Nitric oxide synthases and tubal ectopic pregnancies induced by *Chlamydia* infection: basic and clinical insights

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**ABSTRACT:** Human ectopic pregnancy (EP) remains a common cause of pregnancy-related first trimester death. Nitric oxide (NO) is synthesized from L-arginine by three NO synthases (NOS) in different tissues, including the Fallopian tube. Studies of knockout mouse models have improved our understanding of the function of NOS isoforms in reproduction, but their roles and specific mechanisms in infection-induced tubal dysfunction have not been fully elucidated. Here, we provide an overview of the expression, regulation and possible function of NOS isoforms in the Fallopian tube, highlighting the effects of infection-induced changes in the tubal cellular microenvironment (imbalance of NO production) on tubal dysfunction and the potential involvement of NOS isoforms in tubal EP after *Chlamydia trachomatis* genital infection. The non-equivalent regulation of tubal NOS isoforms during the menstrual cycle suggests that endogenous ovarian steroid hormones regulate NOS in an isoform-specific manner. The current literature suggests that infection with *C. trachomatis* induces an inflammatory response that eventually leads to tubal epithelial destruction and functional impairment, caused by a high NO output mediated by inducible NOS (iNOS). Therefore, tissue-specific therapeutic approaches to suppress iNOS expression may help to prevent ectopic implantation in patients with prior *C. trachomatis* infection of the Fallopian tube.

**Key words:** nitric oxide / nitric oxide synthase isoforms / *Chlamydia trachomatis* / tubal ectopic pregnancy

**Introduction**

Ectopic implantation in the first trimester of pregnancy is a common cause of human maternal morbidity and mortality (Corpa, 2006). It accounts for 1.5–2% of all pregnancies in the western world (Barnhart, 2009), and ~97% of ectopic pregnancies (EPs) are in the Fallopian tube (Mukul and Teal, 2007). Tubal EP is a growing problem in developing countries (Farquhar, 2005; Barnhart, 2009). The mammalian Fallopian tube is a dynamic, steroid-responsive tissue (Jansen, 1984) composed of heterogeneous cell types: ciliated and secretory epithelial cells as well as smooth muscle cells, all of which appear to be specialized to perform different functions (Shao et al., 2007a, b).

Tubal abnormalities and dysfunction (e.g. altered abnormal ciliary activity or contractility) are thought to lead to tubal EP, but the precise aetiology of its initiation and development is unknown (Shao, 2010). A major risk factor is previous pelvic inflammatory disease, which is quite common in women with tubal EP (Brunham and Rey-Ladino, 2005). According to the World Health Organization (WHO) (http://www.who.int/medicentre/factsheets/fs110/en/index.html), 10–40% of women with untreated *Chlamydia trachomatis* infection worldwide develop symptomatic pelvic inflammatory disease associated with complications. Women infected with *C. trachomatis* in the genital tract are highly susceptible to tubal EP (Chow et al., 1990). An untreated *C. trachomatis* infection is likely to injure tissue, cause permanent scarring and obstruct the Fallopian tubes at multiple sites (Paavonen and Lehtinen, 1996; Farquhar, 2005) as a consequence of the host inflammatory immune response (Entrican et al., 2004), ultimately inhibiting or disrupting gamete transport. However, the
pathological mechanisms that induce tubal EP are unknown. Since 70–90% of women with C. trachomatis infection do not have symptoms (Peipert, 2003), the WHO has recommended screening strategies to decrease the C. trachomatis spread and thereby indirectly decrease the tubal EP rate. In many industrialized countries and China, the prevalence of C. trachomatis infection is 2–5% of the population (Fenton et al., 2001; Parish et al., 2003; Miller et al., 2004).

How C. trachomatis infection induces Fallopian tube damage is poorly understood at the molecular level, and a better understanding of the biological basis of infection-induced tubal EP is needed to aid its prevention and treatment. Nitric oxide (NO) is an endogenous short-lived signalling molecule involved not only in multiple physiological processes but also in diseases in different organs/tissues (Moncada and Higgs, 1991; Moncada et al., 1991), including the Fallopian tube (Rosselli et al., 1998). The role of NO in regulating gene expression, enzyme activity, and transcription factor activation has been extensively demonstrated in several experimental models other than Fallopian tube (Moncada and Higgs, 1991; Moncada et al., 1991). NO is a double-edged sword. At low levels, it alters intracellular Ca²⁺ levels and activates uterine smooth muscle relaxation (Telfer et al., 1995). But at high levels, it causes infection-induced immune reaction and inflammation-induced tissue lesions (Moncada and Higgs, 1991; Moncada et al., 1991; Rosselli et al., 1998).

