

INTERRUPTION OF PREGNANCY IN THE RAT BY A UTERINE SUTURE

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Summary. A silk suture inserted into the uterine lumen prevented implantation in the rat. Normal embryos were present in the oviduct until Day 4 of pregnancy but were missing from the tract by Day 5. If the threaded uterus was ligated at the cervix at Day 4, degenerating ova were recovered the next day. This indicates that shortly after the ova entered the uterus they were usually expelled *per vaginam*.

A leucocytic infiltration of the endometrium was consistently observed in cornua from which embryos were missing. The inflammatory reaction was accentuated by anti-inflammatory compounds which stimulated myelopoiesis. Two months after thread insertion, the uterus showed the histologic modifications associated with a chronic inflammation, i.e. a proliferative rather than a marked exudative response.

The inflammatory reaction appeared to be essential for interruption of pregnancy since the absence of leucocytes was always correlated with implantation in the sutured cornu. The location of the thread determined whether a leucocytic reaction occurred. Implantation took place in the treated cornu if the thread had not penetrated into the lumen but instead was lodged in the stroma. This accounted for the presence of embryos in some of the threaded uteri despite the *in situ* location of the suture. Pregnancy continued when the thread was deliberately placed in the stroma, at some distance from the lumen.

INTRODUCTION

There has recently been renewed interest in the use of intra-uterine contraceptive devices (Nelson & Tietze, 1962), but the mechanisms by which these techniques work are unknown. A model possibly simulating their mode of action can be produced by inserting a silk suture into one uterine horn of the rat (Doyle & Margolis, 1963). This procedure prevents implantation in the treated cornu but not in the contralateral uterus. The current study confirms the results of Doyle & Margolis and sheds additional light on the mechanisms by which a uterine foreign body interrupts pregnancy in the rat.

MATERIAL AND METHODS

A total of seventy-five female Holtzman rats (175 to 250 g) was used in the experiments. A uterine foreign body was produced in cyclic animals in the following manner. Under ether anaesthesia, a ventral midline incision was made and the antimesometrial surface of the right uterus was exposed. A silk suture (size 6-0) was inserted into the uterine lumen and brought out approximately 5 mm above the initial site of entry. The free ends of the suture were then cut close to the uterus and knotted. Three to 5 days later, the females were caged with males and removed on the morning that spermatozoa were recovered in the vaginal smear (designated as Day 1 of pregnancy).

TABLE 1

EFFECT OF UNILATERAL INSERTION OF A SUTURE INTO A UTERINE HORN ON PREGNANCY

Female No.	No. embryos at laparotomy* in:		Treatment of sutured cornu at laparotomy	No. embryos at subsequent mating in:	
	Sutured cornu	Control cornu		Sutured cornu	Control cornu
1	0	8	Thread left <i>in situ</i>	0	5
2	0	6	Thread left <i>in situ</i>	0	6
3	0	7	Thread left <i>in situ</i>	0	6
4	0	8	Thread left <i>in situ</i>	0	8
5	0	9	—†	—†	—†
6	0	5	—†	—†	—†
7	0	5	—†	—†	—†
8	0	4	Thread removed	4	7
9	0	4	Thread removed	3	9
10	5	5	Thread removed	9	4
11	2	9	—†	—†	—†

* 8 to 10 days *post coitum*.

† Animal killed at time of laparotomy.

A second laparotomy was usually performed after 8 to 10 days *post coitum* and the number of embryos was counted in the treated and the contralateral uterine horns. Portions of both cornua were removed for histologic study from representative tracts. The specimens were fixed in Bouin's solution, embedded in paraffin, sectioned serially at 10 μ and stained with haematoxylin and eosin.

Another series of mated animals was killed on Days 2 to 5 *post coitum* and ova were flushed from the oviducts and cornua with a 26 gauge needle attached to a 1 ml syringe. Additional experiments are described in appropriate sections of the text.

