

# The reproductive significance of human Fallopian tube cilia

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**Effective tubal transport of ova, sperm and embryos is a prerequisite for successful spontaneous pregnancy. Although there is much yet to be discovered about the mechanisms involved, it is evident that tubal transit is a far more complicated process than initially thought. Propulsion of gametes and embryos is achieved by complex interaction between muscle contractions, ciliary activity and the flow of tubal secretions. Evidence is accumulating of the important and possibly pre-eminent role of ciliary motion in this process; and this review describes current knowledge about ciliary activity and its physiological regulation. There is also a description of the effects on ciliary function of cigarette smoking and various pathological states, including endometriosis and microbial infection, with consideration given as to how altered ciliary activity may impact upon fertility.**

*Key words:* chlamydia/cilia/ectopic pregnancy/Fallopian tube/tubal infertility

## Introduction

With the progress in IVF, the contribution of the Fallopian tube towards successful reproduction has been comparatively overlooked. It is clear from the success of IVF, which of course bypasses tubal transport, that exposure to the tubal milieu is not an absolute requisite for fertilization or implantation to occur. Thus, the Fallopian tube is often now thought of as little more than a mere conduit. However, in fertilization *in vivo*, the Fallopian tube plays an essential role in gamete transport, fertilization and the early development of the embryo.

It is becoming increasingly evident that the mechanism of tubal transport is much more complex than first thought and can be affected by a wide range of factors and conditions that may impair fertility. It is the purpose of this article to review the recent advances in knowledge about tubal transport predominantly within the human Fallopian tube, with particular reference to the role of the mucosal cilia, the physiological control of their action and how this can be disrupted by various pathological states.

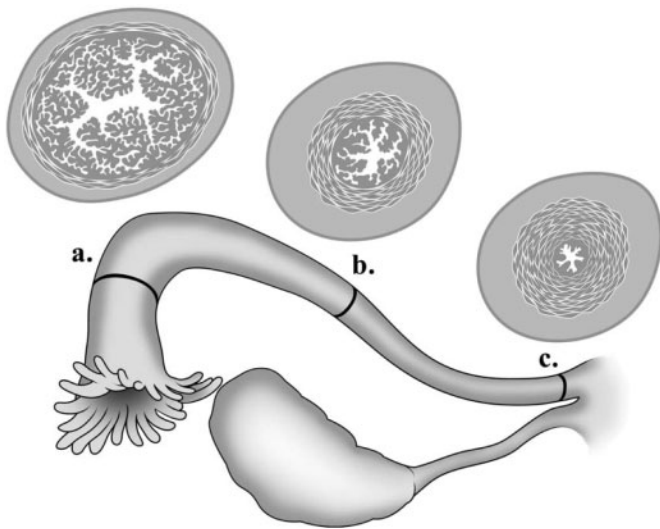
## Structure of the human Fallopian tube epithelium

The tubal mucosa is arranged into longitudinal folds, which become increasingly convoluted towards the distal end of the tube (Figure 1). By light microscopy, the Fallopian tube is seen to have a single layer of cuboidal or columnar epithelium which often appears pseudostratified because of crowding of cells of different height. The two major cell types are the ciliated and secretory cells. Ciliated cells are found predominantly on the apex of the mucosal folds

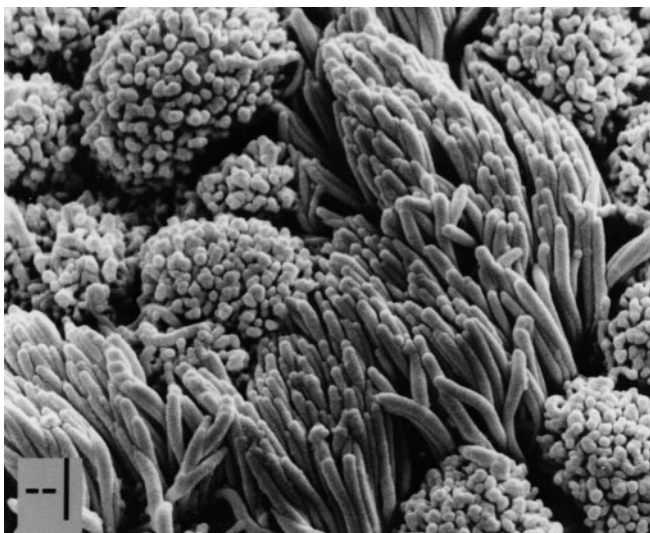
(Ferenczy *et al.*, 1972; Patek, 1974). At the base of each cilium is a basal body from which the cilium originates. These develop from centrioles which have migrated towards the luminal surface of the cell, where they are aligned perpendicular to the cell surface giving a refractile appearance on electron microscopy (Hagiwara *et al.*, 2000). Cilia are about 10 µm long and 0.25 µm in diameter (Satir, 1992). There is a progressive decrease in the proportion of ciliated cells from over 50% in the fimbria to less than 35% in the isthmus (Patek *et al.*, 1972a; Amso *et al.*, 1994; Crow *et al.*, 1994).

