Studies on ciliated epithelia of the human genital tract

I. Swelling of the cilia of Fallopian tube epithelium in organ cultures infected with Mycoplasma hominis

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Summary

Organ cultures of human Fallopian tubes were infected with *Mycoplasma hominis*. Scanning and transmission electron microscopy revealed swelling of the cilia of the tubal epithelial cells in infected cultures. In some, the entire cilia were swollen ; in others, only the tips. Uninfected cultures kept for up to 7 days showed no structural changes in the cilia or other surface structures. *M. hominis* multiplied in organ cultures, but not in culture medium without tissue. A practical organ culture technique for the preparation of specimens for electron microscopy is described.

Introduction

During the past three decades, there have been many studies of genital infection with mycoplasmas (for review see McCormack, Almeida, Bailey, Gardy, and Lee, 1972). *Mycoplasma hominis* can be isolated from the Fallopian tubes of women with acute salpingitis (Mårdh and Weström, 1970a). In such patients, a significant antibody response to the organism has been demonstrated (Mårdh and Weström, 1970b). These findings suggest that *M. hominis* causes tubal infection.

In salpingitis, studies of the pathophysiology of the natural disease are hampered by the inaccessibility of the Fallopian tubes. However, Fallopian tube organ cultures have been used to study experimental infections with *Neisseria gonorrhoeae* (Carney and Taylor-Robinson, 1973; Ward, Watt, and Robertson, 1974) and mycoplasmas (Taylor-Robinson and Carney, 1974). Such cultures have also been used to propagate viruses (Casal, Rubenstein, Votava, and Tyrrell, 1970).

Experimental infections of tubal and other homologous and heterologous ciliated epithelia may lead

to loss of ciliary activity and histological changes of the cells, but as far as we know changes in the ciliary morphology have not been reported in experimental or naturally occurring infections.

The present study concerns swelling of the cilia in Fallopian tube organ cultures after infection of the cultures with *M. hominis*. The phenomenon was demonstrated by means of scanning and transmission electron microscopy.

Material and methods

ORGANISMS

A strain of *M. hominis* recovered from a Fallopian tube of a patient with acute salpingitis was used. It had been subcultured on artificial media only twice before being used in the experiments. In some experiments, the strain had been stored at — 90°C. before being used. In other experiments, strains of *N. gonorrhoeae*, producing T 1 colonies (Kellogg, Peacock, Deacon, Brown, and Pirkle, 1963) were used. These strains were harvested from haematin agar plates that had been inoculated with urethral specimens from patients with acute urethritis and incubated overnight. The test organisms were isolated at our laboratory and the specimens came from patients attending the Departments of Obstetrics and Gynaecology and Venereology, University Hospital, Lund.

MEDIA

Organ culture medium

The organ cultures were maintained in Earle's balanced salt solution (Flow Ltd.) to which amino acids, glutamin, sodium bicarbonate, and vitamins had been added. No antibiotics or sera were used.

Transport medium for Fallopian tubes

This was the same as that used to maintain the organ cultures.

Culture medium for M. hominis

The medium for isolation and maintenance of the strain of *M. hominis* consisted of heart infusion broth (Difco), with 20 per cent. (v/v) horse serum, 10 per cent. (v/v) of a 25 per cent. (w/v) extract of dried yeast, and 1,500 units of penicillin.

ORGAN CULTURE TECHNIQUE

Preparation of organ culture specimens

Fallopian tubes were obtained from patients who were subjected to hysterectomy for non-malignant conditions. All the patients were less than 45 years old and were still menstruating. None had received radiological or hormonal treatment before operation. The operations were usually performed close to mid-cycle. At operation, the uterine tubes were gently dissected free, care being taken to avoid direct handling. The tubes were then directly transferred to bottles containing transport medium.