RESULTS

Eleven animals were bred shortly after the insertion of a suture in one uterine horn; laparotomy at Days 8 to 10 *post coitum* revealed that nine rats had no embryos in the treated cornua whereas two did (Table 1). The embryos in the latter group were located between the utero-tubal junction and the thread (henceforth called 'above' the thread), a site in subsequent experiments where they were invariably found in treated horns containing embryos. The thread

was still *in situ* in tracts having embryos in the sutured cornu. At the time of laparotomy at Days 8 to 10 of pregnancy, the thread was either removed or left in the treated horn; the animals after delivery were remated (Table 1). When killed during the second pregnancy, embryos were present in the cornua from which the thread had been removed but were missing from tracts in which it was still present. A sham operation was performed in four rats by carrying out the regular operative procedure except that the thread was not left *in utero* (animals not included in Table 1). When the animals were killed 8 days *post coitum*, normal embryos were found in the sham operated as well as the untouched cornua.

The failure to find embryonic swellings in the majority of threaded uterine horns logically suggested a study of the morphology and distribution of pre-implantation ova. The results, summarized in Table 2, show that morulae were

TABLE 2
LOCATION OF OVA IN THE REPRODUCTIVE TRACT AFTER
THREAD INSERTION

Day of pregnancy*	Female No.	No. ova in		Location of ova
		Side sutured	Contralateral side	
2	1	4	5	Oviduct
4	2	3	3	Oviduct
	3	5	4	
	4	4	4	
5	5	0	9	Uterus
	6	0	3	
	7	0	3	
	8	0	4	
	9	0	5	
	10	6	3	

* Day of recovery of sperm was Day 1 of pregnancy.

present in both oviducts through Day 4 of pregnancy, thus indicating that fertilization and oviducal transport were unaffected by the uterine foreign body. However, on Day 5, blastocysts were recovered from the untreated cornua of six rats whereas in five out of six cases no ova were found in the threaded uterus or its oviduct. The one treated cornu in which six normal blastocysts were found showed significant histologic differences from the other threaded uteri (see below).

The failure to recover blastocysts from the treated uterus on Day 5 suggested that the ova were possibly being expelled *per vaginam* as a result of increased uterine motility. To test this possibility the uterus containing the foreign body was ligated at the cervical end on the morning of Day 4, when ova are still present in the oviduct. The animals were killed on Day 5 and the oviducts and cornua of the treated and untreated sides were then flushed to recover ova. The results fell into three categories (Table 3). *Degenerating ova* were recovered from

the treated cornua of five rats; there were variable numbers of leucocytes in the uterine washings. *Normal blastocysts* were recovered from two threaded uteri; there was no leucocytic infiltration of the uterine fluid contents. Ova were not recovered from three ligated tracts. A purulent exudate made it difficult to determine whether eggs were present. Attention was next directed to the endometrium to determine whether the presence of the thread modified uterine histology.

A consistent histologic finding in uteri in which the thread penetrated into the lumen was a leucocytic infiltration of the endometrium (Pl. 1, Fig. 1). This was found in unmated cyclic animals as well as in animals killed between Days 2 to 12 *post coitum*. Polymorphonuclear leucocytes of the neutrophil series were especially numerous in the uterine epithelium where they apparently accumulated before migrating into the lumen. Leucocytes were most frequent directly beneath the epithelium, but were also occasionally observed in the deep stroma.

TABLE 3
EFFECT OF LIGATING THE SUTURED CORNU AT DAY 4 ON SUBSEQUENT RECOVERY OF OVA

Female No.	Ova recovered at Day 5 from:		Condition of ova from sutured uterus
	Sutured cornu	Control cornu	
1	5	2	} Degenerating
2	2	2	
3	1	4	
4	5	4	
5	1	4	
6	5	4	} Normal blastocysts
7	5	4	
8	0	5	
9	0	3	} No eggs recovered but a purulent uterine exudate was present
10	0	2	

No decidual reaction was elicited by the thread since it was inserted several days before mating. White blood cells were not restricted to the vicinity of the thread. On the contrary, they were present at all levels of the uterine horn. In most treated tracts, the uterine glands appeared normal and did not contain leucocytes. In contrast to the threaded horns, those of the contralateral cornua were substantially normal (Pl. 1, Fig. 2).