## Cyclical variation in human tubal morphology

The first description of a distinct oviductal cycle in women was made in 1928 (Novak and Everett, 1928). In common with the rest of the female reproductive tract, the Fallopian tube undergoes cyclical changes under the influence of estrogen and progesterone (Critoph and Dennis, 1977a). Cells are low in height during the menstrual phase of the cycle, increasing during the proliferative phase to reach their maximal height in the periovulatory period. At this time, both secretory and ciliated cells are of equal size, with the secretory cells forming domes between the tufts of cilia (Figure 2). Around the time of ovulation, the secretory cells reach peak activity and discharge their contents into the lumen of the tube, consequently reducing in height relative to the ciliated cells (Pauerstein and Eddy, 1979). This results in greater prominence of the cilia and may enable them to move particulate material or viscous secretions more effectively. Subsequently, in the luteal phase, both cell types reduce in height and there is partial deciliation (Patek *et al.*, 1972b; Verhage *et al.*, 1979).



**Figure 1.** Illustration of the human Fallopian tube, showing the longitudinal folds in cross-section at the (A) infundibulum, (B) ampulla and (C) isthmus.



**Figure 2.** Scanning electron microscopic photograph of ciliated and secretory cells within the human Fallopian tube epithelium.

Ovarian hormones affect tubal epithelial structure and expression of cilia. Receptors for estradiol ( $E_2$ ) and progesterone have been identified within the Fallopian tube epithelium, and the expression of these receptors varies according to the stage of the ovarian cycle (Pollow *et al.*, 1981). Tubal  $E_2$  receptors are maximal at midcycle, whereas progesterone receptors are present throughout the cycle, even into the late luteal phase (Amso *et al.*, 1994). Estrogen stimulates epithelial cell hypertrophy, secretion and ciliogenesis, whilst atrophy and deciliation are associated with high levels of serum progesterone (Verhage *et al.*, 1979; Donnez *et al.*, 1985).

### Structure of the cilia

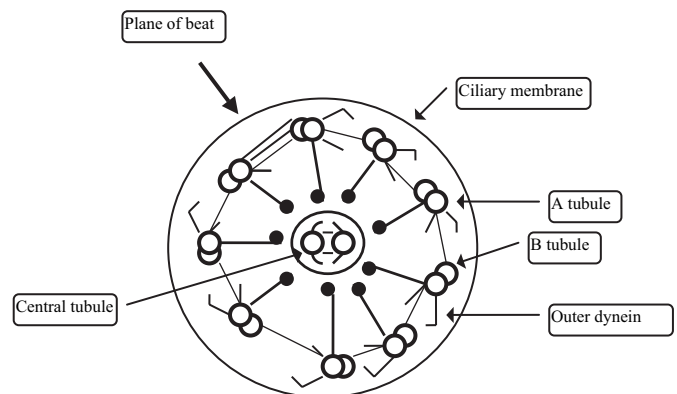
The cilia possess a central bundle of microtubules, called the axoneme, in which nine outer doublet microtubules surround a

central pair of single microtubules (Figure 3). This gives a characteristic '9 + 2' arrangement when viewed in cross-section. Each doublet microtubule consists of A and B tubules, or subfibres, each approximately 200 Å in diameter. Rows of dynein arms reach out from the A tubule to the B tubule of the neighbouring doublet.

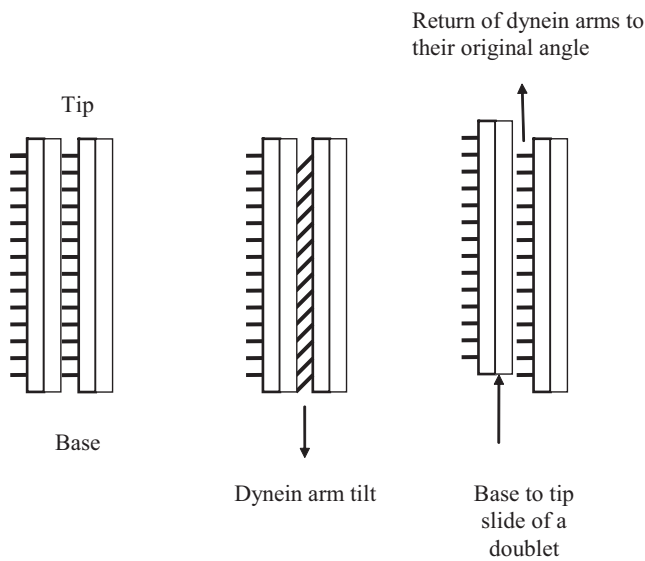
The basis for axonemal movement is the sliding of doublet microtubules relative to one another. This requires hydrolysis of ATP, utilizing ATPase located in the outer dynein arms. Local regulating factors include calcium ions, cyclic adenosine monophosphate and polypeptides (Huitorel, 1988). The successive active formation and breakage of cross-bridges results in the effective 'walking' of the outer dynein arms of the A tubule of one doublet along the B tubule of the adjacent doublet towards its base (Sale and Satir, 1977). Once the dynein arm has become transiently attached to the B tubule, it returns to its original tilt angle, pushing this doublet in a base-to-tip direction (Figure 4). Since microtubule sliding occurs in one direction only, this means that when the doublets on one side of the axoneme are actively sliding, the doublets on the other side are involved in passive movement in the opposite direction. This is termed the switch point hypothesis (Satir and Matsuoka, 1989).

