New insights into the mechanisms underlying hydrosalpinx fluid formation and its adverse effect on IVF outcome

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The adverse effects of hydrosalpinx on the outcome of IVF have been well documented; however, the causes for impaired implantation in patients with hydrosalpinx are poorly understood. Hydrosalpinx fluid has been shown to be toxic to mouse embryos but not human embryos, and this has become a topic of intense debate. An understanding of the mechanisms underlying hydrosalpinx formation following pelvic inflammatory disease appears to be essential in elucidating the causes for reduced implantation in hydrosalpinx patients and providing more rational treatments. This review discusses the mechanisms underlying hydrosalpinx formation and its adverse effect on IVF outcome, with new insights into possible involvement of Fallopian tube epithelial transporters and ion channels, particularly the cystic fibrosis transmembrane conductance regulator (CFTR). Possible links between *Chlamydia trachomatis* in pelvic inflammatory disease and the subsequent CFTR-mediated events in hydrosalpinx formation leading to infertility in hydrosalpinx are proposed. The causes of reduced implantation, particularly in patients with visible hydrosalpinges shown on ultrasound scanning, are re-examined in light of these possible mechanisms.

Key words: CFTR/chlamydial infection/hydrosalpinx/IVF

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Introduction

IVF was initially used to treat patients with tubal factor infertility (Steptoe and Edwards, 1978). It has been assumed for a long time that when the damaged tubes can be bypassed by IVF, the resulting pregnancy rate should be approaching that of a normal population (Lenton *et al.*, 1992). However, the presence of hydrosalpinges appears to be detrimental, even after IVF.

About 30% of infertile patients with tubal problems undergoing IVF have hydrosalpinges diagnosed by hysterosalpingography or laparoscopy (Strandell *et al.*, 1994; Blazar *et al.*, 1997; Murray *et al.*, 1997; Ng *et al.*, 1997). Many retrospective studies (Sims *et al.*, 1993; Andersen *et al.*, 1994; Kassabji *et al.*, 1994; Strandell *et al.*, 1994; Vandrome *et al.*, 1995; Vejtorp *et al.*, 1995; Akman *et*

al., 1996; Fleming and Hull, 1996; Katz *et al.*, 1996) as well as two reviews (Aboulger *et al.*, 1998; Nackley and Muasher, 1998) and two meta-analyses (Zeyneloglu *et al.*, 1998; Camus *et al.*, 1999) have convincingly shown a detrimental effect of hydrosalpinx fluid (HF) on implantation and pregnancy rates. A similar picture was found following the transfer of cryopreserved–thawed embryos (Strandell *et al.*, 1994; Akman *et al.*, 1996) and when donor oocytes were replaced (Cohen *et al.*, 1999). However, not all studies reported that IVF outcome was impaired by the hydrosalpinx (Sharara *et al.*, 1996; Blazar *et al.*, 1997; Ng *et al.*, 1997; Hurst *et al.*, 2001).

Different hypotheses have been put forward to explain reduced implantation of embryos in patients with hydrosalpinx. However, there is little information in the literature regarding the mechanisms of HF formation on which more rational treatments should be based. This review discusses the mechanisms underlying hydrosalpinx formation and its adverse effect on IVF outcome, with new insights into possible involvement of Fallopian tube epithelial transporters and ion channels, particularly the cystic fibrosis transmembrane conductance regulator (CFTR). Possible links between *Chlamydia trachomatis* in pelvic inflammatory disease and the subsequent CFTR-mediated events in hydrosalpinx formation leading to infertility in hydrosalpinx are proposed. The cause for reduced implantation, particularly in
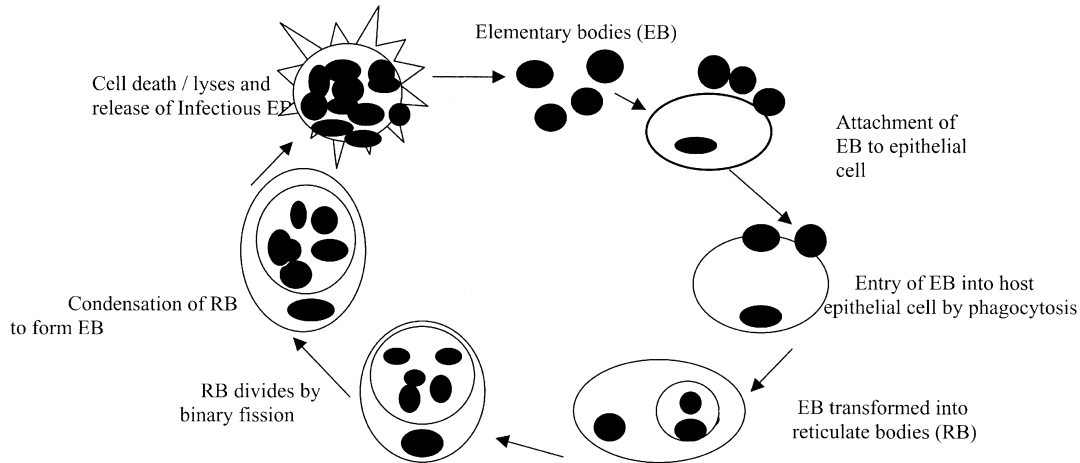
Figure 1. Developmental cycle of chlamydia in a host epithelial cell. The infections form of chlamydia elementary bodies (EB) attach to and are taken up into the host epithelial cell by phagocytosis. Inside the cell, EBs are transformed into reticulate bodies (RB) by condensation. RBs undergo binary fission to produce EBs that are released on cell death by lysis.

patients with visible hydrosalpinges shown on ultrasound scanning, will be re-examined in light of these possible mechanisms. New research on elucidating the role of CFTR in hydrosalpinx is suggested.

