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Tubal Damage, Infertility and Tubal Ectopic Pregnancy: *Chlamydia trachomatis* and Other Microbial Aetiologies

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1. Introduction

Infertility is a worldwide health problem with one in six couples suffering from this condition and with a major economic burden on the global healthcare industry. Estimates of the current global infertility rate suggest that 15% of couples are infertile (Zegers-Hochschild *et al.*, 2009) defined as: (1) failure to conceive after one year of unprotected sexual intercourse (i.e. infertility); (2) continual failure of implantation at subsequent cycles of assisted reproductive technology; or (3) persistent miscarriage events without difficulty conceiving (natural conceptions). Tubal factor infertility is among the leading causes of female factor infertility accounting for 7-9.8% of all female factor infertilities. Tubal disease directly causes from 36% to 85% of all cases of female factor infertility in developed and developing nations respectively and is associated with polymicrobial aetiologies. One of the leading global causes of tubal factor infertility is thought to be symptomatic (and asymptomatic in up to 70% cases) infection of the female reproductive tract with the sexually transmitted pathogen, *Chlamydia trachomatis*. Infection-related damage to the Fallopian tubes caused by *Chlamydia* accounts for more than 70% of cases of infertility in women from developing nations such as sub-Saharan Africa (Sharma *et al.*, 2009). Bacterial vaginosis, a condition associated with increased transmission of sexually transmitted infections including those caused by *Neisseria gonorrhoeae* and *Mycoplasma genitalium* is present in two thirds of women with pelvic inflammatory disease (PID). This review will focus on (1) the polymicrobial aetiologies of tubal factor infertility and (2) studies involved in screening for, and treatment and control of, Chlamydial infection to prevent PID and the associated sequelae of Fallopian tube inflammation that may lead to infertility and ectopic pregnancy.

2. Tubal factor infertility

In the absence of functional Fallopian tubes, couples may only conceive through *in vitro* fertilisation procedures. Women with tubal factor infertility may be defined as women who have either (1) damaged/occluded Fallopian tubes or (2) have history of salpingectomy. Ectopic pregnancy is only relevant if the Fallopian tubes remain *in situ*. Previous studies

have concluded that salpingitis can accompany early intrauterine pregnancy, often with significant foetal loss (Lara-Torre & Pinkerton 2002; Yip *et al.,* 1993) but that upper genital tract infections do not always result in poor reproductive health outcomes (den Hartog *et al.,* 2006). PID, which is diagnosed in greater than 800,000 women each year in the United States is associated with Fallopian tube inflammation, which can lead to tubal factor infertility in women ranging from 5.8% and 60%, depending upon the microbial aetiology of disease and the number of recurrent infections (Soper, 2010; Westrom, 1980). A recent estimate, not including women with 'silent salpingitis' or asymptomatic infections was that the annual cost of caring for women with PID is US $2 billion (Soper, 2010). PID is known to be caused by the sexually transmitted microorganisms *C. trachomatis, N. gonorrhoeae,* and *M. genitalium* as well as bacterial vaginosis-associated microorganisms consisting predominantly of anaerobic Gram-negative bacilli. Investigations into the levels of antimicrobial compounds in Fallopian tubes or antibodies in sera collected from women with ectopic pregnancy, suggest that immune responses to infectious agents may also predispose for this condition (Refaat *et al.,* 2009; Srivastava *et al.,* 2008).

3. Fallopian tube function

The Fallopian tube plays an essential role in gamete and zygote transport. In parallel with the endometrium, the Fallopian tube also undergoes cyclical changes in response to the steroid hormones oestradiol and progesterone, which alter morphology and the frequency of beating of the ciliary (Critoph and Dennis, 1977a).

The transport of gametes and embryos through the Fallopian tubes relies on contractions of the tubal musculature, ciliary activity and the flow of tubal secretions (Jansen, 1984). Distortions of the luminal architecture of the Fallopian tubes have been associated with tubal ectopic pregnancy, predominantly because of failure of the transport mechanisms to move the gametes/embryos through the tube and into the uterus prior to implantation (Mast, 1999). Microbial infection of the Fallopian tubes is one reason for alterations in the tubal epithelial lining. Tubal disease resulting in infertility is the result of an inflammatory process in or around the Fallopian tube (Mastroianni, 1999). The extent of tubal damage is dependent on the severity and duration of the infection. The disease spectrum ranges from complete tubal occlusion with hydrosalpinx to mild intraluminal adhesions (Mastroianni, 1999).