The purpose of this review is to highlight the potential role of nitric oxide synthases (NOS) in Fallopian tube physiology and to discuss how C. trachomatis infection changes the tubal microenvironment by regulating NO production by different NOS isoforms, leading to tubal EP. The current review also suggests future research directions.

**NO signalling and NOS isoforms in the Fallopian tube**

The diverse biological actions of NO as an intra- and inter-cellular messenger are mediated by binding to metal-containing centres of enzymes and by stimulation of the soluble NO-sensitive form of guanylate cyclase (sGC). This event leads to increases in cyclic guanosine monophosphate (cGMP) levels and activation of cGMP-dependent protein kinase (PKG) or cGMP-independent intracellular effects in target cells (Moncada et al., 1991). In the clinic, treatment of vagina with sildenafil citrate, which inhibits cGMP breakdown, increases endometrial thickness (Jerzak et al., 2008), which may increase the chance of successful pregnancy at spontaneous conception or IVF in recurrent miscarriage patients. As far as caution on cardiovascular effects of sildenafil in humans is concerned, there is a need for studies to evaluate toxicities associated with sildenafil treatment, in particular the human risk assessment, before clinical use of sildenafil in women with EP.

The precise role of the NO-cGMP signalling pathway in the Fallopian tube is unclear. Rat Fallopian tube cells express a number of NO-cGMP signalling components, such as sGCx, sGCβ1, PKG1ε and PKG1α (Zhan et al., 2003). The mammalian Fallopian tube is a site of endogenous production of NO (Rosselli et al., 1998), which is quickly metabolized to its stable end product, nitrate (Wennmalm et al., 1993). One clinical study showed a significant increase in circulating nitrite/nitrate levels after the luteinizing hormone surge in the menstrual cycle (Ekerhovd et al., 2001). NO signalling is largely regulated at the level of NO biosynthesis, and the primary source of NO production in vivo is oxidation of L-arginine by NOS enzymes (Alderton et al., 2001).

Three distinct tissue-localized NOS isoforms with predicted molecular weights of 130–160 kDa have been identified: calcium-independent inducible NOS (iNOS), calcium/calmodulin-dependent neuronal NOS (nNOS) and endothelial NOS (eNOS) (Moncada and Higgs, 1991; Moncada et al., 1991; Rosselli et al., 1998). NOS isoforms are encoded by three distinct genes, exhibit 50–60% amino acid sequence similarity, and fulfill different functions (Nathan and Xie, 1994; Alderton et al., 2001). nNOS and eNOS are constitutively expressed. iNOS is expressed in response to stimuli (e.g. immune and inflammatory responses) and increased iNOS expression leads to massive NO production that has both cytotoxic and cytoprotective effects in a wide range of tissues and cells (Mashimo and Goyal, 1999). Moreover, activation of nNOS or eNOS results in transient NO release within seconds or minutes, whereas iNOS generates cellular production of NO that continues for hours or even days (Moncada and Higgs, 1991; Moncada et al., 1991).

Studies of the roles of NOS isoforms in NO production and activity in knockout mouse models indicate that NO activity is maintained at appropriate levels by a potent NOS network of mechanisms (Tranguch and Huet-Hudson, 2003). All nucleated mammalian cells possess at least one of the three conserved NOS isoforms; however, their expressions vary in different tissues and cells in vivo (Bryan et al., 2009) and may reflect their biological roles. The three NOS isoforms are expressed differentially in different cell types of the Fallopian tube in humans (Rosselli et al., 1996; Ekerhovd et al., 1997; Tschugguel et al., 1998; Ekerhovd et al., 1999; Machado-Oliveira et al., 2008; Al-Azemi et al., 2010; Refaat et al., 2009), rats (Bryant et al., 1995; Chatterjee et al., 1996; Zhan et al., 2003; Kilic et al., 2008), cattle (Majewski et al., 1995; Rosselli et al., 1996; Lapointe et al., 2006; Ulbrich et al., 2006) and pigs (Majewski et al., 1995; Andronowska et al., 1999; Gawronska et al., 2000) (Table I). However, only tubal epithelial cells express all NOS isoforms, suggesting that this cell type is a potentially important determinant of the effects of NO in the Fallopian tube (Table I). Tubal iNOS and eNOS are the predominant isoforms in both humans and animals. As discussed below, NOS appears to have physiologically important, isoform-specific functions in the Fallopian tube.