Chlamydial infection and pelvic inflammatory disease

C. trachomatis is an intracellular obligate bacterium that depends on its host for energy substrates for metabolism and reproduction. *C. trachomatis* exists in two forms, alternating between the elementary body (EB), a metabolically inactive infectious form, and the reticulate body, an intracellular metabolically active form responsible for reproduction. Figure 1 illustrates the developmental cycle of chlamydia in a host cell.

C. trachomatis infection is the most common cause of pelvic inflammatory disease (PID) leading to severe tubal damage, hydrosalpinges, ectopic pregnancy and infertility (Walters et al., 1988; Cates and Wasserheit, 1991). Based on the available evidence, ~20% of women with chlamydial lower genital tract infection develop PID, ~4% chronic pelvic pain, 3% infertility and 2% adverse pregnancy outcome (Paavonen and Eggert-Kruse, 1999). Endometritis due to *C. trachomatis* has also been reported (Gump et al., 1981; Mardh et al., 1981; Wolner-Hanssen et al., 1982; Winkler et al., 1984). In a study by Jones et al. 41% of women with evidence of *C. trachomatis* infection had endometrial infections (Jones et al., 1986). Wolner-Hanssen et al. recovered *C. trachomatis* from the uterus and Fallopian tubes of women with acute salpingitis (Wolner-Hanssen et al., 1982). *C. trachomatis* DNA or antigens have been detected in women with post-infectious tubal factor infertility (Patton et al., 1994) and from the endocervix of women with negative cervical culture (Witkin et al., 1999). An increased prevalence of spontaneous abortion in women with a history of chlamydial salpingitis (Toth et al., 1992) or previous exposure to *C. trachomatis* (Licciardi et al., 1992) has been reported. Serological studies on women with salpingitis have demonstrated a strong association of tubal factor infertility with past *C. trachomatis* infection (Jones et al., 1986). The majority of the cases are sub-acute, leading to persistent and/ or chronic infection, which elicits an immune response.

Witkin et al. found increased levels of chlamydial heat shock proteins (HSP) in women with previous chlamydial infections (Witkin et al., 1994). Antibodies to these HSP were more prevalent in women with hydrosalpinx and tubal occlusion than in women with male factor infertility (Spandorfer et al., 1999b). Constant exposure to *C. trachomatis* through persistent infection leads to the production of HSP that elicits intense immune and inflammatory reactions, leading to tissue scarring and endometrial and Fallopian tube damage. These proteins are also thought to be responsible for the induction of local immune responses that lead to an inflammatory reaction, impaired implantation and immune rejection after embryo transfer. The presence of cervical IgA antibody to chlamydial HSP has been strongly correlated with unsuccessful outcome after embryo transfer (Witkin et al., 1994). HSP 10 is associated with tubal factor infertility in a *C. trachomatis*-exposed population (La Verda et al., 2000) and HSP 57 with elementary and reticulate bodies and a delayed hypersensitivity response (Witkin et al., 1994). HSP 60 is capable of eliciting intense mononuclear inflammation and elaboration of inflammatory cytokines (La Verda et al., 1999) and might increase susceptibility to early pregnancy loss (Witkin, 1999). Chlamydial HSP may function in at least two ways to promote chronic disease: first, by direct antigenic stimulation and second as signal transducers that result in macrophage activation (La Verda et al., 1999).

Despite the long recognition of *C. trachomatis* as the most important cause of PID worldwide, the sequence of events linking chlamydia to HF formation and subsequent infertility has not been elucidated to a satisfactory extent.

Possible mechanisms underlying HF formation

Fluid formation in the Fallopian tube

It remains largely unknown why fluid accumulates and distends the Fallopian tubes in post-infectious hydrosalpinx. Fluid movements across secretory epithelia including those lining the Fallopian tube are secondary to ion movements. The importance of ion movements across the oviduct lies in their coupling with the movement of water, which is not actively transported but