3.1 Ovulation and oocyte capture

After ovulation, follicular fluid is the major constituent of the Fallopian tube secretions. The overall composition and viscosity of the tubal secretions (including elevated levels of steroid hormones and prostaglandins) enhances the ciliary beat frequency (Blandau *et al.,* 1975). Ciliary beat frequency is different for each part of the Fallopian tube. Elevations in the progesterone concentration in tubal secretions result in a slowing of the ciliary beat to allow fertilisation to occur, however, if the progesterone levels are too high then deciliation occurs and the prolonged delay in ciliary beat may result in implantation of the embryo within the Fallopian tube mucosa (Diaz *et al.,* 1980).

Prostaglandins within the follicular fluid mix with the tubal secretions and also increase the contractility of the fimbriae and the tubo-ovarian ligaments (Morikawa *et al.,* 1980). A controlled, deliberate movement of the tubal fimbriae ensues, initiating contact between the

point of ovulation and the cumulus-oocyte-complex gently propelling the ovulated oocyte into the Fallopian tube toward the uterus (Lindblom & Andersson 1985). Transportation of the oocyte and then following fertilisation, the embryo, through the Fallopian tubes takes approximately 80 hours (Croxatto *et al.,* 1972; Croxatto *et al.,* 1978). Inhibition of oocyte capture by the Fallopian tube may result from microbial infections of the tube. The subsequent immune response can form adhesions on the fimbrial end of the Fallopian tubes or cause altered pelvic anatomy, which prevents the physical movement of the tube.

3.2 Steroid hormones (oestradiol and progesterone)

The Fallopian tubes undergo cyclical changes under the influence of the steroid hormones, oestradiol and progesterone (Critoph & Dennis, 1977) and Fallopian tube steroid hormone receptors are expressed in response to the ovulatory cycle (Pollow *et al.,* 1981). Changes in the steroid hormone expression within the Fallopian tube contribute to successful transport and ultimately implantation (Horne *et al.,* 2009).

Progesterone has an inhibitory effect in ciliary movement and tubal smooth muscle contractility, resulting in a reduction in contraction frequency (Paltieli *et al.,* 2000) and ciliary beat (Wanggren *et al.,* 2008), capable of causing delayed transport of the embryo and ectopic implantation. Horne *et al.,* (2009) reported a reduced expression of progesterone receptors in the Fallopian tubes of women with previous tubal ectopic pregnancies. They were also unable to detect expression of an oestrogen receptor on the Fallopian tubes from these same women when compared to Fallopian tubes from non-pregnant women. The alterations in steroid hormone expression in response to the ovulatory cycle were discordant in non-pregnant women, compared with those reported in women with tubal ectopic pregnancies (Horne *et al.,* 2009).

The oestrogen receptor is reportedly a dominant regulator of normal Fallopian tube development (Mowa & Iwanaga 2000) however; expression of the oestrogen receptor remains constant throughout the ovulatory cycle (Horne *et al.,* 2009).

Previous investigations have assessed the effect of oral contraceptives on the risk of ectopic pregnancy. The inhibition of fertilisation or ovulation resulted in a decreased incidence of ectopic pregnancy in women with vasectomised male partners, and in women prescribed combined oral contraceptives. In contrast, the incidence of ectopic pregnancy was elevated in women using progesterone only contraceptives, and highest in those women using progesterone only contraceptive and an intra-uterine device (Franks *et al.,* 1990). This may be due to the effect of progesterone on ciliary beat frequency or in the case of an intra-uterine device; there is an increased risk of ascending infection by commensal microflora. Finally, the steroid hormones oestradiol and progesterone are growth factors or inhibitors for various microbial species. It has been suggested that the more frequent diagnosis of specific genital tract infections at various stages of the menstrual cycle is due to the concentrations of each of these hormones (Sonnex, 1998).