The resultant sliding motion is converted into bending by the selective activation and deactivation of protein cross-links, termed radial spokes. In straight regions of the axoneme, the radial spokes are functionally detached from the central sheath, facilitating the sliding of microtubule doublets. However, in bent regions of the cilium, the spokes remain attached to the inner sheath, converting active interdoublet sliding into local bending (Warner and Satir, 1974).

Analysis of serial cross-sections of the cilia reveals consistent structural asymmetries within the axoneme. The outer dynein arm is absent from one doublet for greater than 90% of the axonemal length. This particular doublet is always orientated in the plane of ciliary beat, opposite to the direction of the effective stroke. Thus, it appears to be structural components within the axoneme which determine the direction of ciliary beat (Hoops and Witman, 1983). Cilia often beat out of phase with one another, producing metachronal waves similar to the pattern of a 'field of wheat' (Satir, 1992). However, around the time of ovulation, the strokes of cilia are synchronized and oriented towards the uterus in humans (Gaddum-Rosse *et al.*, 1973).



**Figure 3.** Schematic diagram of a cross-section of a cilium.



**Figure 4.** Schematic diagram representing the mechanism of ciliary movement.

### Cyclical changes in ciliary beat

There have been conflicting reports on the changes in ciliary beat frequency (CBF) throughout the ovarian cycle. In rabbits, the rate of ciliary beat in the oviducts increases by 20% on the 2nd and 3rd days after copulation, corresponding to the time of movement of the ova down the tubes (Borell *et al.*, 1957). Critoph and Dennis detected a significant increase in the CBF of the human isthmus and ampulla after ovulation (Critoph and Dennis, 1977b). In contrast, Westrom found no cyclical or anatomical variation in beat frequencies (Westrom *et al.*, 1977). Recent investigations using analogue contrast enhancement have shown an increase in CBF of the fimbrial section of the tube during the secretory phase (Lyons *et al.*, 2002a). These results agree with those of Critoph and Dennis in confirming a variation in CBF according to the ovarian cycle.

### Methods used to measure CBF

The cilia beat at such high frequencies that direct observation allows only the recognition of dramatic changes in CBF and therefore makes this assessment of limited value (Rusznak *et al.*, 1994). Several techniques for measuring CBF have been developed, but all have various limitations such as poor spatial resolution, vibration artefacts from the immersion fluid and inaccuracy when the cilia are in asynchronous rhythm (Naitoh and Kaneko, 1973; Verdugo *et al.*, 1980a; Kennedy and Duckett, 1981). Recent advances in measurement of CBF involve the use of digital technology. The use of digital cameras allows improvement of the signal/noise ratio, enhancement of the image resolution and direct storage into computer memory (Sanderson, 2000).

Considering the numerous and varied techniques employed to measure CBF, it is not surprising that there is no consensus as to the baseline rate of CBF in the human Fallopian tube. A wide range of frequencies from 5 to 20 Hz has been reported in the literature (Westrom *et al.*, 1977; Paltieli *et al.*, 1995). It is important to note that results obtained using the different techniques cannot be directly compared (Chilvers and O'Callaghan, 2000).

### Role of the cilia in tubal transport

Tubal transport is a precisely timed process, allowing propulsion of sperm in the opposite direction to ova and embryos, and supporting fertilization and early embryogenesis within the tubal lumen. Tubal transit is influenced by contractions of the tubal musculature, ciliary activity and the flow of tubal secretions (Jansen, 1984). Although the relative importance of each of these mechanisms is still unclear, there is evidence that ciliary action may play the dominant role in transfer of gametes and embryos. If muscular activity is inhibited by isoproterenol, a  $\beta$ -adrenergic agonist, there is no difference in total transit times through the ampulla, suggesting that the cilia alone are capable of transporting the ovum to the site of fertilization within a normal time frame (Halbert *et al.*, 1976, 1989). Conversely, women with the 'immotile cilia syndrome', or Kartagener's syndrome, suffer from subfertility (Afzelius *et al.*, 1978; Pederson, 1983; Lurie *et al.*, 1989). Successful pregnancies reported in this condition may be explained by a subgroup of women demonstrating some, albeit dyskinetic, ciliary action (McComb *et al.*, 1986; Halbert *et al.*, 1997).

### Ovum transport

At ovulation, the fimbria comes into close contact with the surface of the ovary and moves back and forth over the ovulatory follicle, disrupting and removing the cumulus mass (Lindblom and Norström, 1986). Although the mechanism involved is unclear, the mesosalpingeal muscle seems to twist the tube and bring the fimbria into close apposition with the ovary (Okamura *et al.*, 1977). It is believed to be the prostaglandins (PGs) within follicular fluid which mediate this action by increasing the contractility of both fimbria and tubo-ovarian ligaments (Sterin-Speziale *et al.*, 1978; Morikawa *et al.*, 1980).