moves in response to osmotic gradients, largely established by the transport of ions (Leese, 1988). Early studies examining ion movements across the oviduct (Brunton and Brinster, 1971; Brunton, 1972; Leese, 1988) have demonstrated that chloride ions move actively in the direction from serosa to mucosa (i.e. into the oviduct lumen) while sodium ions move at equal rates in each direction, although the mechanism by which ions move across the oviduct has not been fully investigated. Active sodium absorption drives chloride ions and fluid from the lumen into the cells, while active chloride secretion drives sodium and water into the lumen in the same way. Adrenergic agonist (isoprenaline) added to the vascular medium stimulated tubal fluid formation, an effect that was abolished by the antagonist (propranolol) (Dickens and Leese, 1994). Using vascular perfusion technique, it was found that cell to lumen movement of chloride ions was associated with fluid secretion in the rabbit oviduct (Gott et al., 1988; Dickens and Leese, 1994). Further studies with rabbit polarized primary epithelial cell culture showed that chloride ion flux from the basal to apical direction was greater than that in the direction from the apical to basal (Gott et al., 1988; Dickens et al., 1993). Human Fallopian tube epithelial cells were reconstituted in a polarized culture and chloride ion movement was found to be responsible for the generation of transepithelial potential difference across the cultured epithelial cells (Dickens et al., 1996; Downing et al., 1997). These chloride fluxes have been shown to be sensitive to the inhibitors of some ion channels and co-transporters (Gott et al., 1988).

Epithelial ion channels and transporters

The loss of membrane polarity may lead to decreased expression of epithelial membrane transporters and ion channels. This could be responsible, at least in part, for the formation of HF following pelvic inflammatory disease. These transporters may include sodium/hydrogen exchangers (NHE), anion Cl^-/HCO_3^- (AE) exchangers, sodium bicarbonate co-transporter (NBC) and sodium epithelial channels (ENaC), aquaporin water channels (AQP) and CFTR, a cAMP-activated chloride channel. NHE and AE are known plasma membrane transporters in mammalian epithelial cells, and have been shown to be involved in homeostatic functions such as intracellular pH regulation and cell volume control. They are also involved in modulating the intracellular concentration of cAMP, which is responsible for several physiological processes and mediating transepithelial transport of salt and water (Yun et al., 1995). It has been demonstrated in our laboratory that NBC is responsible for the transport of HCO_3^- across the basolateral membrane from the blood into cells (Wang et al., 2002), while apically located AE and CFTR transport HCO_3^- into the lumen interdependently in mouse uterine epithelium (X.F.Wang et al., unpublished data).

Post-infectious hydrosalpinx pathology–atrophy of mucosal folds, marked exfoliation and loss of epithelial cells may affect epithelial membrane ion channels and transporters located on the epithelial membrane, which are necessary for electrolyte and fluid transport, therefore leading to abnormal fluid secretion and reabsorption. CFTR, in particular, may also play an important role in the process of HF formation considering the accumulated findings on the properties of CFTR.

CFTR as a chloride channel

CFTR is the only member of the superfamily of ATP-binding cassette (ABC) transporters that has been proven to function as a chloride channel. It has been shown that CFTR chloride channel activation depends on phosphorylation by cAMP-dependent protein kinase A (PKA) and ATP hydrolysis or binding. Phosphorylation occurs in the regulatory domain and ATP hydrolysis in the nuclear binding domain. Transmembrane domain 1 plays a role in the formation of the channel pore. The CFTR chloride channel is an independent chloride channel capable of transporting chloride ions. It has been shown that an increase in the concentration of cAMP leads to an increase in the transepithelial potential difference or current and subsequently increase in fluid secretion, indicating the involvement of the CFTR chloride channel (Sheppard and Welsh, 1999).

CFTR as a regulator

In addition to being a chloride channel, CFTR also functions as a regulator of a number of ion channels and transporters. CFTR regulates outwardly rectifying chloride channels (ORCC) by an autocrine mechanism involving ATP release. Devidas and Guggino proposed that ATP and chloride might be conducted through CFTR (Devidas and Guggino, 1997). ATP may then stimulate ORCC, thereby increasing chloride secretion.

Evidence suggests that CFTR controls the activity of calcium-activated chloride currents, regulates voltage-dependent potassium channels and activates water permeability through AQP (Kunzelmann and Screiber, 1999; review). It has also been reported that CFTR regulates vesicle trafficking in a cAMP-dependent manner, and itself undergoes regulated trafficking (Bradbury, 1990).

The best-known ion channel regulated by CFTR is ENaC, which has been shown to be inhibited by the activation of CFTR in a number of epithelia (Stutts et al., 1995; Briel et al., 1998; Chan et al., 2001). Single channel studies of ENaC expressed in fibroblasts suggested that CFTR alters the sodium current by changing ENaC's open probability (Stutts et al., 1997). It has been suggested that CFTR and ENaC may interact with each other by protein–protein binding (Ismailov et al., 1997; Kunzelman et al., 1997). The co-ordination of proteins involved in transepithelial ion transport could be one of the essential functions of CFTR in epithelial cells. CFTR's regulatory effects on other transporters could be due to its ability to activate GTP-binding proteins and control of exocytotic and endocytoic processes of membrane proteins (Kunzelman et al., 1997). Using in-situ hybridization, Chan et al. have demonstrated the distribution of CFTR and ENaC subunits along the mouse female reproductive tract, including the oviduct (Chan et al., 2002). Evidence has also suggested that ENaC and CFTR are involved in mediating absorptive and secretory activities respectively in the uterine epithelium, and CFTR is a negative regulator of ENaC (Deachapunya and O'Grady, 1998; Chan et al., 2000). The interactions between CFTR and other ion channels may also be important for the balance of secretion/absorption in the oviduct, disruption of which may lead to hydrosalpinx.

