Infertility in mice infected genitally with a human strain of *Chlamydia trachomatis*

M. Tuffrey, P. Falder, J. Gale*, R. Quinn and D. Taylor-Robinson

Division of Sexually Transmitted Diseases, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

Summary. Progesterone-treated C3H and TO mice were inoculated genitally with a human *C. trachomatis* strain, serovar E, designated N.I.1 or with 2SP control medium. Of the C3H mice serving as controls 93% had litters by the end of a 6-month period compared to 31% of mice infected with chlamydiae. This infertility could not be explained by tubal occlusion, since the oviducts appeared normal at autopsy. Some of the mice were induced to superovulate. Eggs were never recovered from the oviducts on the inoculated sides of infertile mice although they were sometimes found in the lumen of the uninoculated oviducts. In contrast, eggs were recovered routinely from both oviducts of control mice. In addition, eggs and/or their accompanying cumulus cells could be seen in the periovarial space of mice inoculated with chlamydiae, indicating a failure of the transportation of eggs to the oviduct. This could explain the high incidence of ectopic pregnancies in women after chlamydial infection.

No adverse effect on fertility was seen in TO mice inoculated genitally with strain N.I.1. Of the mice given 2SP medium, 73% had litters, but 87% of the mice inoculated with chlamydiae were also fertile. There was, however, a significantly greater variation in the birth weights of mice born to infected TO mothers than those born to control mice. This difference in the susceptibility of mouse strains suggests that a genetic predisposition should also be considered for man.

Introduction

Salpingitis caused by *Chlamydia trachomatis* in women may lead to infertility and an increased risk of ectopic pregnancy (Sweet, 1982). Obstruction of the Fallopian tubes is the most common cause of sterility in women, and tubal occlusion is a recognized mechanism for infertility occurring as a sequel to chlamydial salpingitis. However, the problem may be more insidious because serological evidence has suggested that subclinical chlamydial infections may be a significant factor in tubal infertility (Jones, Ardery, Hui & Cleary, 1982; Brunham, Maclean, Binns & Peeling, 1985).

We have developed a mouse model of chlamydial salpingitis by inoculating human strains of *C. trachomatis* into the genital tract (Tuffrey, Falder, Gale & Taylor-Robinson, 1986). This has enabled us to design experiments to study the effect of chlamydial genital-tract infection on reproductive capacity and egg transportation, the results of which are presented in this communication.

Materials and Methods

Mice. Syngeneic C3H mice, 6–8 weeks old, either bred and maintained in the specific pathogen-free (SPF) unit at the Clinical Research Centre (CRC) or obtained from OLAC, were used in the first two experiments. Outbred TO mice from the SPF unit at the CRC were used in a third experiment.

* Present address: Department of Epidemiology, University of Washington, Seattle, WA 98195, U.S.A.

C. trachomatis strain. This strain, of serovar E, designated N.I.1, had been isolated from the endocervix of a contact of a man with non-gonococcal urethritis. It was passed serially in McCoy cells and chlamydiae from the 4th, 5th and 9th passes were suspended in sucrose-phosphate (2SP) medium containing 10% fetal calf serum and stored in liquid nitrogen for use in these experiments. Chlamydiae of these passes were known to cause salpingitis in mice (Tuffrey *et al.*, 1986).

Infection of mice. Progesterone treatment is known to enhance infection with chlamydiae (Tuffrey & Taylor-Robinson, 1981) and so all the mice were given a single dose of 2.5 mg progestagen (Depo-Provera, Upjohn) subcutaneously 6 days before inoculation with strain N.I.1 or Medium 2SP. The animals were anaesthetized and the inoculum was introduced either into the right uterine horn (intrauterine) or under the right ovarian bursa (intrabursal) and thus via an oviduct. Mice were given 10^5 inclusion-forming units (i.f.u.) of strain N.I.1 by the intrauterine route and 10^4 i.f.u. by the intrabursal route. These operative techniques have been described previously (Tuffrey *et al.*, 1986). On Day 14 after inoculation, i.e. 20 days after progesterone had been given, the mice were placed with fertile males. This schedule was selected after the results of preliminary experiments with various doses of progesterone had shown that this dose stopped the oestrous cycle long enough for the infection to become established and then allowed the cycle to restart during the infection so that mating could occur. The first vaginal plugs were detected 2 days after placing with males, confirming that the oestrous cycle had recommenced, and vaginal swabs taken just before this time indicated that 29 of 32 inoculated mice were infected at least on one occasion.