Rodent studies suggest that ovum transit is slow and steady for the first few millimetres beyond the fimbrial end of the tube. Thereafter, the movement of the ovum changes to a complex pattern of rapid forward and backward motion associated with segmental contractions of the oviduct wall. The purpose of these segmental muscular contractions may be to cause stirring of the tubal contents and ensure the mixing of gametes and embryos with tubal secretions (Muglia and Motta, 2001).

It is obviously difficult to study ovum transport in women, although investigations have been performed on patients undergoing salpingectomy for sterilisation. Operations were undertaken at varying time periods following the LH surge, and the site of ovum retrieval was recorded. From this, the researchers concluded that the tubal transit time in humans is approximately 80 h (Croxatto *et al.*, 1978). Once released, the ovum takes around 8 h to reach the ampullary-isthmic junction (AIJ) where fertilization occurs, and the developing embryo then appears to reside in the ampulla for 72 h (Croxatto and Ortiz, 1975; Croxatto *et al.*, 1978). Increased isthmic tone around the time of ovulation, as well as the presence of a dense, tenacious mucus obliterating the isthmic lumen, may be responsible for ovum retention at this site (Jansen, 1980; Anand and Guha, 1982). Once progesterone dominance begins, the isthmic mucus disappears and the muscle tone relaxes because of the down-regulation of  $\alpha$ -adrenergic receptors by inhibitory  $\beta$ -receptors.

As a result, the isthmic lumen opens and allows transport of the early embryo to the uterus (Jansen, 1978).

This delay at the AIJ is believed to be important in exposing the embryo to developmental factors and nutrients within the tubal fluid and in postponing entry into the uterus to the time of maximal endometrial receptivity. The concept that the fertilized ovum is delivered to the uterine cavity at an appropriate time for implantation implies the presence of regulatory mechanisms governing tubal transport. Three possible mechanisms have been suggested: endocrine regulation, mainly by ovarian steroids; neuronal regulation through the autonomic nervous system and paracrine regulation by the embryo itself.

### Sperm transport

Because pregnancy has been shown to result from intercourse which occurs up to 5 days before ovulation (Wilcox *et al.*, 1995), human sperm must be stored at some site in the female genital tract. Although motile sperm have been recovered from the human cervix up to 5 days after insemination (Gould *et al.*, 1984), it is not known whether these sperm could reach the Fallopian tube so long after deposition or indeed whether they might have re-entered the cervix from the uterus. The available evidence suggests the human Fallopian tube itself as the likely candidate for a sperm storage site as the tubal epithelium provides a favourable environment for sperm. Anatomical sperm reservoirs within the tubal isthmus have been detected in a variety of mammalian species (Yanagimachi and Chang, 1963; Hunter, 1981; Hunter and Nichol, 1983), but a distinct tubal reservoir has not been located in humans (Williams *et al.*, 1993).

Nevertheless, motile human sperm have been shown to bind by their heads to the ciliated apical areas of the tubal epithelium *in vitro*, and the density of sperm is greater in the isthmus than the ampulla (Baillie *et al.*, 1997). Species-specific carbohydrate moieties are involved in the binding process in animal studies, but no carbohydrate performing such a function has yet been identified in humans. Recently, however, it has been proposed that human sperm–isthmus attachment results from binding of the amino acid sequence Arg-Gly-Asp of an as-yet-undefined sperm protein to integrins on the tubal epithelium (Reeve *et al.*, 2003). Sperm binding in the human endosalpinx appears to be intermittent and less tight than in other mammalian species studied (Pacey *et al.*, 1995a, b). Despite this, when associated with the convoluted architecture and thick tenacious mucus of the isthmic endosalpinx, these factors may be sufficient to act as a functional sperm reservoir and slow sperm progression through the tube.

Sperm–endosalpingeal contact preserves the viability of sperm. Incubation of human sperm with cultured tubal epithelium preserves viability for longer than culture in medium alone, as does incubation with vesicles prepared from the apical membrane of the human endosalpinx (Kervancioglu *et al.*, 1994; Murray and Smith, 1997). This indicates that the epithelium can enhance sperm survival by direct contact rather than by secretions. Retention of sperm within the tubal isthmus could therefore prolong the availability of viable sperm and increase the chance of successful fertilization.

There is evidence from animal studies that the incidence of polyspermy is increased if sperm is freely available in the tubal ampulla. If porcine sperm is injected directly into the ampulla, or

the isthmus is excised or the smooth muscle of the muscularis is inhibited by direct administration of progesterone, then the frequency of polyspermic fertilization rises (Day and Polge, 1968; Hunter and Leglise, 1971; Hunter, 1973). Thus, a functional isthmic sperm reservoir may also serve to prevent polyspermy by restricting the rate of sperm release into the ampulla to only a few at a time.