CFTR as a receptor

In addition to its role as a chloride channel and a regulator for other ion transporters, CFTR has also been shown to act as a receptor for bacteria in epithelial cells. In pulmonary epithelial cells as well as corneal cells, CFTR is the receptor for *Pseudomonas aeruginosa* internalization (Pier *et al.*, 1997, 1998; Zaidi *et al.*, 1999; Chroneos *et al.*, 2000; Goldberg and Pier, 2000; Pier, 2000). In the gastrointestinal tract epithelial cells, *Salmonella typhi* and *Cholera vibro* also bind to CFTR leading to an increase in cAMP and subsequent diarrhoea due to increased serosa-to-mucosa fluxes of both sodium and chloride ions (Gabriel *et al.*, 1994; Pier *et al.*, 1998; Gerceker *et al.*, 2000).

The first event in *C. trachomatis* infection of a mammalian cell is the attachment of an infectious particle to a receptor on the surface of a host cell. The ability of EBs to bind to and subsequently invade epithelial cells is the key point in chlamydial pathogenesis. Bacterial genus-specific lipopolysaccharide (LPS), major outer membrane protein and HSP are among the chlamydial ligands for adherence. These are also the bacterial ligands identified in other bacteria using CFTR for epithelial cell internalization. *C. trachomatis serova E* infections are largely localized to epithelial cells in various human tissues giving rise to a spectrum of pathological conditions such as pneumonitis, conjunctivitis, keratitis, cervicitis, endometritis and salpingitis. These epithelial cells are also rich in CFTR expression. We
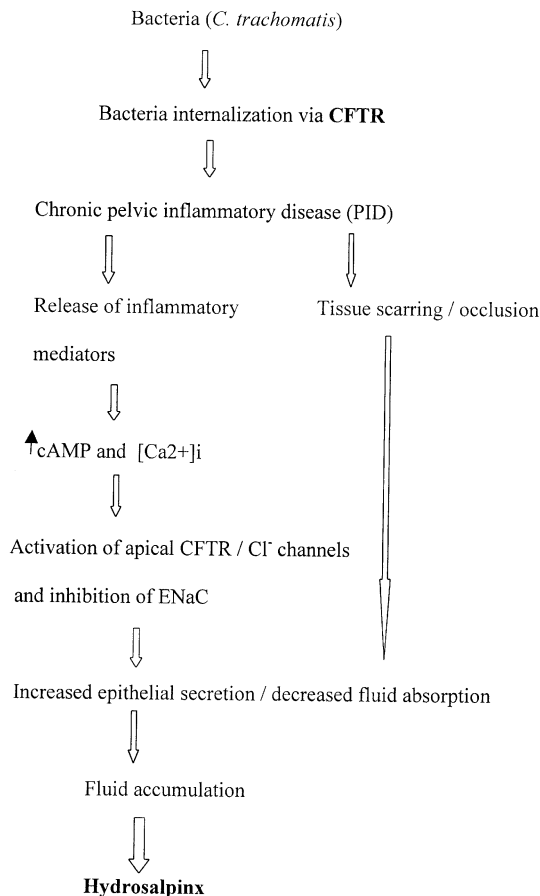
therefore hypothesize that *C. trachomatis* EBs may also use CFTR as a receptor for cellular internalization in the female reproductive tract epithelium as well as other epithelia (Figure 2). *C. trachomatis* infection of cells has been reported to cause an increase in tyrosine phosphorylation of proteins (Bliska *et al.*, 1993; Birkelund *et al.*, 1994; Fawaz *et al.*, 1997 and Hosseinzadeh *et al.*, 2000), an important step in the signal transduction pathway of increased epithelial fluid transport from serosa to lumen. Therefore, bacterial infection may alter the fluid transport profile, via CFTR, in the Fallopian tubes as seen in hydrosalpinx. Figure 3 shows the proposed sequence of events linking *C. trachomatis* infection and infertility in hydrosalpinx.

Effects of inflammatory mediators in oviductal fluid formation

Inflammatory mediators have marked effects on the secondary messengers and secretory responses in a number of epithelia. Prostaglandins, histamine, serotonin (5-HT), platelet-activating factor (PAF) and other products of inflammation may also act on the oviduct epithelium to increase secretion. In epithelial cells of other tissues, histamine has been shown to promote microvascular permeability via an increase in intracellular calcium $[Ca^{2+}]i$ (Huang and Yuan, 1997; Downing *et al.*, 1999). There are also indications that inflammatory mediators alter sodium transport in the airway epithelium (Clarke *et al.*, 1992). In cultured human Fallopian tube epithelial cells, histamine influenced ion movements and therefore may also influence tubal secretion (Downing *et al.*, 1999). In chronically inflamed tubes as in hydrosalpinx, histamine production may increase HF production. PAF promotes the synthesis of prostaglandins and stimulates, either directly or indirectly, myometrial and possibly myosalpingeal contractions that may cause retrograde spillage of HF into the uterine cavity. Occlusion of fimbrial ends of the tubes increases luminal hydrostatic pressure leading to increased fluid accumulation.