Detection of chlamydiae. Vaginal swabs were taken on Days 5 and 13 after inoculation. If the samples were not chlamydia-positive, often the case for mice inoculated by the intrabursal route, they were swabbed again on Days 19 or 22. Thus, only a single swabbing was undertaken after placing with the males in case this procedure interfered with the occurrence of pregnancy. Each swab was expressed in 1 ml of cold Medium 2SP. This was stored in liquid nitrogen until inoculation into cycloheximide-treated McCoy cells for the isolation of chlamydiae, as described previously (Thomas, Evans, Hutchinson & Taylor-Robinson, 1977).

Detection of eggs. Pituitary gonadotrophins administered to adult female mice irrespective of their oestrous phase cause superovulation, and, if placed with a male, mating and implantation. Some of the infected mice and 10 uninfected age-matched C3H mice were induced to superovulate at the end of the experiments to test ovarian function and to study the transportation of eggs through the oviducts. Mice were given 5 i.u. PMSG (Folligon: Intervet, Cambridge, U.K.) intraperitoneally, followed by 5 i.u. hCG (Chorulon: Intervet) about 45 h later. Eggs were sought in the oviducts on the following 2 days. The mice were killed and clumps of eggs could be seen in the oviduct surrounded by cumulus oophorus cells. The oviduct was dissected out and placed in a watch glass in phosphate-buffered saline. Under a dissecting microscope the length of the oviduct was scraped gently with a small scalpel and the contents of the dish were swirled and allowed to settle. Eggs were observed in the bottom of the dish and could be collected with a fine glass Pasteur pipette.

To confirm the developmental capacity of the eggs and to observe their further transport down the oviduct and into the uterus, the animals were placed with a male at the time they were given hCG. Vaginal plugs were sought the following morning and mice that had mated were killed over the next 3 or 4 days. By the 3rd day, fertile eggs should be at the early blastocyst stage and entering the uterus.

Histology. Some mice were killed for histological examination at the end of the experiment. The reproductive tract was dissected out on both sides and ovaries, oviducts and pieces of uterus were fixed in 10% buffered formalin or Bouin's solution before sections were cut (4 μ m thick) through various levels of the paraffin-wax blocks and stained with haematoxylin and eosin. Pieces of oviduct were also examined histologically after collection of blastocysts from the uterus.

Results

C3H (CRC) mice inoculated with Medium 2SP or strain N.I.1 p4 (Exp. 1)

Mice inoculated by the intrauterine route. As shown in Tables 1 and 2, all 6 mice inoculated with Medium 2SP became pregnant during the study period of 5 months and some gave birth to second, third and fourth litters. One mouse was killed while pregnant for the 3rd time and 2 while pregnant for the 4th time, fetuses being found in both uterine horns of each. Two mice that had 3 litters each were killed while not pregnant and appeared normal histologically, while the mouse (No. 1; Table 3) that only had a single litter was induced to superovulate and eggs were found in both oviducts at autopsy 2 days later.

In contrast, only 1 of 4 mice that had been inoculated with N.I.1, all of which were chlamydia-positive, proved fertile over the same length of time (Tables 1 and 2), giving birth to 2 litters. It was superovulated at the end of the experiment and killed 2 days later, at which time eggs were found in the oviduct on the uninoculated side but not on the inoculated side (mouse No. 4; Table 3). The remaining 3 infertile mice (Nos 1–3, Table 3) were treated in the same way but no eggs were found in either oviduct of any of them despite the presence of corpora lutea in all ovaries.

Table 1. Pregnancy rate over 5 months for C3H mice inoculated genitally with Medium 2SP or *C. trachomatis* strain N.I.1

Inoculum	Route of inoculation	No. of mice pregnant/ no. of mice inoculated	
		Exp. 1	Exp. 2
Medium 2SP	Intrauterine	6/6 (100%)	6/7 (86%)
N.I.1	Intrauterine	1/4 (25%)	3/12 (25%)
Medium 2SP	Intrabursal	7/8 (88%)	6/6 (100%)
N.I.1	Intrabursal	2/5 (40%)	4/11 (32%)

Table 2. Number of pregnancies and litter sizes in C3H mice inoculated genitally with Medium 2SP or *C. trachomatis* strain N.I.1