3.3 Salpingitis and alterations to the Fallopian tube luminal epithelium

The most frequent cause of ectopic pregnancy is previous salpingitis (Lehner *et al.,* 2000). The predominant facultative pathogens identified in tubal fluid from women with salpingitis are coliform bacteria (Holmes *et al.,* 1980; Ledger *et al.,* 1994; Swenson *et al.,* 1974) and the predominant anaerobic species originate from the *Bacteroides* genera. Microorganisms and the immune response may result in scar tissue formation, alter the

activity of tubal cilia, result in the partial or complete destruction of cilia, and alter the composition and viscosity of the tubal secretions. Within the Fallopian tube mucosa, the response to microorganisms is not uniform. Each species evokes an individual and specific response (Laufer *et al.*, 1984). For example, *E. coli* cells or lipopolysaccharide cause swelling of the ciliary tips followed by adhesions between shortened and swollen cilia in addition to shortened microvilli on non-ciliated cells (Laufer *et al.*, 1980; Laufer *et al.*, 1984). *C. trachomatis* infection of Fallopian tubes reveals patches of flattened cells mixed with cells with only a single elongated cilium (Patten *et al.*, 1990). The sexually transmitted pathogen, *N. gonorrhoeae* causes invagination in ciliated cells and loss of microvilli in non-ciliated cells (Draper *et al.*, 1980).

4. Effects of microorganisms on the Fallopian tubes

The Fallopian tubes play an integral role in reproduction and undergo cyclical changes in morphology and ciliary activity that are dependent upon ovarian hormones (Lyons *et al.*, 2006). Recent reviews have reported that infection reduces ciliary motion and even destroys cilia within the Fallopian tubes (Lyons *et al.*, 2006; Shaw *et al.*, 2010). Reduced ciliary function can be a cause of infertility and can result in ectopic pregnancy since the embryo relies on cilia to facilitate its propulsion through the Fallopian tubes into the uterus. In addition, inflammation of the lumen of the Fallopian tubes results in tubal occlusion and tubal factor infertility. Whilst much research regarding the microflora associated with Fallopian tube damage continues to focus on sexually transmitted pathogens many other microorganisms have been associated with Fallopian tube pathology and tubal factor infertility.

4.1 Bacterial vaginosis

Bacterial vaginosis is a frequently encountered condition among women affecting from between 10 – 20% of fertile women (Holmes, 2008). Bacterial vaginosis is induced by the change from *Lactobacillus* spp. dominant vaginal flora to vaginal flora dominated by other microorganisms (Holmes, 2008). Several factors contribute to a reduction in the vaginal *Lactobacillus* spp. levels including antimicrobial treatment, hormonal imbalance, douching, use of non-barrier contraception, demographic factors − age and socioeconomic status, and the sexual history of the female − age of commencement of sexual intercourse, and number of previous sexual partners (Tibaldi *et al.*, 2009; Witkin *et al.*, 2007a). Bacterial vaginosis is a polymicrobial condition and an altered immunity hypothesis proposes that bacterial vaginosis develops as a result of the inhibition of Toll-like receptor (TLR) activation. The negative consequences of bacterial vaginosis are facilitated in part by a release and/or inadequate function of the antimicrobial plasma protein, mannose binding lectin (MBL) (Witkin *et al.*, 2007b). Microorganisms that frequently replace the normal *Lactobacillus* dominant lower genital tract flora include *Gardnerella vaginalis, Ureaplasma* spp., *M. hominis, Streptococcus viridans* and anaerobic Gram-negative bacilli from the genera *Prevotella, Porphyromonas, Bacteroides, Fusobacterium* and the coccus, *Peptostreptococcus* (Biagi *et al.*, 2009; Hillier 1993). The quantification of several microorganisms, particularly *G. vaginalis* and *Atopobium vaginae*, allows for a molecular diagnosis of bacterial vaginosis (Menard *et al.*, 2008). Microorganisms infecting the lower genital tract can be transported to the uterus and the Fallopian tubes either by (1) ascending to cause endometritis and subsequent salpingitis or (2) transport by the

lymphatic system (Brook, 2002). Bacterial vaginosis has been associated with genital and obstetric infections, including PID (Catlin, 1992; Hay *et al.,* 1992; Soper, 1994), particularly in the presence of other sexually transmitted infections (Hillier *et al.,* 1996; Wiesenfeld *et al.,* 2002) including human papilloma virus infections (Verteramo *et al.,* 2009).