**Regulation of NOS isoforms in the Fallopian tube**

Although gene expression of NOS isoforms depends on various factors, hormonal regulation is quite important in the Fallopian tube. Expression of NOS isoforms and nicotinamide adenine dinucleotide phosphate-diaphorase (a histochemical, non-specific marker for all NOS isoform activities) varies across the normal reproductive cycle in human, rat, cattle and porcine Fallopian tubes (Bryant et al., 1995; Chatterjee et al., 1996; Gawronska et al., 2000; Lapointe et al., 2006; Ulbrich et al., 2006; Al-Azemi et al., 2010). These results raise the possibility that changes in NOS-derived NO production during fluctuating physiological conditions may be involved in regulation of important Fallopian tube functions, such as ciliary activity or contractility.
Given the complex interactions between fluctuating and declining levels of the major ovarian-derived steroid hormones (17β-estradiol and progesterone) during the reproductive cycle, local expression of NOS isoforms may be regulated by 17β-estradiol and/or progesterone. Indeed, NOS expression and activity in the Fallopian tube are related to the ratio of 17β-estradiol and progesterone (Rosselli et al., 1998). Several clinical and experimental observations suggest a regulatory role for estrogens in the expression of NOS isoforms and endogenous NO production. First, during follicular development, increases in estrogen levels are associated with circulating nitrate levels in reproductive-age women (Rosselli et al., 1994a; Ekerhovd et al., 2001), and circulating estrogen levels correlate with circulating/follicular nitrite/nitrate levels during ovarian stimulation in IVF treatment (Rosselli et al., 1994a; Anteby et al., 1996). Second, in postmenopausal women, administration of 17β-estradiol significantly increases circulating nitrate/nitrate levels (Ramsay et al., 1995; Cinicinelli et al., 1997). Third, in non-pregnant guinea pigs, the experimental manipulation of endogenous estrogen levels induces expression of nNOS and eNOS mRNA and NOS activity in the heart, kidney and skeletal muscle (Weiner et al., 1994). Fourth, treatment with 17β-estradiol selectively up-regulates iNOS mRNA and protein expression in the bovine Fallopian tube during the periovulatory period (Lapointe et al., 2006).

Both genomic and non-genomic effects of estrogen may contribute to the regulation of NOS isoform expression, but the role of tubal estrogen receptors in this regulation is poorly characterized. On the other hand, although the effect of pregnancy on NOS isoform expression does not support progesterone-mediated induction of nNOS and eNOS expression (Weiner et al., 1994), progesterone can up-regulate tissue-dependent iNOS mRNA and protein expression in pregnant rodents in vivo (Maul et al., 2003). Moreover, acute in vitro treatment of bovine oviduct epithelial cells with progesterone rapidly increases iNOS and eNOS mRNA expression (Ulbrich et al., 2006). Thus, multiple steroid-related mechanisms may contribute to the overall regulation of NOS isoform expression and NO production in the Fallopian tube in vivo.

### Table I Summary of reported cell type-specific differences of NOS isoforms in the Fallopian tube.

| Species | nNOS                      | iNOS                              | eNOS                              | References                     |
|---------|---------------------------|-----------------------------------|-----------------------------------|--------------------------------|
| Human   | Epithelial cells (ampulla) | Smooth muscle cells (AIJ, isthmus) | Endothelial cells (AIJ, isthmus)  | Al-Azemi et al. (2010)          |
|         | Smooth muscle cells       | Endothelial cells                 |                                   | Ekerhovd et al. (1997, 1999)    |
|         |                           | Endothelial cells (AIJ, isthmus)  |                                   | Machado-Oliveira et al. (2008)  |
|         |                           |                                    |                                   | Refaat et al. (2009)            |
|         |                           |                                    |                                   | Rosselli et al. (1996)          |
|         |                           |                                    |                                   | Tschugguel et al. (1998)        |
| Rat     | Epithelial cells          | Epithelial cells                  | Epithelial cells                  | Bryant et al. (1995)            |
|         |                           |                                    | Smooth muscle cells               | Chatterjee et al. (1996)        |
|         |                           |                                    | Endothelial cells                 | Kilic et al. (2008)             |
|         |                           |                                    |                                   | Zhan et al. (2003)              |
| Bovine  | Epithelial cells*         | Smooth muscle cells*              | Smooth muscle cells*              | Lapointe et al. (2006)          |
|         | Smooth muscle cells*      | Endothelial cell                  | Endothelial cell                  | Majewski et al. (1995)          |
|         |                           |                                    |                                   | Rosselli et al. (1996)          |
|         |                           |                                    |                                   | Ulbrich et al. (2006)           |
| Porcine | Nerve fibre (isthmus)     | Epithelial cells (ampulla, isthmus)| Endothelial cells (ampulla, isthmus)| Andronowska et al. (1999)       |
|         |                           | Endothelial cells (IF, ampulla, isthmus)|                                   | Gawronska et al. (2000)         |
|         |                           |                                    |                                   | Majewski et al. (1995)          |