Inoculum	Route of inoculation	Experiment 1			Experiment 2		
		No. of mice in group	No. of mice pregnant (and parity)	No. of young (range) in litter	No. of mice in group	No. of mice pregnant (and parity)	No. of young (range) in litter
Medium 2SP	Intrauterine	6	6 (1st) 5 (2nd) 4 (3rd) 2 (4th)	2–6* 4–6* 5–10 8	7	6 (1st) 2 (2nd) 1 (3rd)	1–6 4, 6 2
N.I.1	Intrauterine	4	1 (1st) 1 (2nd)	2 4	12	3 (1st) 2 (2nd)	2–6 4, 8
Medium 2SP	Intrabursal	8	7 (1st) 7 (2nd) 6 (3rd) 1 (4th)	1–6* 2–7 4–12 2	6	6 (1st) 2 (2nd)	1–7 5
N.I.1	Intrabursal	5	2 (1st) 2 (2nd)	5, 5 4, 7	11	4 (1st) 1 (2nd)	2–4 4

*Signs of cannibalism amongst these litters; litter sizes could have been larger.

Mice inoculated by the intrabursal route. Of 8 mice inoculated with Medium 2SP, 7 became pregnant (Table 1) on one or more occasions (Table 2). Five of the pregnant mice were killed and had implantations in both uterine horns. One mouse was killed while non-pregnant and the reproductive tract found to be normal. The remaining animal (No. 4; Table 4), which did not become pregnant, was induced to superovulate and eggs were found in both oviducts.

In contrast, only 2 of 5 mice inoculated with N.I.1 became pregnant. Chlamydiae were re-isolated from vaginal swabs taken from all these mice 19 days after inoculation. The 2 fertile animals were killed at the end of the study when they were pregnant for the second time and both had unilateral implantations on the uninoculated side. One of them had 7 fetuses and the other had 4 fetuses. Of the 3 mice still infertile after 5 months, 2 were killed and the oviducts in both appeared normal histologically. The remaining infertile mouse (No. 4; Table 4) was induced to superovulate but, despite the presence of corpora lutea in the ovaries, no eggs were found in the oviducts or uterus.

C3H (OLAC) mice inoculated with Medium 2SP or strain N.I.1 p9 (Exp. 2)

Mice inoculated by the intrauterine route. As shown in Tables 1 and 2, 6 of 7 mice inoculated with Medium 2SP gave birth to 1–3 litters during the study period of 6 months. The infertile mouse (No. 7; Table 3) was induced to superovulate before autopsy, when corpora lutea were seen in the ovary of the uninoculated side and eggs were found in the ipsilateral oviduct. The ovary on the inoculated side was necrotic and no corpora lutea or eggs were seen. Two of the fertile mice (Nos 8 and 10; Table 3) were also induced to superovulate and eggs were recovered from each of the 4 oviducts.

In contrast, only 3 of 12 mice inoculated with N.I.1 proved fertile over the same study period (Tables 1 and 2). Two of these mice (Nos 8 and 10; Table 3) each gave birth to 2 litters and at autopsy eggs were recovered from both oviducts after superovulation. A few chlamydiae were isolated from the vagina of one of these mice (No. 8) 22 days after inoculation but never from the other mouse. The remaining fertile mouse (No. 5; Table 3) was chlamydia-positive 5 days after inoculation and delivered its only litter 3 weeks after being placed with a male. However, when killed 5 months later, after superovulation, mating and detection of a vaginal plug, blastocysts were not found in either uterine horn, despite the fact that corpora lutea were present in the ovaries and the oviducts appeared normal histologically. The 9 mice that were infertile were also induced to superovulate before being killed and observations on the recovery of eggs and the histological findings are summarized in Table 3. Corpora lutea were present in both ovaries of all the mice except one. Ovaries and associated oviducts were dissected out from 3 of these mice (Nos 9, 11 and 12; Table 3) killed the day after hCG injection, when eggs and surrounding cumulus cells are normally in the ampulla, and histological sections were prepared from the complete specimens. The sections from one mouse were unsatisfactory for examination, but those from the other two appeared normal histologically. The ampullae from both mice were empty but follicular cumulus cells were found in the periovarial space on both sides in one mouse (No. 9; Table 3). In contrast, in two hormone-treated mice from the group given Medium 2SP by the intrauterine route, killed at the same time, eggs and cumulus cells were seen in the ampullae on both sides of one mouse and cumulus cells were detected in the ampulla on the uninoculated side of the other (Nos 13 and 12; Table 3).