Gaudoin *et al.,* (Gaudoin *et al.,* 1999) reported a strong association between bacterial vaginosis and tubal factor infertility and in a study by Wilson and colleagues (2002) it was concluded that women with tubal infertility were three times more likely to have bacterial vaginosis than women with male factor or unexplained infertility. In a retrospective analysis of a population of 952 women investigated over two years, it was recently reported that the genital discharges of asymptomatic women with infertility consisted of an overgrowth of several aerobic bacteria especially *G. vaginalis* (19.7%), Enterobacteriaceae or Enterococci (12.1%) and *Streptococcus agalactiae* (8.6%) noting a prevalence of *C. trachomatis* of only 0.5% in this cohort of women (Casari *et al.,* 2010).

4.2 Upper genital tract infections

The most frequent method of female upper genital tract infection is by ascension of members of the lower genital tract endogenous microflora, which may first cause disruption to the normal balance such as that seen in cases of bacterial vaginosis or vaginal candidiasis (Population Council, 2003). Following medical intervention, iatrogenic infections may result from the direct inoculation of microorganisms from the lower genital tract into the upper genital tract. Iatrogenic procedures associated with tubal ectopic pregnancy are tubal surgery and trans-vaginal oocyte retrieval for *in vitro* fertilisation (IVF).

Seminal fluid is also reportedly a mechanism of microbial transfer to the female upper genital tract. Furthermore, some microorganisms have the propensity to attach to the surface of spermatozoa, whilst others are obligate intracellular parasites within the spermatozoa *C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma* spp., *Ureaplasma* spp., and *E. coli* have all been shown to adhere to the surface of spermatozoa or form intracellular inclusions within the spermatozoa (Friberg *et al.,* 1987; Hickey *et al.,* 2009; James-Holmquest *et al.,* 1974; Murthy *et al.,* 2009; Sanchez *et al.,* 1989; Wolner-Hanssen & Mardh 1984). Further, female partners of infected men with spermatozoa in their ejaculate had a significantly higher incidence of upper genital tract infection compared to infected men who have been vasectomised (Toth *et al.,* 1984). Interestingly it was recently reported that a significantly higher incidence of sperm-immobilizing antibodies (6.4%) was found in sera collected from 273 infertile women with a past *C. trachomatis* infection compared to that found in women without a past chlamydial infection (1.5%) (Hirano & Hoshino 2010). Thus, it may be that the production of sperm-immobilizing antibodies in infertile women is the result of a past *C. trachomatis* infection in these women and this may contribute to their infertility.

4.3 Infections of the female upper genital tract (the endometrium, Fallopian tubes and ovaries)

It is becoming increasingly accepted that the female upper genital tract is not a sterile site, but likely in fact to be asymptomatically colonised or infected with microorganisms (Horne et al 2008; Wira et al 2005). Endometritis, a persistent inflammation of the endometrial lining, has been reported in up to 19% of women (Farooki 1967). Endometritis is frequently asymptomatic, but similarly to other gynaecological infections, endometritis has been shown to reduce conception rates (Feghali et al 2003; Taylor & Frydman 1996). Excessive

inflammation in the endometrium at the time of implantation may be a cause of infertility. Endometritis represents an early stage in the continuum from lower genital tract infection through to salpingitis, the most serious form of female genital tract infection with respect to fertility.

Endometritis is a polymicrobial infection caused by the ascension of endogenous microorganisms or sexually transmitted infections. Endometritis is frequently reported in association with an altered lower genital tract microflora, such as that seen in women with bacteria vaginosis (Hillier et al., 1992; (Jacobsson et al 2002) or PID (Centres for Disease Control and Prevention, 2002). Alterations of the lower genital tract microbial milieu are not the only cause of endometiritis as this infection has been reported in women with 'normal' levels of lower genital tract microorganisms (Lucisano et al 1992). Microorganisms can also be introduced into the endometrium iatrogenically, during gynaecological investigations and treatment (Kiviat et al 1990).