Organ culture preparation began within 30 minutes of removal. All subsequent procedures were performed in a laminar flow cabinet. The lumen of the tube was identified, and the tube was cut open and spread onto a sterile cotton towel with the mucosal surface upwards. The mucosal surface was washed with culture medium, and the ampullary end of the tube was cut into 3–4 mm. squares. The tissue pieces, mucosal surface upwards, were nailed with glass nails to sterile silicone-rubber discs, 8 mm. in diameter, and placed upside down 'standing' on the glass nails in 50-mm. diameter Petri dishes containing 10 ml. medium (Fig. 1). The medium was changed every second or third day. In some preliminary experiments, cork was used instead of silicone-rubber discs; in others, the tissue pieces were placed directly on to the bottom of the Petri dishes, the plastic surface of which had been scored with a scalpel as described by Hoorn and Tyrrell (1965). The Petri dishes were incubated at 37° C. in an airtight plastic box. The box was flushed with 5 per cent. carbon dioxide in air.
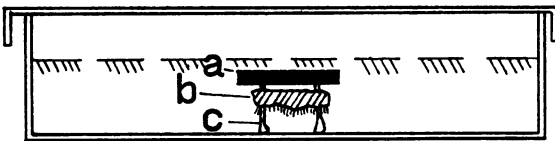

FIG. 1 *Schematic drawing showing mounting of tissue specimens (b) with glass nails, (c) on to silicone-rubber discs, (a) with the mucosal surface facing downwards in a Petri dish containing medium*

Infection of the organ cultures with M. hominis

The broth culture of *M. hominis* used to infect the organ cultures contained 10^5 colony-forming units (CFU)/ml. The broth cultures were centrifuged at 16,000 rpm (30,000 G) for 1 hour. The pellet obtained was resuspended to its original volume in the organ culture medium. This mixture was used to infect the organ cultures.

Titration of M. hominis

In each experiment titration of the number of *M. hominis* organisms in the organ culture medium was performed by removing samples daily. The samples were diluted in 10-fold steps in liquid mycoplasma medium, and the mixtures seeded on to agar plates. The number of colonies was counted. The re-isolated organisms were identified as *M. hominis* by growth-inhibition tests with specific antisera. The culture media were checked for possible bacterial and fungal contamination.

Ciliary activity

The ciliary activity of the epithelium of the Fallopian tube organ cultures was observed daily by means of a stereomicroscope (50 ×) using oblique illumination. This method did not permit quantitative estimation of the ciliary activity.

Histology

Organ culture specimens were fixed in 10 per cent. formalin. The fixed specimens were processed by routine histological methods, including staining with haematoxylin and eosin.

Preparation for scanning electron microscopy (SEM)

The tissue specimens mounted on the silicone-rubber discs were fixed for 24 hrs, in a solution of 2·5 per cent. glutaraldehyde in 0·1 M phosphate buffer, pH 7·2, with an osmolarity of 499 mOs. After rinsing in a 0·2 M sucrose solution in 0·1 M phosphate buffer pH 7·2, for a further 24 hrs (Patek, Nilsson, and Johannisson, 1972), they were dehydrated in ethyl alcohol of increasing concentration (50, 70, 80, 96 per cent., and finally in absolute alcohol). They were then immersed in pure amyl acetate for 24 hrs. The tissue specimens, still mounted on the silicone-rubber discs, were placed in a bomb for critical point drying. The bomb was flushed with liquid carbon dioxide at room temperature until all amyl acetate was replaced. The system was sealed and its temperature raised to 37° C. This transformed the liquid carbon dioxide into a gaseous state and increased the pressure in the system to well above the critical point pressure for CO_2 at 37° C. (*i.e.* 72 atm). The gaseous carbon dioxide was allowed to escape slowly. When the pressure in the system was equal to the atmospheric pressure, the system was cooled to room temperature.

The tissue specimens were removed from the silicone-rubber discs, glued (Bostik 555) to specimen stubs, and finally coated in a vacuum evaporator with gold-palladium and examined in a Cambridge Stereoscan Mark II A scanning electron microscope.

Transmission electron microscopy (TEM)

Some specimens prepared for SEM were cut on an LKB Ultratome and stained with lead citrate or uranyl acetate. The sections were examined in a Zeiss EM 10 electron microscope.

Results

UNINFECTED ORGAN CULTURES

Comparison of cultures nailed to cork discs, to silicone-rubber discs, and placed directly on the bottom of the Petri dishes

The use of cork discs was unsatisfactory, as these apparently were toxic to the ciliated epithelium. The ciliary activity of cork-mounted specimens ceased within 48 hours. Silicone-rubber discs, on the other hand, did not affect the cultures, and the ciliary activity of these specimens continued for as long as that of specimens placed directly on the scored bottom of the Petri dishes, *i.e.* for at least 10 days.