Both oviducts from 5 (Nos 1–4 and 7; Table 3) of the 6 remaining superovulated infertile animals were washed out 1 and 2 days after hCG treatment but eggs were not recovered. The 6th mouse (No. 6; Table 3) was placed with a male at the time of hCG treatment and a vaginal plug was detected the following day. The mouse was killed 3 days later and 5 blastocysts were recovered from the uterine horn on the uninoculated side only.

Mice inoculated by the intrabursal route. All 6 mice inoculated with Medium 2SP were fertile (Table 1) and 3 had second pregnancies (Table 2). One mouse (No. 2; Table 4) killed 2 days after superovulation and mating had blastocysts in both uterine horns.

Table 3. Recovery of eggs from superovulated C3H mice inoculated by the intrauterine route with Medium 2SP, or strain N.I.1 or from untreated mice

Mouse		Method of observation	Recovery of eggs from oviduct or uterus after superovulation		Histological findings	
			Inoculated side	Uninoculated side	Ovaries	Oviducts
Fertile 2SP (Exp. 1)	No. 1	W	+	+	nt	nt
Fertile N.I.1 (Exp. 1)	No. 4	W	—	+	nt	nt
Infertile N.I.1 (Exp. 1)	No. 1	W	—	—	nt	nt
	No. 2	W	—	—	nt	nt
	No. 3	W	—	—	N	nt
Infertile 2SP (Exp. 2)	No. 7	W	—	+	Necrotic inoc. side	N
Fertile 2SP (Exp. 2)	No. 8	W	+	+	nt	nt
	No. 10	W	+	+	nt	nt
	No. 13	H	+	+	N	N
	No. 12	H	—	Cells in ampulla	N	N
Fertile N.I.1 (Exp. 2)	No. 8	W	+	+	nt	nt
	No. 10*	W	+	+	nt	nt
	No. 5	W	—	—	Cystic both sides but corpora lutea present	N
Infertile N.I.1 (Exp. 2)	No. 9	H	— (cells P/S)	— (cells P/S)	N	N
	No. 11	H	Unsatisfactory sections		N cystic inoc. side	nt
	No. 12	H	—	—	N cystic inoc. side	N
	No. 1	W	—	—	No corpora lutea	nt
	No. 2	W	—	—	nt	nt
	No. 3	W	—	—	nt	nt
	No. 4	W	—	—	nt	nt
	No. 7	W	—	—	N	nt
	No. 6	W	—	+	N	N
				(5 blasts)		
Untreated	Nos 1–10	W	+	+	nt	nt

* Chlamydiae not isolated on any occasion.

W = eggs washed from oviduct or uterus.

H = eggs observed in histological sections.

N = normal histology; functional ovary. Corpora lutea present in all ovaries.

nt = not tested.

cells P/S = freshly ovulated cumulus cells in periovarial space.

blasts = blastocysts recovered from uterus.

In contrast, only 4 of 11 mice inoculated with N.I.1 p9 were fertile (Table 1), one giving birth to a second litter (Table 2). These 4 mice were induced to superovulate before autopsy and corpora lutea were detected in all ovaries. One of them (No. 2; Table 4) was killed 2 days after hCG treatment and 4 eggs were recovered from the oviduct on the uninoculated side. None was found on the inoculated side. The other 3 mice (Nos 1, 4 and 5; Table 4) were mated successfully and 1 or 2 blastocysts were recovered from each of the uninoculated uterine horns. None was found on the

Table 4. Recovery of eggs from superovulated C3H mice inoculated by the intrabursal route with Medium 2SP or strain N.I.1 or from untreated mice

Mouse		Method of observation	Recovery of ova from oviduct or uterus after superovulation		Histological findings	
			Inoculated side	Uninoculated side	Ovaries	Oviducts
Infertile 2SP (Exp. 1)	No. 4	W	+	+	nt	nt
Infertile N.I.1 (Exp. 1)	No. 4	W	—	—	nt	nt
Fertile 2SP (Exp. 2)	No. 2	W	+ (4 blasts)	+ (3 blasts)	N	N
	No. 4	H	+ (and cells)	Cells in ampulla	N	N
	No. 6	H	+ (and cells)	Cells in ampulla	N	N
Fertile N.I.1 (Exp. 2)	No. 2	W	—	+	nt	nt
	No. 1*	W	—	+ (2 blasts)	N	N
	No. 4	W	—	+ (1 blast)	N	N uninoc. side Hydro inoc. side
	No. 5	W	—	+ (1 blast)	N	N
	No. 6	W	—	+	nt	nt
Infertile N.I.1 (Exp. 2)	No. 3*	W	—	—	nt	nt
	No. 7	W	—	—	N	nt
	No. 8	H	—	— (ova and cells P/S)	N	N
	No. 9	H	— (cells P/S)	— (ova and cells P/S)	N	N
	No. 10	H	— (cells P/S)	—	N (cystic inoc. side)	N
Untreated	Nos 1–10	W	+	+	nt	nt