Effect of ovarian stimulation hormones on fluid formation

Ovulation induction is associated with formation and sudden increase in HF (Hill *et al.*, 1986; Schiller and Tsuchiyama, 1995; Bloechle *et al.*, 1997; Sharara and McClamrock, 1997). Some hormones, including those for ovarian hyperstimulation, are known to stimulate an increase in the cellular concentra-

Figure 2. Hypothetical involvement of the cystic fibrosis transmembrane conductance regulator (CFTR) as a receptor in the formation of hydrosalpinx.
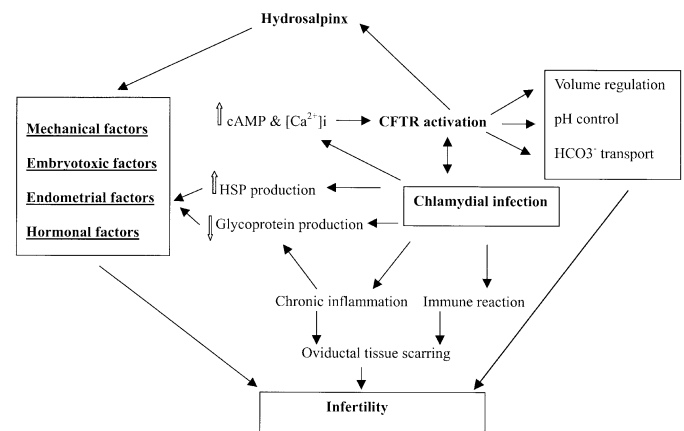

Figure 3. Proposed sequence of events linking chlamydial infection and infertility in hydrosalpinges.

tion of cAMP, which also plays an important role in the stimulation of epithelial water and electrolyte secretion. Dickens *et al.* observed a transient increase in potential difference across an epithelial cell monolayer when adrenaline was added to the basal side and noticed a small increase in basal to apical chloride transport across the cells (Dickens *et al.*, 1993). In ewes, estrogen treatment increased fluid secretion, while progesterone decreased fluid secretion (Mastroianni *et al.*, 1961). Pregnant mare serum gonadotrophin and estrogen, both *in vivo* and *in vitro*, have been shown to up-regulate CFTR (Rochwerger and Buchwald, 1993; Rochwerger *et al.*, 1994). The presence of a forskolin-sensitive anion current in the oviductal epithelia of normal but not CFTR knockout mice indicates CFTR involvement in mediating hormone-regulated electrolyte and fluid secretion.

Other hormones, including those for ovulation induction, may also up-regulate CFTR and this may be responsible for the sudden increase in HF production observed in patients with tubal infertility undergoing ovarian stimulation for IVF treatment. Their actions may be via one or more of the second messengers involved in the signal transduction pathway of CFTR activation. There is no report yet of hydrosalpinx in cystic fibrosis (CF) patients. The decreased fertility rate in CF women has been attributed to thick cervical mucus leading to decreased sperm penetration. However, CFTR-mediated bicarbonate transport may also be affected, which could contribute to reduced fertility in CF women (H.C.Chan *et al.*, unpublished data). Figure 4 is a schematic illustration of possible CFTR involvement in the mechanisms underlying HF formation.

Possible causes for impaired implantation in hydrosalpinx

Mechanical factors

Mansour *et al.* and Andersen *et al.* suggested that reflux of HF into the uterine cavity from the Fallopian tubes could produce a 'flushing effect' that inhibits implantation (Mansour *et al.*, 1991; Andersen *et al.*, 1996). The reflux of HF also prevents appropriate contact between the embryo and endometrial epithelium (Vandrome *et al.*, 1995). Fluid in the uterine cavity during implantation may directly exert a detrimental effect. Even the presence of a tiny amount of fluid inside the uterine cavity may interfere with the implantation process, since the ultramicroscopic pinopodes on the luminal surface of the endometrial epithelial cells may have the function of extracting fluid from the microenvironment of the embryo, thus facilitating its contact with the endometrium during implantation (Edwards, 1992).