Keeping the specimens with the mucosal surface downwards did not affect the results of the experiments; similar results were obtained with the ciliated surface directed upwards.

Ciliary activity

The ciliary activity of the specimens mounted on to silicone-rubber discs was maintained apparently unaffected throughout the maximum experimental period of 10 days.

Histology

During the first 48 hrs, no changes were observed. After 72 hrs, slight oedema was noted in the subepithelial tissue and the beginning of pyknotic degeneration of the cells in the deeper parts of *tunica mucosae*. No changes were observed in the surface epithelial cells. From the third day onwards, increasing hydropic degeneration of the subepithelial tissue cells was noted. Necrosis of the cells in parts of the subepithelial tissue was observed after 5 days in culture. The epithelial surface cells were still intact. The first signs of cell degeneration and desquamation of the epithelial surface cells were noted after 7 days in culture.

Scanning electron microscopy

No morphological changes in the surface structures of uninfected organ tissue specimens were observed by SEM during the first 7 days of incubation. The epithelial surface of a specimen fixed within half an

FIG. 3 *Uninfected Fallopian tube specimen after 7 days incubation as an organ culture. No significant morphological changes are observed in ciliated or secretory cells. Scanning electron micrograph.* × 1,800

hour of removal of the tube from the patient may be seen in Fig. 2. A piece of tubal mucosa from the same patient after incubation in culture for 7 days is seen in Fig. 3. In these uninfected cultures, the cilia were well preserved and neither cilia nor surface structures of the secretory cells showed any morphological changes throughout the experimental period. The proportion of ciliated/secretory surface epithelial cells varied with the source of the specimen, i.e. the relative number of ciliated cells increased towards the abdominal orifice of the tube, but was not related to the incubation period as organ culture.

Transmission electron microscopy

TEM revealed degenerative changes similar to those observed in the histological preparations after the same time-schedule. The epithelial cells appeared normal during the first 5 days.

ORGAN CULTURES INFECTED WITH *Mycoplasma hominis*

Ciliary activity

The infection of the Fallopian tube organ cultures with *M. hominis* did not appear to affect the activity of the ciliated epithelium, as judged from the presence or absence of such activity observed by stereomicroscopy. With this technique, however, it was impossible to determine the exact proportion of ciliated cells still showing activity.
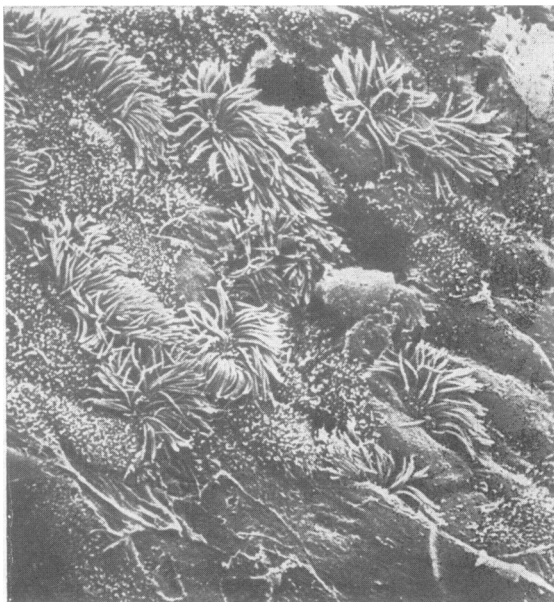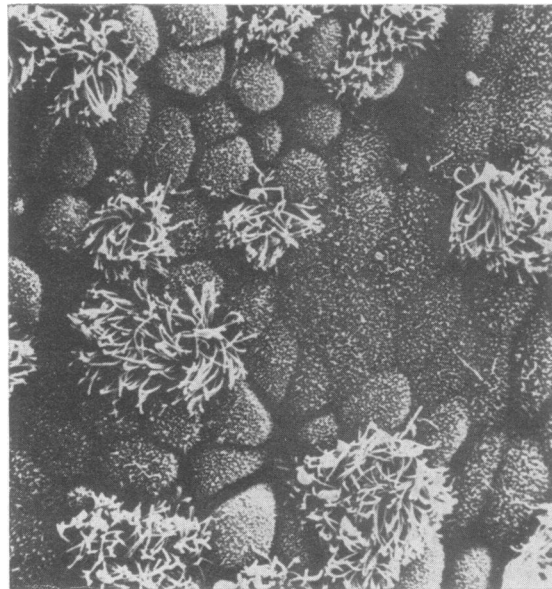

FIG. 2 *Uninfected Fallopian tube specimen prepared immediately after its removal from the patient, showing ciliated cells and secretory cells with microvilli. Scanning electron micrograph.* × 1,800

Histology

No significant histological differences were observed in the specimens infected with *M. hominis* compared with the uninfected controls.