The leucocytic infiltration noted in most of the threaded uteri prompted a series of experiments to determine whether an even greater response could be produced by treatment with anti-inflammatory compounds. Such agents might be expected further to increase the concentration of white blood cells by stimulating myelopoiesis in the bone marrow (Gordon, 1955). Accordingly, immediately after thread insertion four rats were injected subcutaneously daily with 10 mg of phenylbutazone (Butazolidin) in physiologic saline until killed at 8 to 10 days *post coitum*. None of the threaded cornua contained embryos whereas they were present in all of the contralateral uterine horns. The uteri containing

PLATE 1

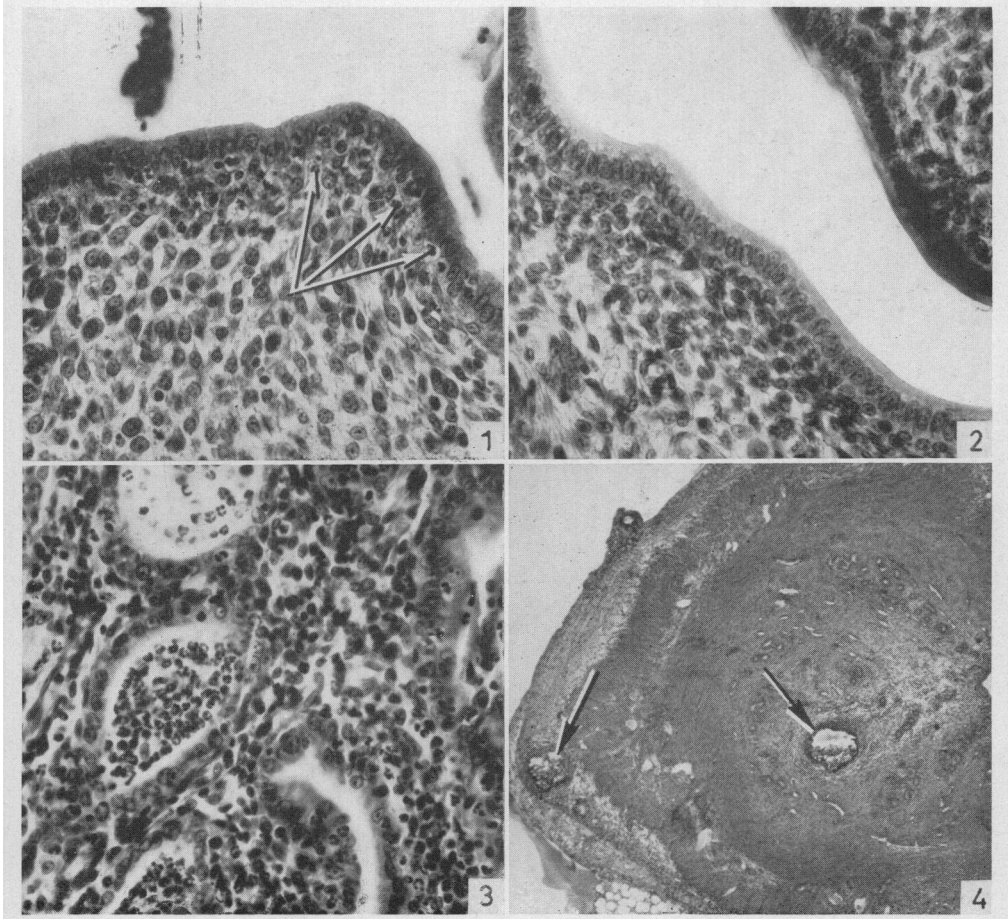


FIG. 1. Endometrium from threaded uterine horn. Note infiltration of polymorphonuclear leucocytes (indicated by arrows). $\times 480$.

FIG. 2. Endometrium from other cornu. Specimen was removed from region between two implantation sites. $\times 480$.

FIG. 3. Effect of treatment with phenylbutazone on the threaded cornu. The compound has accentuated the inflammatory response, especially in the uterine glands and stroma. $\times 480$.

FIG. 4. Example of a threaded uterus in which the suture did not reach the uterine lumen. The arrows indicate the position of the thread. Note the distance between the thread and the uterine lumen (far right of picture). $\times 42$.

the thread had an accentuated leucocytic response not only in the uterine epithelium and stroma but also in dilated uterine glands (Pl. 1, Fig. 3). The control cornua were not infiltrated with leucocytes. Two of four animals receiving daily injections of saline for 10 days after thread insertion had embryos in the threaded uterus above the site of the foreign body.