Once sperm have undergone the processes of capacitation and hyperactivation in preparation for fertilization, they appear to lose their binding affinity. Capacitation modifies the proteins on the surface of the sperm cell membrane, which could alter or reduce binding sites. Uncapacitated boar and bull sperm binds more avidly to tubal epithelium than after capacitation (Lefebvre and Suarez, 1996; Fazeli *et al.*, 1999). Hyperactivation can supply the force necessary for the physical disruption of sperm–endosalpingeal attachment and may assist in the detachment of human sperm (Pacey *et al.*, 1995b). Thus, maturational changes within the sperm themselves appear to regulate their binding and subsequent release from the tubal epithelium. Once released, ciliary motion may assist in transport to the site of fertilization within the ampulla, as co-culture of sperm with tubal epithelial cells has been shown to increase CBF (Morales *et al.*, 1996).

### Regulation of ciliary beat

Ciliary reactivity is affected by a variety of hormonal and neuronal stimuli. CBF is a calcium-dependent process which requires hydrolysis of ATP. In the absence of calcium in the culture medium, ciliary motility *in vitro* ceases (Verdugo, 1980). ATP increases CBF in a dose-dependent manner *in vitro*. It has been suggested that ATP is released by the secretory cells *in vivo* and subsequently acts upon the ciliated cells in a paracrine manner (Villalon and Cardina-Danovaro, 1994). Beta-adrenergic stimulation increases ciliary activity, an effect which can be blocked by the  $\beta$ -adrenergic receptor blocker, propranolol, confirming the receptor specificity of this response (Verdugo *et al.*, 1980b).

Angiotensin II in nanomolar concentrations has been shown to stimulate CBF *in vitro*. This effect is inhibited by the specific type I angiotensin II receptor antagonist, losartan (Saridogan *et al.*, 1996). The presence of a renin–angiotensin system has been described in the human Fallopian tube, and angiotensin II receptors are present in the tubal mucosa, with immunostaining being most prominent in the proliferative phase. The role of angiotensin II in tubal function has not yet been elucidated.

It has been hypothesized that the increase in CBF after ovulation is because of rising progesterone levels in an estrogen-rich environment, which may increase the release of ATP from apically situated mitochondria within the ciliated cells, and thus increase CBF (Jansen, 1984). However, high-dose progesterone has been shown to inhibit CBF *in vitro* by up to 63% (Mahmood *et al.*, 1998; Paltieli *et al.*, 2000). This inhibition can be reversed by the progesterone receptor antagonist, mifepristone (Mahmood *et al.*, 1998). The ovarian hormones are present in the Fallopian tube mucosa in much higher concentrations than in the general circulation, because of a countercurrent exchange mechanism between the ovarian artery and the venous plexuses along the mesosalpinx (Bendz *et al.*, 1982). Also, the Fallopian tube cilia are directly exposed to high levels of ovarian steroids at midcycle with the influx of follicular fluid. During the secretory phase,

estrogen and progesterone levels remain high in the peritoneal fluid, which is in direct communication with the tubal lumen, thus prolonging the ciliary exposure to raised ovarian hormone levels. Because high concentrations of progesterone decrease CBF, these findings suggest that alternative factors may be responsible for the increase in CBF detected post-ovulation.

PGE<sub>2</sub> and F<sub>2α</sub> both stimulate fimbrial CBF in the rabbit oviduct *in vitro*, an effect believed to be mediated by PG-induced release of calcium ions (Verdugo, 1980; Verdugo *et al.*, 1980a). It has been hypothesized that PGs released locally in the tubal mucosa or synthesized by the cumulus complex surrounding the transported ovum stimulate ciliary activity, acting through the release of calcium ions from intracellular storage sites or the extracellular space (Jansen, 1984).

Follicular fluid exerts a significant stimulatory effect on CBF of human Fallopian tube explants *in vitro* (Lyons *et al.*, 2006). The follicular fluid of human pre-ovulatory ovarian follicles contains high concentrations of E<sub>2</sub>, progesterone and PGs (Edwards *et al.*, 1972; McNatty *et al.*, 1979; Seibel *et al.*, 1984). At ovulation, the fimbrial end of the Fallopian tube is in close apposition to the dominant ovarian follicle (Doyle, 1956). Once the follicle ruptures, the ovum is transported into the Fallopian tube by the flow of follicular fluid (Harper, 1982). Follicular fluid thus becomes the major constituent of tubal fluid immediately post-ovulation. It appears that PGs or other factors in follicular fluid may provide the stimulus for the increase in CBF observed in the secretory phase and that this may aid ovum pick-up and transport.

## Pathological effectors of CBF

### Ectopic pregnancy

Fallopian tubes containing an ectopic pregnancy demonstrate a marked reduction in the number of ciliated cells in comparison with those of women with an intrauterine gestation. Marked deciliation is also sometimes seen subsequent to an ectopic pregnancy and in biopsies from women undergoing tubal surgery who later develop a tubal pregnancy (Vasquez *et al.*, 1983). This effect suggests that pathological processes affecting the tubal cilia may predispose to ectopic gestation.