*Demonstrated in the ampulla, AIJ and isthmus.

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**Probing the biological functions of NOS isoforms**

In mammals, NOS activity is highly controlled because its product is a potent regulator of many physiological processes (Moncada and Higgs, 1991; Moncada et al., 1991; Rosselli et al., 1998). Tubal transport of the embryo is a complex and highly regulated process, which spans several days. One line of evidence suggests that NO helps regulate tubal contractility and relaxation under physiological conditions (Rosselli et al., 1996, 1998). In *vitro* treatment with two NO donors (nitro-glycerin and spermine NONOate) and 8-bromo cGMP decreased the contractility of the human Fallopian tube (Ekerhovd et al., 1999). In line with these findings, the opposite effect was observed in human and bovine Fallopian tubes treated with N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis (Rosselli et al., 1994b; Ekerhovd et al., 1997). Moreover, local administration of spermine NONOate abolished the effects of L-NAME, reducing the transport speed of ovulated oocyte-cumulus complexes in rat Fallopian tubes (Perez Martinez et al., 2000).

Use of pharmacological blockers of NO synthesis does not distinguish the competitive inhibitory properties of the three NOS isoforms or the diversity of specific NOS-derived NO production that
may result in cell-specific responses in the Fallopian tube under physiological conditions. Interestingly, the transiently increased iNOS expression (Shao et al., unpublished data) corroborates reported increases in circulating NO production (Ekerhovd et al., 2001) and frequency of contraction (Ekerhovd et al., 1999). Taken together, these results imply that iNOS, rather than nNOS and eNOS, is the major contributing NOS for local NO production, which might be needed to suppress tubal transport in the specific period. Since Fallopian tubal transport is under precise temporal control by specific hormonal and signalling pathways (Jansen, 1984), research is needed to determine whether tubal transport requires iNOS, and if steroid hormonal regulation of tubal transport are independent of NOS-derived NO network.

Key insights into the physiological significance of each NOS isoform have come from gene knockout studies in mice. For example, central but not ovarian nNOS activity is crucial for ovulation in nNOS-deficient female mice (Gyurko et al., 2002), whereas studies of iNOS-deficient female mice have shown that iNOS contributes to the maintenance of decidual cellular integrity for implantation and embryo survival (Burnett et al., 2002). In contrast, eNOS-deficient female mice have a reduced ovulatory capacity, a prolonged oestrous cycle, impaired early embryonic viability and decreases in implantation during pregnancy (Tempfer et al., 2000; Heffler and Gregg, 2001; Heffler et al., 2001; Pallares et al., 2008). However, typically the deletions remove simultaneously more than one NOS gene, hindering the establishment of genotype-phenotype correlations. In fact, nNOS-iNOS-, nNOS-eNOS- and iNOS-eNOS-double homozygous mutant female mice show insufficient alterations of oestrous cyclicity and fertility (Tranguch and Huet-Hudson, 2003), suggesting that, when one NOS isoform is deleted, compensatory responses of other NOS isoforms maintain overall NOS activity in female reproductive events in vivo.

However, it is not clear how deletion of NOS isoforms, individually or in combination, affects Fallopian tube function. Furthermore, isoform-specific NOS-deficient mouse models show diverse immune responses (Liu and Huang, 2008). For example, nNOS-deficient mice are resistant to neuronal inflammatory injury, while eNOS-deficient mice show increased susceptibility to neuronal and vascular inflammatory injury. More interestingly, iNOS-deficient mice show decreased neuronal inflammatory injury and increased susceptibility to bacterial and viral pathogens (MacMicking et al., 1995; Wei et al., 1995). These provocative findings shed light on the mechanisms that regulate the dynamic expression of different NOS isoforms under pathophysiological conditions.