A similar increase in the number of leucocytes was observed in threaded uterine horns of animals treated with cortisone acetate. After thread insertion, nine animals were injected subcutaneously with 5 mg of cortisone acetate daily for 7 days. When killed at 9 days *post coitum*, there were no embryos in any of the treated cornua whereas embryos were present in all of the contralateral uteri (range: two to eight embryos). The uteri containing the foreign body showed the same histologic features noted in the phenylbutazone treated tracts. In all instances, the control cornua contained embryos, and the endometrium between the embryonic swellings was histologically normal.

The preceding experiments demonstrated a leucocytic reaction after the insertion of a silk suture; the response was further enhanced by treatment with anti-inflammatory compounds. Attention was next directed toward the use of a material which is even more inert than silk thread, i.e. a monofilament stainless steel wire. If the inflammatory response was responsible for loss of embryos, it was reasoned that substitution of wire for thread might increase the percentage of mated animals having embryos in both cornua. A stainless steel wire was therefore inserted into one uterine horn of five cyclic rats and the animals subsequently mated. At laparotomy, embryos were present in all of the untreated cornua (range: three to five embryos) but missing from three of the treated uteri. Each of the remaining cornua contained one embryo implanted above the level of the thread. Histologic examination of the treated uterine horns lacking embryos revealed a leucocytic infiltration of the endometrium to the same extent as tracts in which a silk suture had been inserted.

The endometritis caused by thread insertion might be expected to impair the decidual reaction produced by uterine trauma. To test this possibility, *both* uterine horns were traumatized along their entire length at Day 4 *post coitum* and the animals killed at Day 9. In three rats treated in this manner, the threaded uterine horn weighed on the average 399 mg, whereas the contralateral uteri weighed 936 mg. Histologic examination revealed a definite decidual reaction in the threaded uteri but to a much more limited extent than in the contralateral horns.

As previously mentioned, several threaded uteri contained embryos at the time of laparotomy (e.g. Table 1, Nos. 10 and 11). In almost every experiment, some of the treated cornua contained embryos although the thread was still *in situ*. These uteri differed histologically in several respects from the non-pregnant treated cornua. The endometrium was not infiltrated with leucocytes and, perhaps of greater significance, the inserted thread had not entered into the uterine lumen but instead was lodged in the stroma at some distance from the lumen (Pl. 1, Fig. 4). Although leucocytes were found surrounding the thread, the reaction was localized and none were migrating into the uterine lumen.

These observations suggested that variations in the depth of thread insertion might account for pregnancy in the treated cornua. This could be tested by

deliberately placing the thread in a superficial position in the stroma, thus avoiding its entry into the uterine lumen. Following this procedure, embryos were found in the threaded uteri of four animals (Table 4). Moreover, the inflammatory response was restricted to the area adjacent to the thread and elsewhere the endometrium was devoid of leucocytes.

All of the previous experiments were carried out as *acute* studies, with the rats usually killed at most 2 to 3 weeks after a uterine foreign body was established. It appeared likely that the inflammatory reaction induced by the foreign body was responsible for most of the functional derangements of the threaded uteri, e.g. loss of blastocysts at Day 5, cytolysis of ova trapped in the uterus by a cervical ligature, and the impaired decidual reaction. A chronic inflammation is normally characterized by a different histologic picture than the acute phase (Robbins, 1962). Therefore, it was of interest to determine what effects the thread would cause if it was left *in situ* for several months.

TABLE 4
EFFECT OF SUPERFICIAL INSERTION OF A
THREAD ON PREGNANCY

Female No.	No. embryos in:	
	Sutured cornu	Contralateral cornu
1	5	7
2	4	7
3	7	5
4	5	5

For these studies, females were isolated for at least 2 months after thread insertion before being caged with fertile males. Four animals were killed at Day 5 of pregnancy and the reproductive tract flushed to recover ova. Blastocysts were absent from all of the threaded uteri whereas normal ova were recovered from the contralateral cornua. Eleven rats were killed at 10 days *post coitum*; in eight tracts, no embryos had implanted in the threaded horn but the other uterus was pregnant (range of embryos: five to eight). In the remaining three tracts, embryos were present in the treated and control uteri, and in the former, as usual, they were located above the thread.