Scanning electron microscopy

In tissue specimens infected with *M. hominis* and kept in organ culture for 48 hrs or less, no significant morphological changes of the cilia were observed by SEM. After 72 hrs or more, swelling and clubbing of the cilia were observed (Figs 4 to 6). These changes were always patchy and appeared to affect cilia from separate cells, neighbouring cells being apparently normal (Fig. 5). In some instances, only the tips of the cilia showed marked club-like swellings (Fig. 4*a*); in other instances, the entire length of the cilia was thickened (Fig. 4*b*). Swelling of the cilia was never observed in uninfected cultures from the same Fallopian tube or in organ cultures that had been infected with *N. gonorrhoeae*. No difference was observed in the proportion of ciliated to secretory cells between infected and uninfected tubal specimens.

Transmission electron microscopy

In the re-embedded and sectioned material, the ciliary swellings could be identified by TEM and were seen to contain tubular-like structures (Fig. 6, overleaf).

As the sections were of low contrast, their finer arrangement could not be assessed. Thus it could not be established whether or not these structures represented intraciliary mycoplasmas. The ciliary origin of the swellings could be confirmed in the cross-sections of the lower parts of the swellings, but whether or not the swellings were due to mycoplasmas remained uncertain.

Isolation of M. hominis *from the organ culture media*

There was little variation in the counts of organisms in the organ culture medium and the number of CFU of *M. hominis* per ml. of medium obtained by daily isolations in a representative experiment in which SEM showed clubbing of the cilia (Fig. 7). The organisms multiplied in the organ culture, and the number of organisms rapidly decreased in pure organ culture medium incubated under the same conditions as the organ cultures.

Cultures for bacteria and fungi from the organ culture media were negative.

Discussion

Examination of Fallopian tube specimens kept in organ culture for 2 or 3 days revealed degenerative changes in the subepithelial tissue in both uninfected and infected cultures, but the surface epithelium, as seen in SEM, TEM, and histological preparations, did not show any such changes even after 5 to 6 days culture. Thus, the organ culture model used in this
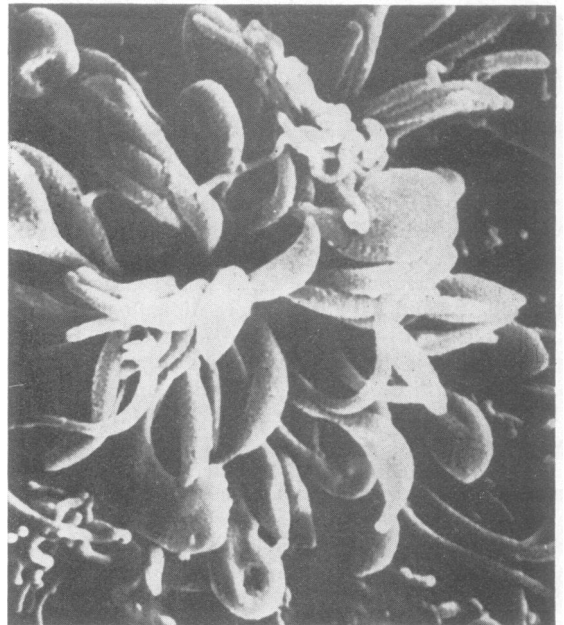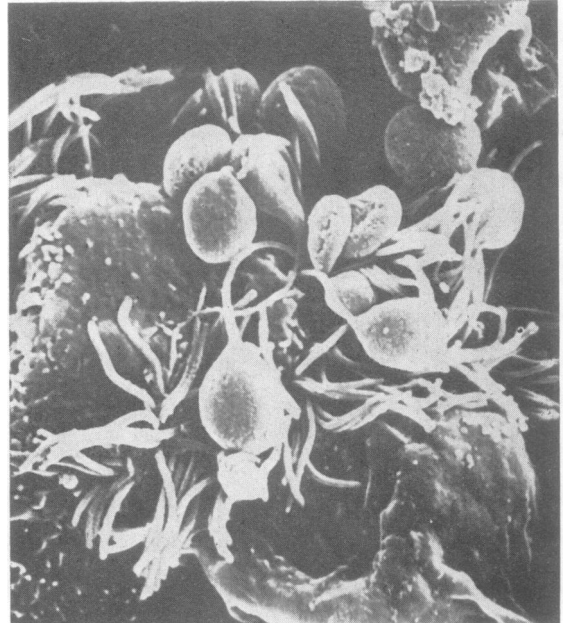