**C. trachomatis infection induces an inflammatory microenvironment in the Fallopian tube**

*Chlamydia* are unique obligate intracellular pathogens that can exist in two forms: the elementary body (EB) and the reticulate body (RB). EBs, the transmissible form of the organism capable of extracellular survival, attach to susceptible host cells to initiate infection. After entering the host cell, the EB induces endocytosis into a host-derived, membrane-bound vacuole designated as an inclusion. Inside the inclusion, the EB rapidly differentiates into the non-infective, intracellular and replicative RB (Muschiol et al., 2006). *C. trachomatis* has a unique biphasic developmental cycle, alternating between metabolically inert EBs and metabolically active RBs (Moulder, 1991). RBs divide exponentially by binary fission before condensing back into EBs, which are released after lysis of infected cells, allowing for further propagation of the infection (Moulder, 1991).

*Chlamydia* shares morphological and structural properties of gram-negative bacteria (Bush and Everett, 2001) and is presented in two ways: cellular lysis and/or reminiscent exocytosis in host cells (Hybiske and Stephens, 2007). Human *Chlamydia* genital infection causes a wide range of pathologies. In women undergoing microtuboplasty for surgical correction of damaged Fallopian tubes, tubal biopsies contain *Chlamydia* (Henry-Suchet et al., 1981; Shepard and Jones, 1989), and women with tubal factor infertility often have *C. trachomatis* DNA or its antigen in the Fallopian tubes (Campbell et al., 1993). In human Fallopian tube organ culture, *C. trachomatis* replicates within both ciliated and non-ciliated epithelial cells (Cooper et al., 1995; Wei et al., 1994). The infected epithelial cells trigger local innate immune responses for the recognition of invading pathogens through pattern recognition receptors such as Toll-like receptors (Hart et al., 2009). A polymorphonuclear leucocyte response is initiated; these cells produce interleukin (IL) 8 and other proinflammatory cytokines (Rasmussen et al., 1997; Iqietseme et al., 1998) and may serve as the first line of defence against such infection by regulating both innate and adaptive immune responses (Wira et al., 2005a, b). The infected epithelial cells trigger local innate immune responses for the recognition of invading pathogens through pattern recognition receptors such as Toll-like receptors (Hart et al., 2009). A polymorphonuclear leucocyte response is initiated; these cells produce interleukin (IL) 8 and other proinflammatory cytokines (Rasmussen et al., 1997) and stimulate the initial neutrophilic response, which is followed by tissue infiltration of macrophages, lymphocyte, plasma cells and eosinophils (Kuo, 1988).

The important feature of innate immunity is cytokine production, and regulation of inflammation-induced cytokine expression in the Fallopian tube has been demonstrated by several groups. Hess et al. (2009) demonstrated that epithelial specific-IL-1β expression in the Fallopian tubes is higher in women with EP than in those with a normal menstrual cycle; however, the number of women with EP who had a *Chlamydia* infection was not determined. Hvid et al. (2007) reported that *C. trachomatis* infection induces epithelial IL-1β and IL-1 receptor Type I synthesis, which could be blocked by treatment with IL-1 receptor antagonist in human Fallopian tubes. This group, as well as Prantner et al. (2009), reported that either inhibition of IL-1β signalling or deletion of IL-1β can prevent pathology in human Fallopian tubes after infection with *C. trachomatis in vitro* or in mouse Fallopian tubes after infection with *Chlamydia muridarum*, a murine strain of *Chlamydia in vivo*. Finally, potential sites for IL-1β synthesis and secretion are present in macrophages in mouse Fallopian tube (Prantner et al., 2009). Infections favour macrophage recruitment and stimulate cytokine synthesis and secretion (La Verda and Byrne, 1994).

Another proinflammatory cytokine, tumour necrosis factor-α (TNF-α), an inflammatory central modulator, is also increased in response to *C. trachomatis* infection in human Fallopian tubes in vivo and in vitro (Toth et al., 1992; Ault et al., 1996). Moreover, TNF-α has an apoptotic role in mouse Fallopian tubes infected with *C. trachomatis in vivo* (Perfettini et al., 2000). Thus, epithelial cells
and macrophages appear to co-operate to regulate IL-1 and TNF-α expression and activation, which may be pathogenic in Fallopian tubes infected with *Chlamydia*.