Uterine histology revealed the characteristic modifications produced by a chronic inflammation, i.e. a proliferative rather than an exudative response. The thread along its path through the stroma was surrounded by numerous plasma cells, lymphocytes and fibroblasts. Polymorphonuclear leucocytes were still entering the uterine lumen but in fewer numbers than during the acute phase. The uterine glands, located at the base of the stroma, contained pus and inspissated secretion and the surrounding epithelial cells were distorted and compressed. On the other hand, glands bordering on the uterine lumen contained no pus and had a normal cuboidal epithelium.

DISCUSSION

In confirmation of the results of Doyle & Margolis (1963), the present experiments demonstrate that a properly placed uterine suture prevents implantation in the rat. The former authors observed no significant histologic alterations in uterine tissue from the sutured areas. However, in the current study, a consistent polymorphonuclear leucocytic infiltration of the endometrium was a characteristic finding in sutured cornua lacking embryos. The acute inflammatory response was noted in tracts of cyclic females as well as of animals killed 2 to 17 days *post coitum*. Thus, it differed in duration from the transitory post-ovulatory influx of leucocytes into the uterine lumen which occurs in several species (for references see Yanagamachi & Chang, 1963). The leucocytic invasion was not limited to the area adjacent to the thread but rather involved the entire uterine horn. Furthermore, the inflammation was exacerbated by treatment with anti-inflammatory compounds which increase the production of leucocytes by bone marrow. In all instances, the contralateral uterine horn was histologically normal and pregnancy was unaffected.

An additional set of experiments involving strict aseptic procedures of sterile thread insertion in one uterine horn have been completed. When killed 8 to 10 days *post coitum*, embryos were present in all the untreated cornua of four rats whereas embryos were found in only one of the sutured cornua. Histologic observations revealed that the non-pregnant threaded uteri had a leucocytic infiltration of the endometrium. In contrast, the sutured uterus containing embryos was devoid of numerous white blood cells.

The inflammatory reaction appeared to be essential for the interruption of pregnancy since the absence of leucocytes was always correlated with implantation in the sutured cornu. The continued presence of the thread was necessary to establish a uterine reaction as shown in the series of sham operations. The necessity of the suture was further demonstrated by the fact that after its removal, implantation occurred at subsequent remating (Table 1). In agreement with these observations, Doyle & Margolis found that tracts from which the thread had been lost contained embryos.

The current study also indicated that the *location* of the thread is important in determining whether a leucocytic response is elicited. This was shown by the presence of embryos in some of the threaded cornua although the suture was still *in situ*. The common denominator in these animals was that the thread had not reached the uterine lumen but instead, at its deepest penetration, was embedded in the endometrial stroma. By deliberately inserting the thread superficially into the uterus, it was shown that pregnancy continued in the threaded uterus (Table 4), and that the uterine epithelium was not invaded by white blood cells.

Implantation, in varying numbers, of threaded uteri is probably attributable to the initial insertion of the suture at various stages of the oestrous cycle. During pro-oestrus and oestrus, the rat uterus is distended with fluid and the endometrium is consequently stretched and thinned whereas the uterine lumen is enlarged. On the other hand, during the late stages of dioestrus the fluid content of the uterus is drastically reduced and the relative proportion of

endometrial thickness to luminal size becomes approximately equal. Thus, random selection of cyclic animals may lead to greater difficulty in inserting the thread into the uterine lumen of animals operated on during dioestrus than the preovulatory period. Unfortunately, vaginal smears were not taken to determine the stage of the cycle at the time of thread insertion.

It is perhaps significant that none of the rats injected with phenylbutazone or cortisone acetate had embryos in the threaded uterus. The greater mobilization of leucocytes in the treated cornua after administration of anti-inflammatory compounds could mask the significance of the location of the thread *in utero*.