Footnotes as for Table 3; Hydro = hydrosalpinx.

* Chlamydiae not isolated on any occasion.

inoculated side. Of the 7 infertile mice in this group, 6 were induced to superovulate and killed 1 or 2 days after hCG treatment. The oviducts were removed from 3 of these mice and 4 eggs were recovered from the uninoculated side of one of them (No. 6; Table 4). However, eggs were not recovered from the inoculated side of this mouse, or from either side of the other 2 mice (Nos 3 and 7; Table 4). The ovaries and attached oviducts were dissected from the other 3 mice and newly liberated eggs and cumulus cells were sought in histological sections. Ova and surrounding cumulus cells were seen in the periovarial space on the uninoculated side of 2 of the mice (Nos 8 and 9; Table 4), while cumulus cells alone, also indicative of ovulation, were seen in the periovarial space on the inoculated side of one of them (No. 9; Table 4) and also on the inoculated side of the third mouse (No. 10; Table 4). None of the oviducts was overtly blocked or showed histological signs of damage. Two of the mice given Medium 2SP by the intrabursal route were treated in the same way and eggs and cumulus cells were found in the ampullae of both of them on the inoculated side (Fig. 1) and cumulus cells were present in the ampullae of both on the uninoculated side (Nos 4 and 6; Table 4). Follicular cells were not found in the periovarial spaces. The remaining infertile mouse in this group which did not receive hormone treatment, and a normal C3H mouse, were killed and the ampullae examined by scanning electron microscopy. There were no apparent differences in the ciliated epithelia of the infundibuli of these mice.

Fig. 1. Section through the ampulla from a 2SP-inoculated control C3H mouse a day after superovulation (hCG) in which eggs and cumulus cells are seen within the oviducal lumen. $\times 275$.

TO mice inoculated with Medium 2SP or strain N.I.1 p5

Fifteen mice were inoculated with Medium 2SP and 15 with 5×10^6 i.f.u. of chlamydiae, both groups by the intrauterine route. At 7 days after inoculation of chlamydiae, they were isolated from the vagina of 13 animals. As in the previous experiments, males were placed with females 14 days after inoculation but the breeding performance of these mice was followed for only 3 months since those in both groups became pregnant. Eleven (73%) of the mice given Medium 2SP had litters and 5 gave birth to second litters during the period of investigation, the number of fetuses in the litters ranging from 3 to 11. Of the mice given chlamydiae, 13 (87%) also delivered litters, 5 of them having second litters and 1 having a third, the number of fetuses in the litters ranging from 4 to 13. Three of the infected mice were killed while pregnant with a second litter and fetuses were distributed in both uterine horns.

The offspring of the infected and control mice were weighed at birth to see whether infection had exerted an effect. Pregnant mice were allowed to litter on a raised mesh grid so that the young dropped through to the bottom of the cage and were, therefore, prevented from sucking before being weighed. Care was taken to weigh the offspring directly after birth. Litters from 8 infected mothers and from 6 controls were weighed. There was a significantly greater variation in the birth weights of young born to infected mice (1.23 ± 0.14 to 1.73 ± 0.06 g; mean $\pm$ s.d. 1.475 ± 0.179 g) than of those born to control mice (1.31 ± 0.09 to 1.45 ± 0.07 g; mean $\pm$ s.d. 1.38 ± 0.057 g) (F test: $P = 0.01$). There was, however, no difference in litter sizes.