FIG. 4 *Fallopian tube specimen in organ culture 3 days after infection with* Mycoplasma hominis. (*above*) *Swelling of tips of some cilia clearly visible. Neighbouring cilia unaffected. Scanning electron micrograph.* ×4,500. (*below*) *Cilia swollen along their entire length. Scanning electron micrograph.* ×4,500

FIG. 5 *Fallopian tube specimen in organ culture 5 days after infection with* M. hominis. *Swelling of cilia of one ciliated cell is seen, whereas cilia from nearby ciliated cells appear to be intact, as do neighbouring secretory cells. Scanning electron micrograph.* ×9,000

study, appears to be valuable for studying the effect of micro-organisms on the surface epithelial cells.

In studies of surface structures, SEM is often superior to TEM. This is especially so in studies of ciliated epithelia. The application of both SEM and TEM makes the interpretation of such structures easier. A phenomenon such as this, *i.e.* swelling of the cilia, would have been more difficult to observe and interpret if only TEM had been used. Critical point drying is the method of choice for the study of ciliated surfaces (Ludwig, Wolf, and Metzger, 1972), as both air-drying and freeze-drying may cause artefacts.

The introduction of cork into the organ culture systems had a toxic effect on the cells, but silicone-rubber was inert. The technique of nailing the tissue specimens to silicone-rubber discs proved to be useful when the specimens were prepared for SEM and TEM, which included a great number of preparative steps. Similar results were obtained in experiments in which the pieces of tissue were kept with the ciliated surface upwards or downwards as indicated in Fig. 1. However, the latter position was found by experience to be more convenient.

In a recent study, Taylor-Robinson and Carney (1974) showed that *M. hominis* multiplies in organ cultures of human Fallopian tube experimentally infected with the organism. Edén and Mårdh (1970)
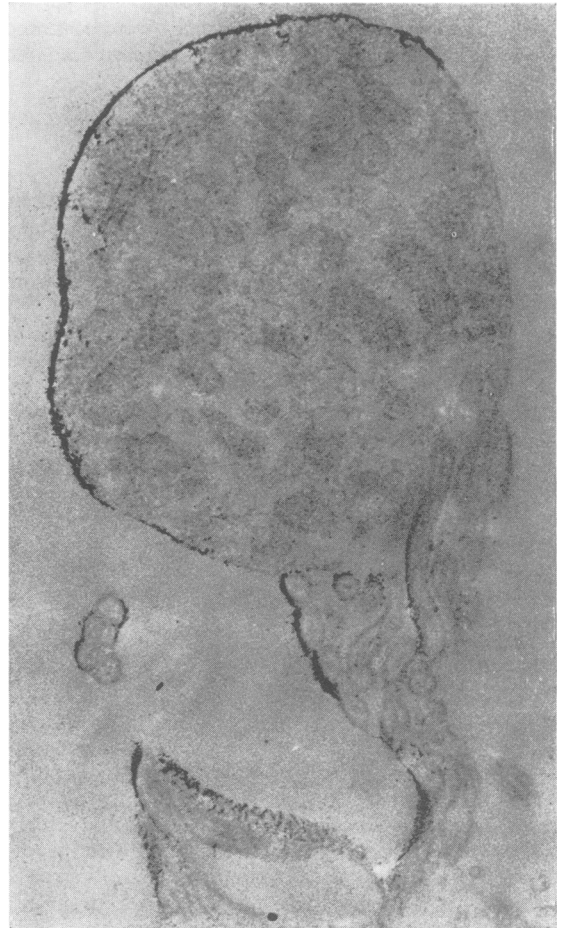

FIG. 6 *Fallopian tube specimen in organ culture 3 days after infection with* M. hominis. *Section of ciliary swelling with tubular-like structures. Transmission electron micrograph.* ×7,600

found that in addition other species of *Mycoplasma*, including *Ureaplasma urealyticum* (T-mycoplasmas) multiplied in *foetal* Fallopian tube organ cultures. In that and the present study a rapid decline in the number of organisms was seen in pure organ culture medium inoculated with the same number of organisms; no viable organisms were found after 3 to 4 days.