PID results from ascension of microorganisms from the vagina to the upper genital tract (Holmes, 1984) causing post-infectious inflammation with potentially long-term sequelae including tubal infertility, ectopic pregnancy and pelvic pain (Cherpes *et al.*, 2006). Reportedly, up to 20% of women will be rendered infertile following a single diagnosis of PID (Westrom *et al.*, 1992) increasing to 50% following multiple episodes (Westrom *et al.*, 1980). In addition, women with a history of PID were twice as likely to have experienced an ectopic pregnancy when compared to women without any history of upper genital tract infection (Miller *et al.*, 1999). Similar to bacterial vaginosis, PID is also frequently polymicrobial. Opportunistic pathogens comprising anaerobic and facultative aerobic bacteria from the normal microflora of the lower genital tract, or species implicated in genital tract infections cause up to 50 % of PID, not the sexually transmitted bacteria *C. trachomatis* or *N. gonorrhoeae* (Soper, 2010).

Salpingitis is an infection in the Fallopian tube(s). A polymicrobial microflora has been reported for Fallopian tube tissue from women with salpingitis (Eschenbach *et al.*, 1975; Soper, 1994). Tubo-ovarian abscesses represent an extension of salpingitis and reportedly occur in up to 16% of women diagnosed with salpingitis.

Tubo-ovarian abscesses are usually complications of PID and represent inflammation of both the Fallopian tubes and the ovaries (Landers & Sweet 1983). The microbial aetiology of tubo-ovarian abscesses is predominantly polymicrobial (Landers & Sweet 1983; Wiesenfeld & Sweet 1993). Microbial invasion of the Fallopian tube(s) initiates an inflammatory response which results in oedema, increased pressure and restricted blood supply to the affected Fallopian tube(s) causing abscess formation and survival of the pathogens in a 'protected' environment (Osborne, 1986).

Numerous sexually transmitted and non-sexually transmitted pathogens have been isolated from infected upper genital tract tissues. Bacterial vaginosis associated bacteria have been detected independently of *C. trachomatis* and/or *N. gonorrhoeae* suggesting that investigations regarding causes of upper genital tract infection, but more specifically in the context of tubal ectopic pregnancy, and tubal damage, should focus on a diverse range of microorganisms.

4.4 *Chlamydia trachomatis*

C. trachomatis is an important pathogen in the aetiology of acute PID and has been isolated from the upper genital tracts of approximately one quarter of patients with this disease. In

addition to causing symptomatic PID, *C. trachomatis* is also associated with subclinical upper genital tract disease in women (Horne *et al.*, 2008). The potentially serious sequelae of cervical infection with *C. trachomatis* can include infertility, ectopic pregnancy, pelvic pain and recurrent PID and these have recently been reviewed (Batteiger *et al.*, 2010; Darville and Hiltke, 2010; Haggerty *et al.*, 2010;). The extent to which disease sequelae eventuate following cervical infection with *C. trachomatis* is probably also significantly linked to natural processes that occur in the reproductive tract and include coitus-related phenomena and cyclical hormonal conditions. A novel paradigm that includes consideration of these and other aspects of reproductive biology particularly when using animal models to investigate potential vaccines for chlamydial genital tract infections in women has recently been proposed (Lyons *et al.*, 2009a). Continued investigations into the mechanisms of *Chlamydia*-induced tissue damage are required to further develop our understanding of the pathogenesis of genital tract disease caused by this organism, and to direct research into effective ways to control *C. trachomatis* infection, including vaccine development.