Discussion

Chlamydial salpingitis has been induced in grivet monkeys (Ripa, Møller, Mårdh, Freundt & Melsen, 1979; Møller & Mårdh, 1980), pig-tailed macaques (Patton *et al.*, 1982a) and rabbits

(Patton, Halbert & Wang, 1982b) as well as mice (Swenson, Donegan & Schachter, 1983), although subsequent infertility has been described only for mice. Swenson & Schachter (1984) demonstrated infertility as a consequence of infection of the upper genital tract with the mouse pneumonitis (MoPn) strain of *C. trachomatis*, although in most of their experiments the mice were infected and killed before term at a time when inflammatory exudates were present in the oviducal lumen. Fertility was assessed by the numbers of implanted fetuses. However, infertility was observed also in mice mated 36 days after intrauterine inoculation with the MoPn strain, when organisms could no longer be recovered from the genital tract and acute inflammatory cells had ceased to occlude the oviducts. Although areas of focal dilatation were observed in the oviducts of 8 of 12 mice, the remainder appeared normal and led these workers to remark that "chlamydial infection may have caused some undetected morphologic or physiological change that resulted in infertility". We showed in earlier experiments (Tuffrey *et al.*, 1986) that C3H mice developed salpingitis when the human strain of *C. trachomatis* N.I.1 was inoculated by the intrabursal or intrauterine route, and severe inflammatory changes causing tubal occlusion were still being seen 41 days after inoculation. In the present experiments, genital infection with the same chlamydial strain had a considerable effect on the fertility of C3H mice: 93% of the uninfected mice had litters, whereas only 31% of the infected animals did so (Table 5). The infertile state lasted up to 6 months after inoculation with chlamydiae and continued after it was known that any inflammatory changes due to infection had resolved. Indeed, at autopsy 5 or 6 months after inoculation, the oviducts were usually normal and so continued infertility at this time could not be explained by tubal occlusion. Of the 32 mice given chlamydiae, 27 were induced to superovulate before autopsy and, apart from 2 exceptions (Nos 8 & 10; Table 3), eggs were not recovered from the oviducts on the inoculated sides of any of them, although corpora lutea were present in the ovaries, confirming successful superovulation. The two exceptional mice were fertile although they had been inoculated with chlamydiae. However, attempts to recover the organisms indicated that infection had not become established. A few chlamydiae were detected in a single swab from one mouse only.

Eggs were recovered from the oviduct of the uninoculated side of about half (5 of 11) of the mice given chlamydiae by the intrabursal route and from a quarter (4 of 16) inoculated by the intrauterine route, whereas they were recovered routinely from both oviducts of control mice. This would account for the litters born to mice in the infected group, presumably as a result of implantation on the uninoculated side. Inoculation by the intrabursal route did not affect the

Table 5. Summary of the effect of infection by *C. trachomatis* N.I.1 on fertility and on recovery of ova from the oviducts or uterus of superovulated C3H mice

Group	Total no.	Fertile*	Infertile	% Fertile	No. of mice with eggs recovered from oviduct or uterus			
					Fertile		Infertile	
					Inoculated side	Uninoculated side	Inoculated side	Uninoculated side
N.I.1 (superovulated)	32 (27)	10 (8)	22 (19)	31	2/8	7/8	0/19	2/19
Medium 2SP (superovulated)	27 (10)	25 (8)	2 (2)	93	7§/8	8/8	1‡/2	2/2
Normal controls (superovulated)	10 (10)				10/10†	10/10†		

* Mice having littered.

† Fertility not tested.

‡ One mouse had no ova because of a necrotic ovary.

§ Ova not apparent in histological section of one mouse.

opposite oviduct so often probably because the infection had further to travel from the original site. These findings are consistent with impairment of transportation of the newly liberated eggs to the oviducts. This hypothesis is substantiated by the finding of eggs and/or accompanying follicular cumulus cells in the periovarial space but never in the oviduct of infected mice. It is possible that chlamydial infection impairs ciliary activity in the infundibulum in some way so that ova remain in the periovarial space and are not transported to the ampulla. We are attempting to investigate this further using organ culture techniques. It seems reasonable to suppose that the same explanation could be put forward to account for the much increased incidence of ectopic pregnancies in women after chlamydial infection.

An impairment in transportation of eggs due to earlier chlamydial infection also could explain why tubal infertility has been associated with sub-clinical infection. Punnonen, Terho, Nikkanen & Meurman (1979) first noted that infertility patients with 'tubal factor' have chlamydial antibody more frequently than do controls and a strong correlation between chlamydial antibodies and tubal factor in infertile women with no known history of salpingitis has been reported by Jones *et al.* (1982). However, chlamydial antibodies were also found in 21% of infertile women with no tubal disease. Our findings in mice suggest that such women may have recovered from chlamydial salpingitis in that their tubes are apparently normal, but have a residual impairment of ovum transportation which has left them infertile. If, as seems likely, infertility can result from a self-limited salpingitis which may leave no apparent tubal damage, early diagnosis and treatment of chlamydial infections in women should be of the utmost importance.