Steroids are thought to affect secretions in the Fallopian tubes and uterine cavity. Alterations in their levels and/or receptor expression could contribute to a poor uterine environment. Ultramicroscopic pinopodes on the luminal surface of the endometrial epithelial cells are reported to be influenced by progesterone (Martel *et al.*, 1991). Their malfunction (Edwards, 1992) under altered progesterone levels would leave the transferred blastocysts stranded in a mass of uterine fluid. Hill *et al.* and Schiller and Tsuchiyama demonstrated that hydrosalpinx might actually enlarge during ovarian stimulation in some women undergoing ovulation induction who had no evidence of hydrosalpinx on their initial ultrasonography (Hill *et al.*, 1986; Schiller and Tsuchiyama, 1995). Bloechle *et al.* reported a case where a patient developed bilateral hydrosalpinges during ovarian
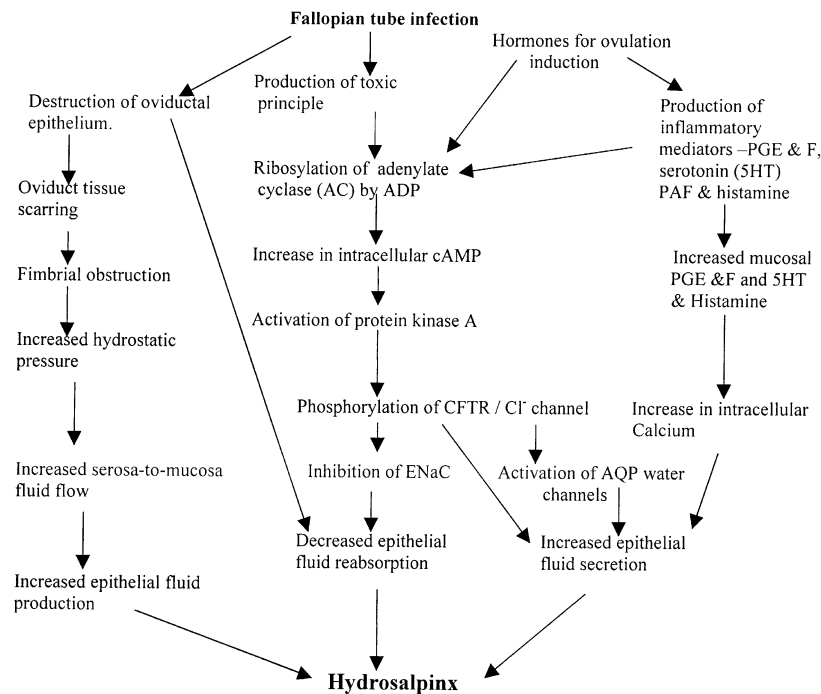

Figure 4. A schematic illustration of proposed mechanisms underlying HF formation. AC = adenyl cyclase; ADP = adenosine diphosphate; AQP = aquaporin water channels; cAMP = cyclic AMP; CFTR = cystic fibrosis transmembrane conductance regulator; Cl⁻ channel = chloride channel; ENaC = epithelial sodium channel; PAF = platelet activating factor; PGE&F = prostaglandins E and F; 5HT = serotonin.

stimulation that rapidly refilled within 2 days after aspiration (Bloechle *et al.*, 1997). Moreover, Sharara and McClamrock showed reflux of HF into the endometrium after HCG administration (Sharara and McClamrock, 1997). As mentioned above, it is likely that these hormones for ovulation induction further stimulate the production of cAMP, on which activation of CFTR depends, hence leading to increased fluid production. Together with the inhibition of fluid reabsorption due to inhibition of Na^+-reabsorbing ENaC by CFTR, a large volume of HF presents a mechanical factor physically interfering with embryo contact with the endometrium during implantation.

Another factor may involve the endometrial wave pattern, which is normally associated with pregnancy in IVF and spontaneous natural cycles, but becomes more pronounced in stimulated cycles (Ijland, *et al.*, 1996, 1997, 1998, 1999). It is possible that HF might influence the wavelike activity of the endometrium. Eytan *et al.* used a mathematical model to show that hydrosalpinx generates a pressure gradient between the cervix and uterine fundus (Eytan *et al.*, 2001). This pressure may adversely counter the cervix-to-fundus intrauterine peristalsis that may thrust embryos away from implantation sites.

Embryotoxic factors

HF toxicity has been documented on mouse embryos (Mukherjee *et al.*, 1996; Beyler *et al.* 1997; Murray *et al.*, 1997; Rawe *et al.*, 1997; Sachdev *et al.*, 1997; Koong *et al.*, 1998; Roberts *et al.*, 1999; Spandorfer *et al.*, 1999a; Arrighi *et al.*, 2001; Carrasco *et al.*, 2001) with one exception, that is the study by Saito *et al.* which did not show mouse embryotoxicity (Saito *et al.*, 2000). Limited studies on human embryos (Granot *et al.*, 1998; Strandell *et al.*, 1998) and placenta cells (Sawin *et al.*, 1997) did not demonstrate embryotoxicity. Studies on mouse embryo culture in HF showed that, in all studies in which 1-cell stage embryos were used, embryo development was impaired at low HF concentrations, while 2-cell stage embryo development was only affected at very high concentrations. Therefore, HF may have an adverse influence on mouse embryo development from the pronuclear to 2-cell stage, but not thereafter, particularly at low concentrations (Ajonuma *et al.*, 2001). The adverse effect of HF on mouse but not human embryos may be related to inter-species differences with respect to optimal culture conditions (Ng *et al.*, 2000; Ajonuma *et al.*, 2001).

The embryotoxic effects may be due to its altered chemical composition. Chemical analysis of HF showed a similar composition to serum with respect to most electrolytes, but lower in calcium, glucose, protein and osmolality (Ng *et al.*, 2000). HF composition is also not very different from that of normal human tubal fluid (Lippes *et al.*, 1972; David *et al.*, 1973; Borland *et al.*, 1980; Dickens *et al.*, 1995; Tay *et al.*, 1997). However, despite the similarities, some variations do exist and may affect embryo development. Schenk *et al.* reported that eight of the nine HF samples they analysed did not meet the standard of a good culture medium (Schenk *et al.*, 1996).