The other major proinflammatory cytokines and chemokines, including IL-2, IL-6, IL-8, IL-10, interferon gamma (IFN-γ) and granulocyte/macrophage-colony stimulating factor (GM-CSF), are likely involved in the propagation and progression of inflammation in the Fallopian tubes after *Chlamydia* infection (Rasmussen et al., 1997; Van Voorhis et al., 1997; Enrican et al., 2004; Strandell et al., 2004; Hvid et al., 2007). Analysis of patients according to prior exposure to genital *C. trachomatis* infection has revealed that the TNFα 308A allele and the IL-10 1082A allele are risk factors for Fallopian tube damage in infected women (Ohman et al., 2009), further illustrating the possibility that cytokines and chemokines mediate tubal damage induced by *C. trachomatis* infection.

Inflammation is a physiological process used by the organism in response to infection, tissue damage or cellular irritants (Stutz et al., 2009). Inflammation progresses through different stages. Under normal circumstance the dynamics of acute inflammation are tightly regulated and self-limited. However, the onset of an unfavourable chronic inflammation by abnormal persistence of a stimulus may induce tissue damage and impair tissue function. The mechanisms responsible for Fallopian tube occlusion are not fully understood, but presumably they involve a combination of chronic inflammation and scarring induced by infections.

**Inappropriate regulation of iNOS-derived NO production facilitates tubal EP**

Little is known about the molecular pathogenesis of tubal EP, and in particular, infection-induced factors (Shao, 2010). Epithelial and smooth muscle cells are key players in tubal transport (Jansen, 1984; Shao, 2010). Indeed, it has been suggested that initial alterations in tubal cell mobility, which result in abnormal ciliary activity, epithelial secretion and contractility, may be responsible for EP. Epithelial cells and macrophages are the targets of *C. trachomatis* infection in the Fallopian tube (Henry-Suchet et al., 1996; Rasmussen et al., 1997; Igietseme et al., 1998), highlighting the major cellular sites for the disease. This link has been strengthened by studies demonstrating that *C. trachomatis* infection directly induces loss of microvilli and cilia in epithelial cells, tubal oedema and extensive disruption of the mucosal surface in human Fallopian tubes in vitro (Cooper et al., 1990) and in mice in vivo (Tuffrey et al., 1990). Notably, in mice with *C. muridarum* infection, the transport of ovulated oocyte-cumulus complexes is inhibited by loss of spontaneous contractile activity and up-regulation of iNOS protein expression in Fallopian tubes in vivo (Dixon et al., 2009).

iNOS is important because its expression and activity are enhanced in inflammatory immune processes associated with tissue damage (Nathan and Xie, 1994; Mashimo and Goyal, 1999). Although the mechanisms linking *C. trachomatis* infection, iNOS and tubal tissue/cell destruction are not completely understood, there is evidence that chronic, intense inflammation contributes to tissue remodelling and scarring in the Fallopian tube (Stephens, 2003; Enrican et al., 2004), increasing the risk for tubal EP. *C. trachomatis* infection stimulates the expression of a number of proinflammatory cytokines that reflect the induction of inflammatory processes, thereby resulting in Fallopian tube pathology and female infertility (Rasmussen et al., 1997; Stephens, 2003; Enrican et al., 2004). It is tempting to hypothesize that inflammation is a mechanism that links *C. trachomatis* infection to tubal EP.

Cytokines produced in the Fallopian tube in response to *Chlamydia* infection may regulate iNOS and subsequently generate NO. In fact, NO is considered to be part of the innate immune response because it is a bactericidal agent that is lethal to intracellular pathogens such as *C. trachomatis*. NO production is a feature of innate immune cells, such as macrophages and dendritic cells, but NO is also produced in epithelial cells (Bogdan, 2001). NO production in response to *C. trachomatis* infection in the Fallopian tube can be both protective and pathogenic. NO can participate in microbe-triggered immune responses within the Fallopian tube; however, their pathological expression may lead to tubal damage and EP (Refaat et al., 2009). The ability of *C. trachomatis* to target both immune and non-immune cell responses may explain its tendency to establish chronic, latent infection and cause tissue damage (Stephens, 2003).