At present, I cannot explain why the thread has to enter the uterine lumen to produce an acute inflammatory response. It is possible that the infection induced by the foreign body becomes somehow localized and 'walled-off' when the thread is lodged in the stroma. The possible role of the uterine luminal flora in accentuating the inflammatory response cannot be overlooked. Recently, Fainstat (1963) showed that a decidual reaction did not develop in rats when a suture failed to reach the level of the luminal epithelium. It is unknown whether the induction of the decidual and inflammatory reactions share some common features.

The primary mechanism affected by thread insertion appears to be disruption of uterine transport of ova. Developing morulae were present in both oviducts on Day 4 of pregnancy, but were missing from the oviduct and cornu of the threaded side by Day 5 (Table 2). When the sutured uterus was ligated at the cervical end on Day 4, degenerating ova were recovered the next day (Table 3). This indicates that the ova are usually lost *per vaginam* as a result of increased motility of the treated cornu. In several instances, no ova were found in the ligated threaded uterus at Day 5. This could be due to improper flushing of the uterus. In other tracts, leucocytes were so numerous in the uterine washing that vision was obscured to the extent that ova—although present—might not have been detected. Acceleration of egg transport and subsequent loss of ova occurred in the chronically threaded uterus as well as in the acute phase. Doyle & Margolis also reported the absence of ova from the operated horn on the 5th day but they did not ligate the uterus at Day 4 to recover ova.

It is unlikely that hyperactivity of the threaded uterus results from hormonal factors in view of the normal egg transport in the contralateral uterus. The most plausible explanation is that myometrial activity is increased by inflammation, acting via increased neural stimulation of the uterus or by a myogenic response.

Ova recovered from almost all of the threaded ligated horns were undergoing cytolysis probably as a result of the proteolytic enzyme activity of the neutrophils as well as their phagocytic properties (Sieracki, 1955). Ligating the uterus *per se* did not necessarily destroy the eggs as shown by the recovery of normal blastocysts at Day 5 from two threaded uteri in which an inflammatory reaction did not develop (Table 3, females 6 and 7). The reduced decidual response of the threaded uterus may also be attributed to the endometritis caused by the foreign body. Both the ovicidal effect and impaired decidualization appear to be secondary to the expulsion of ova from the reproductive tract.

Intrauterine devices in the human may have entirely different modes of

action than in the rat. For one thing, the Graefenberg ring lies free in the uterus and is not embedded in the endometrium. It is of interest, however, that in a series of 109 patients, 10.1% of the endometrial biopsies examined had microscopic evidence of inflammation (Jessen, Lane & Greene, 1963). It remains to be established whether intrauterine foreign bodies in the human also lead to the loss of ova *per vaginam* as a pre-implantation phenomenon.

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REFERENCES

- DOYLE, L. L. & MARGOLIS, A. J. (1963) Intrauterine foreign body: effect on pregnancy in the rat. *Science*, **139**, 833.
- FAINSTAT, T. (1963) Extracellular studies of uterus. I. Disappearance of the discrete collagen bundles in endometrial stroma during various reproductive states in the rat. *Amer. J. Anat.* **112**, 337.
- GORDON, A. S. (1955) Some aspects of hormonal influences upon the leukocytes. In *Leukocytic Functions*. Ed. R. W. Minor. *Ann. N.Y. Acad. Sci.* **59**, 907.
- JESSEN, D. A., LANE, R. E. & GREENE, R. R. (1963) Intrauterine foreign body. *Amer. J. Obstet. Gynec.* **85**, 1023.
- NELSON, W. O. & TIETZE, C. (1962) *Intrauterine contraceptive devices*. Preliminary Report. Issued by The Population Council.
- ROBBINS, S. L. (1962) *Textbook of pathology with clinical implications*, 2nd edn. Saunders, Philadelphia.
- SIERACKI, J. C. (1955) The neutrophilic leukocyte. In *Leukocytic Functions*. Ed. R. W. Minor. *Ann. N.Y. Acad. Sci.* **59**, 690.
- YANAGAMACHI, R. & CHANG, M. C. (1963) Infiltration of leucocytes into the uterine lumen of the golden hamster during the oestrous cycle and following mating. *J. Reprod. Fertil.* **5**, 389.