Murray *et al.* showed that mouse embryo development was inhibited when cultured in 100% HF, but this inhibition was reversed upon addition of 10 mmol/l lactate (Murray *et al.*, 1997). We have shown that low osmolality, low lactate and low protein levels of HF affected sperm motility and survival *in vitro* (Ng *et al.*, 2000) and embryo development (L.C.Ajonuma *et al.*,

unpublished observation). Electrolytes are primarily responsible for maintaining the osmolality of a fluid. CFTR, which has been shown to be a receptor to a number of bacteria in addition to being a regulator of ion channels and transporters, may be constantly phosphorylated during epithelial infection, leading to an increase in transepithelial fluid secretion. In our previous study (Ng *et al.*, 2000), HF samples with low osmolality were also found to have very low electrolyte concentrations, and human sperm motility was virtually zero within 30 min of incubation with these samples. This study was not alone. Strandell *et al.* also had two HF samples with extremely low osmolality values with very low sodium and potassium concentrations (Strandell *et al.*, 1998). In that study, these samples showed poor human embryo development *in vitro*. This may also be the fate of transferred embryos in the presence of HF with low osmolality.

The HF with low osmolality may be due to up-regulation of AQP by CFTR (Kunzelmann and Schreiber, 1999) leading to enhanced water permeability. In hydrosalpinx, this may produce a 'dilution effect' on the electrolytes important for embryo development.

Low protein values in HF may reflect the impaired secretory activity of the epithelial lining of post-infectious hydrosalpinx. Low bicarbonate levels of HF might affect the development of newly ovulated and fertilized oocytes as well as sperm (David *et al.*, 1969). Damaged cells of both the tubes and endometrium may not produce adequate embryotrophic substances needed for early embryo development.

A number of other factors, including cytokines, may also contribute to embryo toxicity in hydrosalpinx. David *et al.* suggested that macrophages, plasmocytes and other cellular elements involved in the late inflammatory reaction might release cytokines, prostaglandin, leukotrienes and other compounds that could be deleterious to intrauterine oocytes (David *et al.*, 1969). Hydrosalpinges may secrete cytokines that adversely affect pregnancy outcome (Grifo *et al.*, 1989; Toth *et al.*, 1992) via haematogenous and lymphatic routes. Chen *et al.* studied cytokines in HF and reported that their concentrations were not predictive of subsequent IVF outcomes (Chen *et al.*, 2002). However, cytokine concentrations have been shown to be higher in severe pelvic adhesions (Cheong *et al.*, 2002). The effect of reactive oxygen species concentration in HF on mouse embryo development has also been reported (Bedaiwy *et al.*, 2002), indicating their possible involvement.

Endometrial factors

The endometrium could be damaged simultaneously with the acute-phase damage of the Fallopian tubes, leaving the endometrium with a permanently diminished capacity for implantation (Strandell *et al.*, 1994).

Bioactive substances such as tumour necrosis factor and other growth hormones associated with salpingitis (Toth *et al.*, 1992) have been shown to alter both endometrial stromal and epithelial cells. Catecholamines and peptidergic transmitters that participate in the regulation of tubal and uterine motility may alter endometrial receptivity (Owman *et al.*, 1992). The presence of hydrosalpinges may affect endometrial receptivity by affecting the endometrial integrins (Lessey *et al.*, 1994). Meyer *et al.* have demonstrated that integrins associated with the window of implantation $\alpha_v\beta_3$ are found to be decreased in women with

hydrosalpinges compared with controls (Meyer *et al.*, 1997). These women's endometria were described as 'out of phase' endometria due to the presence of gland/stromal dyssynchrony. However, 70% (14/20) of these women showed increased integrin expression after surgical correction of their hydrosalpinges. Bildirici *et al.* reported that surgical removal of communicating hydrosalpinges increased the expression of $\alpha_v\beta_3$ and therefore may improve endometrial receptivity (Bildirici *et al.*, 2001). Illera *et al.* demonstrated that blockage of $\alpha_v\beta_3$ resulted in impaired implantation in the mouse (Illera *et al.*, 2000). Putting these results together, it clearly shows that HF may contain yet unknown substance(s) that interfere with the expression of integrins. Dysregulated expression of ebaf (a transforming growth factor) in the endometria of a subset of infertile women has been reported to affect uterine receptivity (Tabibzadeh *et al.*, 2000). Sharara *et al.* suggested that chronic endometritis due to chlamydia or the effect of protein expression might directly affect endometrial receptivity (Sharara *et al.*, 1996). Cells infected with *C. trachomatis* may produce a reduced amount of major structural proteins but increased levels of 57 kDa HSP (Beatty *et al.*, 1993). Epithelial cell exfoliation, thinning and transformation from columnar to flat cuboidal epithelium in hydrosalpinx may reduce the apically located CFTR that regulates vesicular trafficking of epithelial cell-synthesized sulphated glycoproteins, which are essential for early embryo development.