Chlamydial infection did not seem to affect the maturation of ova, or their ability to fertilize and develop. All the ovaries, except one which was necrotic, were functional and histologically normal. Furthermore, a superovulated infertile infected mouse mated successfully and 5 normal blastocysts were recovered from the uninoculated uterine horn.

The fact that infection by chlamydial strain N.I.1 caused infertility in C3H mice but not in TO mice suggests that a genetic predisposition to the pathogenic effects of various strains of *C. trachomatis* should also be considered for women. Although infection had no effect on the reproductive capacity of TO mice in terms of lower birth weight and litter size, there was a significant variation in the birthweights of young born to infected mothers. In view of the suspected association between chlamydial infection and prematurity and still-birth in women, further experiments bearing on the effect of chlamydial infection on fetal development need consideration.

We thank the Department of Comparative Histology for the histological preparations and Janet MacKenzie for help with photomicrography. R.Q. was a sandwich student from Sunderland Polytechnic.

References

- Brunham, R.C., Maclean, I.W., Binns, B. & Peeling, R.W. (1985) *Chlamydia trachomatis*: its role in tubal infertility. *J. infect. Dis.* **152**, 1275–1282.
- Jones, R.B., Ardery, B.R., Hui, S.L. & Cleary, R.E. (1982) Correlation between serum antichlamydial antibodies and tubal factor as a cause of infertility. *Fert. Steril.* **38**, 553–558.
- Møller, B. & Mårdh, P.-A. (1980) Experimental salpingitis in grivet monkeys by *Chlamydia trachomatis*. *Acta path. microbiol. scand.* **B88**, 107–114.
- Patton, D.L., Brunham, R.C., Halbert, S.A., Wang, S.-P., Kuo, C.-C. & Holmes, K.K. (1982a) Salpingitis in the pig-tailed macaque. In *Chlamydial Infections*, pp. 399–402. Eds P.-A. Mårdh, K. K. Holmes, J. D. Oriel, P. Pilot & J. Schachter. Elsevier Biomedical Press, Amsterdam.
- Patton, D.L., Halbert, S.A. & Wang, S.-P. (1982b) Experimental salpingitis in rabbits provoked by *Chlamydia trachomatis*. *Fert. Steril.* **37**, 691–700.
- Punnonen, R., Terho, P., Nikkanen, V. & Meurman, O. (1979) Chlamydial serology in infertile women by immunofluorescence. *Fert. Steril.* **31**, 656–659.
- Ripa, K.T., Møller, B.R., Mårdh, P.-A., Freundt, E.A. & Melsen, F. (1979) Experimental acute salpingitis in grivet monkeys provoked by *Chlamydia trachomatis*. *Acta path. microbiol. scand.* **B87**, 65–70.
- Sweet, R.L. (1982) Chlamydial salpingitis and infertility. *Fert. Steril.* **38**, 530–533.
- Swenson, C.E. & Schachter, J. (1984) Infertility as a consequence of chlamydial infection of the upper genital tract in female mice. *Sex. Transm. Dis.* **11**, 64–67.

- Swenson, C.E., Donegan, E. & Schachter, J. (1983) *Chlamydia trachomatis*-induced salpingitis in mice. *J. infect. Dis.* **148**, 1101–1107.
- Thomas, B.J., Evans, R.T., Hutchinson, G.R. & Taylor-Robinson, D. (1977) Early detection of chlamydial inclusions combining the use of cycloheximide-treated McCoy cells and immunofluorescence staining. *J. clin. Microbiol.* **6**, 285–292.
- Tuffrey, M. & Taylor-Robinson, D. (1981) Progesterone as a key factor in the development of a mouse model for genital-tract infection with *Chlamydia trachomatis*. *FEMS Microbiol. Lett.* **12**, 111–116.
- Tuffrey, M., Falder, P., Gale, J. & Taylor-Robinson, D. (1986) Salpingitis in mice induced by human strains of *Chlamydia trachomatis*. *Br. J. exp. Pathol.* (in press).

Received 28 January 1986