3 and a fall on Days 4 and 5 of pregnancy were also seen in the horn with the device.

Changes in the stroma

Stromal mitotic counts remained low in the control horn till Day 3 of pregnancy ($36.8 \pm \text{S.D. } 39.8$) and increased to $370.8 \pm \text{S.D. } 121.7$ on Day 4 of pregnancy. It again decreased to 167.0 ± 77.1 on Day 5 of pregnancy. In the horn with the device, however, the low mitotic count ($31.4 \pm \text{S.D. } 16.1$) on Day 2 of pregnancy increased to $100.9 \pm \text{S.D. } 73.1$ on Day 3. This increase was maintained on Days 4 ($431.5 \pm \text{S.D. } 128.6$) and 5 of pregnancy.

Similar results have been obtained during the first 5 days of pregnancy in the mouse by Finn & Martin (1967), who demonstrated many mitoses in the luminal and glandular epithelium on the 2nd and 3rd days of pregnancy and an abrupt reversal on the 4th and 5th days of pregnancy, when mitoses appeared to be confined to the stroma. In the present investigation also, the number of mitoses in the stroma increased on Days 4 and 5.

Comparison of the mitotic figures in the control and experimental horn on different days of pregnancy

The results clearly indicate the difference between the luminal epithelial mitotic figures in the control horn and the horn with the IUD on Days 4 and 5 of pregnancy. The mean luminal mitotic count in the control horn was $160.0 \pm \text{S.D. } 164.4$ on Day 4 of pregnancy, as against $397.4 \pm \text{S.D. } 134.5$ in the experimental horn. On Day 5 of pregnancy, the counts were $1.7 \pm \text{S.D. } 2.5$ and $421.0 \pm \text{S.D. } 149.8$, respectively, in the control and experimental horn. A similar decrease in the number of mitotic figures in the luminal epithelium of the mouse on Day 5 of pregnancy was observed by Finn & Martin (1967). The difference in the luminal epithelial counts between the two horns on Days 4 and 5 of pregnancy was statistically significant. It appears, therefore, that while there was no difference in the mitotic pattern of the control and experimental horns on Days 1, 2 and 3 of pregnancy, there was a significant difference on Days 4 and 5. This change appeared just before implantation and it is possible that it may be partly responsible for the antifertility effect of the device.

When the mean glandular mitotic counts in the control and experimental horns on different days of pregnancy were compared on Days 1 to 5 of pregnancy, no significant difference was seen.

The results obtained for stromal mitotic counts indicated no significant difference on Days 1 and 2 of pregnancy. The increased mitotic count in the stroma of the rat uterus with the IUD on Day 3 ($P < 0.05$) and Day 5 ($P < 0.01$) of pregnancy, but not on Day 4, is difficult to explain.

Martin & Finn (1968) have observed that, whereas oestradiol given alone to the mouse produced many mitoses in the luminal and glandular epithelia, treatment with progesterone reversed the pattern. They also observed that oestrogen induced more mitoses in the stroma but very few in the epithelia.

These investigators further noted that administration of progesterone greatly reduced the number of mitotic figures in the luminal epithelium. Similar studies have been carried out in the present experiments on rats. If this effect of progesterone is also present in rats, the fact that there was no decrease in the number of mitoses in the luminal epithelium on Day 5 of pregnancy in the horn with an IUD suggests that the presence of the device made the rat uterus unresponsive to the effect of progesterone. The IUD appears to act, therefore, by nature of an anti-progesterone effect. It would be interesting to observe whether administration of large doses of progesterone on Days 4 and 5 of pregnancy would decrease the number of mitotic figures seen in the luminal epithelium of the rat uterus with an IUD on those days. It is possible, therefore, that the IUD in rats may cause inhibition of implantation by an anti-progesterone effect on the luminal epithelium. In the presence of such large numbers of mitotic figures in the luminal epithelium, there would be no implantation just as there is very little or no decidual response when large doses of oestrogen are administered. It is interesting to note, however, that the effect of endogenous progesterone on stromal mitosis was not altered by the presence of the IUD.

Another possibility, however, is that the decrease in the mitosis in the luminal epithelium, most marked on Day 5 of pregnancy, is not related to the endogenous secretion of progesterone but to the implantation of the fertilized ova. If this is the cause of the decreased mitosis, then it is very possible that, since in the horn with an IUD there was no implantation, there was no decreased mitosis in the luminal epithelium. This can be investigated by transplanting fertilized ova to the horn with the device and studying the effect of this on the mitotic pattern.

A third possibility is that the uterotrophic effect of the IUD demonstrated by Parr & Segel (1966) was responsible for the increased mitosis in the horn with the device, and the reversal seen on Days 4 and 5 of pregnancy in the control horn was prevented because of this activity. This possibility does not, however, appear to be tenable as there was no increased mitosis in the luminal epithelium of the horn with the device on Day 1 of pregnancy.

NOTE ADDED IN PROOF

Studies carried out on groups of five rats to determine the mitotic activity of the uterus at the oestrous and di-oestrous phases of the cycle indicated that the mean luminal epithelial mitotic activity at di-oestrus ($173.1 \pm \text{S.D. } 93.47$) was significantly ($P > 0.01$) higher than the activity at oestrus ($38.4 \pm \text{S.D. } 17.64$). The results obtained in another series of experiments indicated that there was no significant difference in the luminal epithelial mitotic activity at either the oestrous or di-oestrous phase between the uterine horn with a device and the contralateral control horn.

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