### Smoking

Women who smoke have a markedly increased risk of ectopic pregnancy and increased incidence of tubal infertility. Compared with women who have never smoked, women who smoke more than 20 cigarettes a day have an almost four-fold risk of ectopic gestation, similar to the increase in risk associated with a past history of pelvic inflammatory disease (Bouyer *et al.*, 2003). Even prenatal exposure to tobacco smoke has been postulated to increase the prevalence of tubal disease, suggesting that tobacco smoke has a permanent detrimental effect on the developing Fallopian tubes (Matthews *et al.*, 2002).

In animal models, nicotine alters tubal motility (Neri and Marcus, 1972) and decreases tubal blood flow (Mitchell and Hammer, 1985). Exposure of hamsters to doses of cigarette smoke within the range received by active or passive human smokers causes a small but significant increase in the secretory-to-ciliated cell ratio within the infundibulum (Magers *et al.*, 1995). Acute *in vitro* exposure of the hamster infundibulum to smoke solutions

causes a rapid reduction in CBF which is reversible upon washout of the smoke solution (Knoll *et al.*, 1995).

Oocyte cumulus pick-up rate by the hamster oviduct is inhibited in a dose-dependent manner by smoke solutions, and this effect is not easily reversed by washout of the solution, demonstrating that the effect of smoking on ovum pick-up is separate to the effect on CBF. The likely explanation for this is that smoke solutions disrupt the adhesion between the negatively charged tips of the cilia and the oocyte cumulus complex, probably by the binding of an as-yet-undetermined smoke component (Knoll and Talbot, 1998).

Animal data demonstrating reduced efficacy of ovum pick-up and delayed transport along the tube because of decreased CBF may explain the higher rates of infertility and ectopic gestation seen in women who smoke. It is of concern that even fetal exposure to tobacco appears to affect tubal function, suggesting that the consequent tubal damage may be long-term, if not permanent.

### Endometriosis

The association between endometriosis and infertility is well recognized, although whether or not endometriosis causes infertility remains controversial. It is easy to understand how severe endometriosis with the formation of ovarian endometriomas, adhesions and gross distortion of the pelvic anatomy may be associated with infertility. However, the link between mild endometriosis and infertility is as yet unexplained.

The peritoneal microenvironment in women with endometriosis differs from normal fertile controls. There is substantial evidence that the levels of activated macrophages in the peritoneal cavity are raised in women with endometriosis (Halme *et al.*, 1983, 1987; Zeller *et al.*, 1987). There appears to be an inverse relationship between stage of endometriosis and the presence of inflammatory mediators, with a tendency towards higher peritoneal macrophage levels in women with minimal-to-mild disease (Olive *et al.*, 1985; Haney *et al.*, 1991). Cytokines secreted from endometriotic deposits or activated macrophages have been detected at higher concentrations in the peritoneal fluid of women with endometriosis and have been shown to have a deleterious effect on fertility parameters (Fakih *et al.*, 1987; Sueldo *et al.*, 1990).

There is a marked inhibitory effect of peritoneal fluid from women with mild-and-moderate endometriosis on CBF *in vitro* (Lyons *et al.*, 2002b). Additionally, a macromolecular ovum capture inhibitor has also been detected in the peritoneal fluid of women with endometriosis (Suginami and Yano, 1988). This forms a membrane over the fimbrial cilia, causing a complete loss of ovum capture activity. It is possible that a similar mechanism of deposition of filamentous material may inhibit the activity of cilia, and thus tubal transport, in women with endometriosis. Furthermore, one or more of the constituents of the proinflammatory peritoneal fluid found in women with endometriosis may affect ciliary beat directly. The factor(s) in endometriosis peritoneal fluid responsible for this reduction in CBF are unknown. Possible mediators include macrophages or their various secretion products.

Interestingly, human sperm-endosalpingeal interaction *in vitro* appears to be altered in explants of tubal epithelium from women with a diagnosis of endometriosis, with more sperm bound in tissue from affected women (Reeve *et al.*, 2005). This suggests that a reduction in free and motile sperm within the tubal lumen may

reduce fertilization rates. Although little is known about sperm–endosalpingeal interaction in humans, tubal integrins have been implicated in the process (Reeve *et al.*, 2003), and integrin expression is known to be aberrant within the endometrium and endometriotric deposits of sufferers (Lessey *et al.*, 1994; Puy *et al.*, 2002). The impairment of tubal transport of gametes and embryos may thus represent one mechanism by which endometriosis exerts its detrimental effect on fertility.

### Effects of micro-organisms on the Fallopian tube

#### *Neisseria gonorrhoeae*

Exposure of the human Fallopian tube epithelium *in vitro* to fresh isolates of *N. gonorrhoeae* and to gonococcal endotoxin results in reduction and subsequent cessation of ciliary activity. This ciliostatic effect occurs before any ultrastructural changes are apparent on scanning electron microscopy (Mardh *et al.*, 1979). Gonococci invade the non-ciliated cells of the tubal mucosa, but destroy predominantly the ciliated cells, primarily by causing sloughing (McGee *et al.*, 1981).