In a rabbit model, preovulatory ova were transferred into chronic sterile hydrosalpinges induced by infundibular ligation in four rabbits and into another four with chronic infective hydrosalpinges. Normal pregnancies implanted in the uterine horns of all the sterile hydrosalpinx rabbits, but none in the chronic infective hydrosalpinx group. This points to a sequel of infection, rather than simple dilation due to fluid accumulation of the oviduct, as the cause of infertility (McComb *et al.*, 1983).

Management of hydrosalpinges

HF represents a heterogeneous fluid. Individual variations exist and these yield different influences. This may be the reason why there are variations in the different studies on IVF outcome. However, whatever the pathogenesis might be, one of the major concerns for treatment options should be geared toward maximizing IVF outcome. Currently, only two major types of treatment are mentioned in the literature: surgical (salpingectomy, aspiration of HF, Fallopian tube ligation and salpingostomy) and medical treatment. However, the latter has not been widely explored.

Surgical treatment

Aspiration of HF prior to ovarian stimulation or before embryo transfer during oocyte retrieval has been reported to be of little success (Russel *et al.*, 1991; Sharara *et al.*, 1996; Van Voorhis *et al.*, 1998). HF aspiration before ovulation induction produced a better ovarian response but did not improve pregnancy rate (Aboulghar *et al.*, 1990) and no improvement on pregnancy rate was also observed in another larger scaled study (Sowter *et al.*, 1997). However, Van Voorhis *et al.* reported improved pregnancy and implantation rates after ultrasound-guided aspiration of hydrosalpinges at the time of oocyte retrieval (Van Voorhis *et al.*, 1998).

Salpingectomy before IVF was suggested to eliminate the negative effects of hydrosalpinges (Vandrome *et al.*, 1995: Shelton *et al.*, 1996; Freeman *et al.*, 1998; Strandell, 2000). A randomized multicentre study in Scandinavia (Strandell *et al.*, 1999) was unable to demonstrate a statistically significant benefit of salpingectomy for hydrosalpinx prior to IVF, except for a subset of women with hydrosalpinx large enough to be visible by ultrasonography. More recent cumulative results after salpingectomy in a randomized control trial from the same group have showed a statistically significant effect (Strandell *et al.* 2001). Another recent report based on a Cochrane review published in the Cochrane library has also provided evidence that laparoscopic salpingectomy should be considered for all women with hydrosalpinges due to undergo IVF (Johnson *et al.*, 2002). A significant improvement of pregnancy rates after bilateral salpingectomy in patients with bilateral hydrosalpinges shown on scanning may be related to much lower osmolality of HF in these patients.

Medical treatment

Hydrosalpinges and infertility are sequels of *C. trachomatis* infection in most cases after a persistent silent or chronic endometritis and salpingitis. It has been proposed that increased exposure to chlamydial antigens, such as through reinfection or persistent infection, results in chronic inflammation, tissue scarring and contributes to the pathogenesis of endometrial and Fallopian tube damage. This immunopathological damage is believed to be the principal cause of tubal factor infertility (La Verda *et al.*, 1999). Unfortunately, little has been done towards using medical treatment, even when it is known that *C. trachomatis* infection is the major cause of tubal infertility.

Sharara *et al.* attempted medical treatment prior to IVF (Sharara *et al.*, 1996). They reported no significant difference between hydrosalpinx and non-hydrosalpinx groups on implantation, pregnancy and early pregnancy loss rates among patients undergoing IVF after pretreatment with 100 mg doxycycline twice daily for 10 days. No randomization was done and the authors could not rule out any treatment effect. In a more recent retrospective study, Hurst *et al.* reported no detrimental effect of hydrosalpinx with extended doxycycline treatment prior to IVF (Hurst *et al.*, 2001). Persistence of *C. trachomatis* was shown in Fallopian tubes after treatment of chlamydia and, in future studies, extended antibiotic treatment may reduce or eradicate the remaining chlamydial infection and prevent further fluid accumulation in the hydrosalpinx.

Conclusions

In summary, chronic inflammation of human Fallopian tubes following chlamydial infection leads to subsequent HF formation and tubal infertility. Inflammatory mediators may act on Fallopian tube epithelial cells to directly cause microvascular permeability and via secondary messengers, leading to abnormal fluid production. The interactions between CFTR and other epithelial transporters are important for the balance of secretion/absorption, disruption of which may lead to hydrosalpinx. During inflammation, CFTR in oviductal cells may be continually activated, leading to increased fluid secretion and decreased fluid absorption through CFTR inhibition of ENaC. Occlusion of the fimbrial ends

prevents the fluid from draining into the pelvic cavity, hence the retrograde spillage of HF into the uterine cavity, which may act as a mechanical barrier between embryo and the endometrium. Decreased glycoprotein production and enhanced CFTR-mediated water permeability may produce a dilution effect on essential embryotrophic substances. A combination of all or some of these factors may explain the low implantation rate following IVF treatment in women with hydrosalpinges. New research focusing on the involvement of CFTR in HF formation may provide crucial information for a better treatment strategy to enhance IVF outcome for patients with hydrosalpinges.

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