In the present experiments, a freshly isolated strain of *M. hominis* was used ; this may have affected the outcome of the experiments. Like Taylor-Robinson and Carney (1974), no significant effect was seen on the ciliary *activity* of the Fallopian tube epithelium in the organ cultures after infection with *M. hominis*, but ciliary swelling was a prominent feature.

Organ cultures of Fallopian tubes, and even cultures of entire tubes, have been used to study the
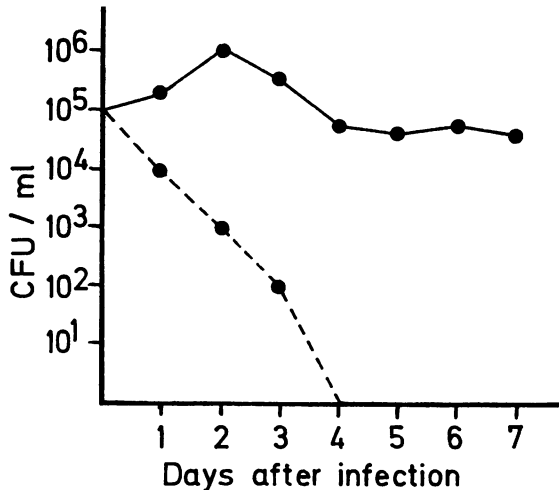
FIG. 7 *Number of colony-forming units of* Mycoplasma hominis *recovered from culture media of Fallopian tube organ cultures* (——•——•——) *and from pure culture medium* (---•---•---) *during 7 days incubation*

effect on the epithelium of gonococcal infections (Ward and others, 1974). These studies demonstrated no effect on the cilia of the Fallopian tubes, similar to that found in the present study. We also studied the effect of *N. gonorrhoeae* in organ cultures set up in the same way as described here. Such cultures, and also uninfected controls, did not show the phenomenon of swelling of the cilia as seen in the cultures infected with *M. hominis*. The fact that uninfected specimens and specimens infected with *M. hominis* and with *N. gonorrhoeae*, all from the same Fallopian tubes, were processed by the same methods and only those specimens infected with *M. hominis* showed any effect on the cilia, suggests that the observed effect on the cilia was due to the infection with *M. hominis*.

We do not yet know what causes the cilia to swell after experimental infection with *M. hominis*. Ciliary swelling is known to occur as a physiological phenomenon on the coronet cells in the *saccus vasculosus* of teleost fishes (Bargmann, 1954). Moreover, after exposure to heat, the very tips of the cilia of rabbit trachea swell (von Mecklenburg, Mercke, Håkansson, and Toremalm, 1974), a phenomenon morphologically distinct from those observed in our study.

After experimental infection with *M. hominis*, tubular-like structures were observed inside the

ciliary swellings, whereas the swellings of the cilia of rabbit trachea after exposure to heat were vesicle like and structureless.

Hydrogen peroxide production by *M. pneumonia* has been suggested as a reason for this mycoplasma causing loss of ciliary activity of respiratory tract epithelium (Taylor-Robinson, 1973). However, production of peroxide by *M. hominis* has not been reported. It is possible that depletion of arginine from the culture medium because of the metabolism of *M. hominis* could be responsible for the swelling of the cilia.

The recovery of *M. hominis* directly from the Fallopian tubes in women with acute salpingitis (Mårdh and Weström, 1970a) and the demonstration of an antibody response to the organism in the same patients (Mårdh and Weström, 1970b) suggests that *M. hominis* can cause tubal infection. Although clubbing of the cilia was observed in the present study, we do not yet know whether this is found in naturally occurring tubal infections with *M. hominis*. Scanning electron microscopy of small pieces of tubal mucosa from patients with salpingitis might answer this question.

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