The two compounds likely to mediate this damage are gonococcal lipopolysaccharide (LPS), released from the outer membrane of the organism, and monomers of peptidoglycan, which are generated by the bacterium (Melly *et al.*, 1984; Woods and McGee, 1986; Stephens *et al.*, 1987). Immediately after addition to human Fallopian tube organ cultures, gonococcal LPS aggregates on the tips of the cilia. By 2 h after exposure, LPS is evident in the cytoplasm of both ciliated and non-ciliated cells in vesicle-like structures, and, by 12 h, sloughing of the ciliated cells has occurred (Cooper *et al.*, 1986). Neutralization of the lipid A moiety of LPS abolishes this effect, confirming that the destructive action of *N. gonorrhoeae* is mediated indirectly by the release of toxins (Stephens *et al.*, 1987).

Gonococcal infection of the human Fallopian tube mucosa results in production of tumor necrosis factor (TNF)- $\alpha$  by the mucosa. The extent of loss of ciliated cells from the tubal epithelium correlates with the mucosal tissue concentration of TNF- $\alpha$  (McGee *et al.*, 1999), and blocking gonococcal production of TNF- $\alpha$  limits epithelial damage (McGee *et al.*, 1992). Direct treatment of tubal epithelial cultures with recombinant TNF- $\alpha$  also causes sloughing of ciliated cells in the manner typical of gonococcus (McGee *et al.*, 1992).

This suggests that induction of this proinflammatory cytokine by gonococcal infection may be another mechanism by which tubal damage occurs. As cytokines, including TNF- $\alpha$ , have already been implicated in the reduction in CBF produced by endometriotric peritoneal fluid, this is an area worthy of further investigation.

#### *Chlamydia*

Fallopian tube damage and tubal factor infertility are common sequelae of upper genital tract infection with *Chlamydia trachomatis*. This pathogen causes a direct cytotoxic effect on the mucosa of the human Fallopian tube, which results in loss of microvilli and disruption of cell junctions, associated with rupture of the epithelial cells (Cooper *et al.*, 1990). However, it is believed to be the consequent immune response which results in permanent tissue scarring, with persistent or repeated infection exacerbating this destruction. Although the precise mechanism by which prolonged

exposure to chlamydial antigens causes tubal damage is not fully elucidated, the 60 kDa chlamydial heat shock protein (hsp60) is believed to be the major antigen triggering this pathogenic immune response. Several studies have demonstrated a correlation between the level of immune response to chlamydial hsp60 and the extent of tubal damage (Brunham and Peeling, 1994; Eckert *et al.*, 1997; Peeling *et al.*, 1997). The detection of antibodies to hsp60 is strongly correlated to the presence of tubal factor infertility (Toye *et al.*, 1993; Ault *et al.*, 1998).

Additional chlamydial antigens have been implicated in the immunopathological mechanism of tubal damage. Immune response to hsp10 has been associated with tubal infertility in a population of women exposed to chlamydia (LaVerda *et al.*, 2000). Recently, evidence of genetic protection against chlamydia-mediated tubal damage has emerged, with the description of two class II alleles detected less commonly in chlamydia-seropositive women with tubal infertility (Cohen *et al.*, 2003).

Chlamydial hsp60 is capable of provoking both a humoral and cell-mediated immune response, with intense macrophage activation (LaVerda *et al.*, 1999). Activated macrophages secrete proinflammatory cytokines, and chlamydial infection induces significantly higher secretion of TNF- $\alpha$ , interleukin-1 $\beta$  and interferon (IFN)- $\gamma$  (Toth *et al.*, 1992; Ojcius *et al.*, 1998; Kinnunen *et al.*, 2003). Apoptosis of both infected epithelial cells and macrophages occurs, releasing more proinflammatory cytokines and exacerbating the host inflammatory response (Ault *et al.*, 1996; Ojcius *et al.*, 1998).

Under different conditions, *C. trachomatis* appears to be capable of either promoting or inhibiting apoptosis. Chlamydial proteins in infected cells possess diverse antiapoptotic properties, including inhibition of caspases, the enzyme system primarily responsible for triggering apoptosis (Fan *et al.*, 1998). Macrophage-released IFN- $\gamma$  has also been shown to inhibit apoptosis of infected cells (Dean and Powers, 2001; Perfettini *et al.*, 2002). This inhibition of apoptosis of infected cells is likely to contribute to the development of persistent infection (Witkin, 2002).

The pathways leading to permanent tubal damage have not yet been fully elucidated. The production of endothelial cell adhesion molecules within the Fallopian tube can be induced directly by chlamydial LPS or by the cytokines of the inflammatory response (Kelly *et al.*, 2001). These cell adhesion molecules mediate the extravasation of lymphocytes from the circulation into the site of infection and thus promote the inflammatory response (Butcher *et al.*, 1999). Enhanced production of PGs and collagen, with increased expression of integrin and transforming growth factor- $\beta$ , contributes to fibrosis and scarring (Perfettini *et al.*, 2000). Hsp60 also causes the induction of matrix metalloproteinases, which may produce tubal scarring as a result of increased turnover and repair of the extracellular matrix (LaVerda *et al.*, 1999; Ault *et al.*, 2002).