In macrophages and other cell types, iNOS expression is transcriptionally induced by stimulation with cytokines or exposure to microbial products (Xie et al., 1992; Nathan and Xie, 1994). Since both epithelial and smooth muscle cells in the human Fallopian tube express iNOS (Table I), it is assumed that the tubal cells targeted for cytokine action by NO production can be the cytokine-producing cell itself (e.g. epithelial cells) or, through direct release, neighbouring cells such as macrophages. iNOS-synthesized NO is equally important in human immune regulation and disease (Nathan and Xie, 1994) but in large amounts is thought to disrupt cellular signalling cascades, resulting in anti-inflammatory or immunosuppressive effects. Interestingly, iNOS-deficient mice show greater inflammation, more dissemination of *C. trachomatis* infection (Igietseme et al., 1998; Ramsey et al., 2001) and impaired clearance (Perry et al., 1998), demonstrating that iNOS may contribute to infection-induced immunopathology, rather than the host immunity against *Chlamydia*.

It has been suggested that NO is important for implantation and early pregnancy and activation of inflammatory responses in mice (Rosselli et al., 1998; Purcell et al., 1999). If optimally balanced, NOS-derived NO accumulation is crucial for implantation in rodents (Barroso et al., 1998; Ota et al., 1999; Purcell et al., 1999; Novaro et al., 2002). In the Fallopian tube, iNOS is expressed in multiple cells, where it regulates NO production through autocrine or paracrine effects. iNOS participates in the growth associated with the decidual response in the rat uterus (Spencer et al., 1998). The effects of NO are highly dependent on the local microenvironment, tissue/cell type and redox state (Rosselli et al., 1998). Although the cause of tubal EP seems to be multifactorial, NO and iNOS are likely to contribute to tubal EP because tubal iNOS mRNA and protein expression is increased in the ampullary region, a site of fertilization, of women with tubal EP (Al-Azemi et al., 2010).

The reason why high levels of NO favour permanent tubal damage, resulting in tubal EP may be that large and sustained fluxes of NO can induce cytotoxicity and transcriptional disturbance in various tissues/cells (Moncada and Higgs, 1991; Moncada et al., 1991). Thus, it is most likely that cytokine/chemokine-triggered iNOS-derived NO production in women infected with *Chlamydia* causes Fallopian tube scarring and blocks tubal transport by inhibiting ciliary beats and smooth muscle contractions, ultimately resulting in EP (Fig. 1). If this
there must be research priority to discern how *C. trachomatis* infection promotes tubal scarring and/or fibrosis so that appropriate prevention and treatment strategies may be adopted.

**Concluding remarks and perspective**

Tubal EP induced by *C. trachomatis* infection is still a clinical problem and the diagnosis remains a challenge. As the complexity of Fallopian tubal transport under physiological conditions is being revealed (Jansen, 1984; Shao, 2010), so is the complexity of the mechanisms responsible for NOS regulation and inflammation-related EP after *Chlamydia* infection. To our knowledge, obligatory participation of the different NOS isoforms in the Fallopian tube in a physiological context has not been defined.

In this brief review, we have attempted to present NOS isoforms, in particular iNOS, as a component of the NO signalling pathway that is invoked when tubal cells undergo chronic inflammatory processes. Moreover, cytokines and chemokines produced and released in response to *Chlamydia* infection may cause progressive damage and functional decline of the Fallopian tube, resulting in tubal EP. Although estrogen can exhibit both anti-inflammatory and immunomodulatory properties in a tissue/cell-specific manner (Straub, 2007), there is little evidence for the importance of steroid hormone interactions with cytokines after *Chlamydia* infection. However, in contrast to progesterone (Tuffrey et al., 1990), high levels of endogenous estrogen or treatment with 17β-estradiol increases resistance to *C. trachomatis* infection in mice (Brunham and Rey-Ladino, 2005) and the host estrogen receptor complex influence *C. trachomatis* attachment in human endometrial epithelial cells (Davis et al., 2002). Because estrogen acts through activation of estrogen receptors in the Fallopian tube (Shao et al., 2007a, b), targeted deletion of estrogen receptor α/β lowers iNOS expression in female mouse vascular smooth muscle cells (Liang et al., 2003). Since estrogen up-regulates iNOS expression in the Fallopian tube, studies of the molecular mechanisms by which estrogen modulates cell-specific pro-inflammatory cytokine secretion and NO production are increasing our understanding the role of estrogen in Fallopian tubal pathology after *Chlamydia* infection. Such knowledge might facilitate the development of therapeutic approaches to mitigate tubal dysfunction.

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