Salpingitis is associated with distal occlusion of the Fallopian tube and deciliation, which can be extensive (Patton *et al.*, 1983, 1987; Westrom and Wolner-Hanssen, 1993). This deciliation appears to be permanent (Donnez *et al.*, 1984). It is possible that the function of the remaining cilia may not be affected (Cooper *et al.*, 1990). In women with tubal infertility, serological evidence of chlamydial infection is not associated with changes in CBF. However, there is preliminary evidence that particular chlamydial serotypes, such as serotypes C and E, may be associated with zero

or reduced levels of CBF respectively, although the numbers of subjects sampled are small, and these results must be interpreted with caution (Leng *et al.*, 1998). CBF is significantly lower in the surviving cilia of Fallopian tubes showing evidence of oedema, erythema or distal obstruction (Patton *et al.*, 1989; Leng *et al.*, 1998). Therefore, it appears that chlamydia causes deciliation and the associated chronic inflammation reduces CBF, although it is possible that certain serotypes may affect CBF directly and may presage a particularly poor prognosis for subsequent fertility.

#### Other pathogens

Inoculation of *Escherichia coli* into the Fallopian tubes of rabbits results in a dose-dependent deciliation, with the remaining cilia being swollen, shortened and adherent throughout their entire length. The secretory cells demonstrate loss of microvilli. However, regeneration does occur, commencing 2 weeks after the initial injury, and being complete by eight weeks (Laufer *et al.*, 1984).

Bacterial vaginosis (BV) is characterized by an overgrowth of vaginal anaerobic and facultative bacteria. It is associated with the development of endometritis and pelvic inflammatory disease, especially in the presence of other sexually transmitted diseases (Soper *et al.*, 1994; Hillier *et al.*, 1996; Wiesenfeld *et al.*, 2002). BV is strongly associated with tubal factor infertility (Gaudoin *et al.*, 1999). Some of the micro-organisms involved in the condition, including *Mycoplasma hominis*, *Mobiluncus* and *Bacteroides ureolyticus*, have been shown to cause direct tubal damage or altered ciliary activity.

Infection with *M. hominis* results in ciliostasis and swelling of the cilia (Mardh *et al.*, 1976). Infected human tubal mucosa cultures demonstrate either zero or reduced ciliary activity (Baldetorp *et al.*, 1983). Species of *Mobiluncus* (*Mobiluncus curtisii* and *Mobiluncus mulieris*) produce cytotoxins that reduce ciliary activity within 60 h in cultures of bovine oviduct. This is associated with loss of cilia and bloating and detachment of ciliated cells (Taylor-Robinson *et al.*, 1993). LPS endotoxins released from *B. ureolyticus* (*Prevotella*) also damage the mucosa of the human Fallopian tube, causing sloughing of cells and loss of ciliary activity (Fontaine *et al.*, 1988).

These various micro-organisms ascend into the upper genital tract where they frequently cause subclinical pelvic inflammation. There is an associated destruction of the ciliated cells combined with a general ciliostatic effect because of the release of endotoxins. These could be mechanisms by which BV contributes to the pathogenesis of tubal disease and infertility.

#### Conclusion

The Fallopian tube plays an essential role in tubal transport of both gametes and embryos and in early embryogenesis. The tube undergoes cyclical changes in morphology and ciliary activity in response to ovarian hormones. Whilst the varying contributions to tubal transport of ciliary activity, muscle contractions and secretory activity remain undetermined, there is emerging evidence that muscle contractions may play a role in mixing of secretions rather than in propulsion of gametes and embryos.

Ciliary activity is more vigorous in the secretory phase of the menstrual cycle. Many pathological conditions associated with

infertility and ectopic pregnancy have been shown either to destroy cilia or to reduce ciliary motion or both. Gonococcal infection produces both destruction of the ciliated cells and reduced ciliary activity, whereas chlamydia also destroys the tubal mucosa. Although the micro-organism itself does not appear to alter ciliary beat, the inflammation and oedema associated with chlamydial salpingitis has been shown to reduce CBF. Peritoneal fluid from women with mild-and-moderate endometriosis reduces CBF significantly *in vitro*. An 'ovum capture inhibitor' has been described in the peritoneal fluid of women with endometriosis, which covers the fimbrial cilia resulting in a complete but reversible loss of ovum capture ability. The 'immotile cilia syndrome' is known to be associated with subfertility. Deciliation is found in Fallopian tubes of women with a past history of ectopic gestation. These women are at increased risk of future tubal pregnancies. This evidence suggests an important role for the tubal cilia in the mechanism of gamete and embryo transport.

Further research needs to be undertaken to investigate the functioning of the cilia *in vivo*. Only one study has measured physiological CBF *in vivo* (Paltieli *et al.*, 1995), and this needs to be extended to the effect of pathological states on CBF. Direct examination of the effect of conditions such as endometriosis or pelvic inflammatory disease on ovum transport may be possible in animal models using laparoscopy to investigate ovum pick-up and fallopscopy to study ovum transit along the tube. It is only as we begin to understand more about the complex interactions of the effectors of tubal transport that we approach the possibility of being able to improve tubal transport in women afflicted with tubal infertility.

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