4.4.1 Chlamydia, tubal pathology and tubal factor infertility

In women it has previously been reported that a single chlamydial infection of the genital tract does not result in tubal scarring (Paavonen and Eggert-Kruse, 1999); however, prolonged exposure to *Chlamydia* due to a chronic persistent infection or frequent re-infection has been associated with (1) an autoimmune response to Chlamydial heat shock protein (which shares homology with human heat shock protein) and (2) the chronic inflammation associated with tubal factor infertility (Brunham and Peeling, 1994; Mardh, 2004; Ness *et al.*, 2008). The severity of the inflammatory response to chlamydial infection is enhanced during re-infection, causing inflammation, tissue damage and scarring. Recent studies by Hvid *et al.*, (2007) have concluded that damage to the Fallopian tubes is disproportional to the number of *C. trachomatis* infected cells, suggesting that Fallopian tube cell lysis does not occur as a direct result of infection. In their study, they instead demonstrated that IL-1 had a toxic effect on ciliated Fallopian tube cells.

Based on the results of epidemiological studies, prospective data from studies of infertile women and on results from animal models, *C. trachomatis* infection of the female reproductive tract is known to be causally associated with tubal infertility. In the murine model, a primary chlamydial infection is sufficient to induce tubal damage and infertility (Swenson *et al.*, 1983) with Toll-like receptor 2 being identified as essential for oviduct pathology in this model (Darville *et al.*, 2003; Phillips *et al.*, 1984;). Derbigney and colleagues (2007) reported that *C. muridarum* infection of murine oviduct epithelial cell lines induced a beta-interferon response and implicated Toll-like receptor 3 as the source of this interferon. In female guinea pigs, long-term tissue damage was also caused following the host response to a primary chlamydial infection (Rank and Sanders, 1992), with chlamydial salpingitis also reported in female guinea pigs receiving oral contraceptives (Barron *et al.*, 1988). By contrast, in macaque monkeys a single upper genital tract infection with *Chlamydia* is usually self-limiting with tubal scarring only resulting from repeated episodes of salpingitis (Patton *et al.*, 1987; VanVoorhis *et al.*, 1997). It has been shown that *C. trachomatis* infection in monkeys induced delayed hypersensitivity, which is proposed to be the pathogenic mechanism of tubal damage in this species (Patton *et al.*, 1994).

There have been reports of serologic evidence of past chlamydial infections (Ness and Brooks-Nelson, 1999; Patton *et al.*, 1994b; Robertson *et al.*, 1987) in women with tubal infertility, and it has been reported that interleukin-1 (IL-1) initiates Fallopian tube

destruction following a *C. trachomatis* infection (Hvid *et al.,* 2007). In a retrospective study of 84 infertile women with tubal occlusion, the sera collected from 28% of these women were positive for chlamydial anti-IgG antibody, compared to only 11% positivity to the chlamydial anti-IgG antibody in sera collected from 253 infertile controls (Merki-Feld *et al.,* 2007). A study of 114 women with laparoscopically-verified tubal factor infertility (of which 96 cases showed evidence of past infection with *Chlamydia*) was undertaken to further elucidate the mechanisms of tubal damage in women with *Chlamydia*-associated infertility (Ohman *et al.,* 2009). The functional polymorphisms in selected cytokine genes [including IL-10, interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α] revealed an increase in severe tubal damage in women with infertility caused by *Chlamydia* when certain IL-10 and TNF-α alleles were present (Ohman *et al.,* 2009). In terms of cytokine secretions in *Chlamydia*-positive infertile women, it has been reported that *Chlamydia*-stimulated cervical cells secreted significantly higher levels of IL-1ß, IL-6, IL-8 and IL-10. This indicated that the cytokine secretion profile of cervical cells may produce vital information to indicate the outcome (i.e. fertile or infertile) of a chlamydial infection of the female genital tract (Agrawal *et al.,* 2009). Others have reported that IL-1β, IL-4, IL-5 and IL-6 as well as IL-10 levels were found to be higher in *Chlamydia* membrane protein (Inc protein)-stimulated cervical cells of *C. trachomatis*-positive infertile women compared to fertile women infected with *Chlamydia* (Gupta *et al.,* 2009). More recently a unique link between elevated levels of anti-Chlamydial caseinolytic protease P (ClpP) and tubal factor infertility was identified in 21 tubal factor infertility patients (Rodgers *et al.,* 2010).

Host genetic factors are known to modulate the immune defence mechanisms to a *Chlamydia* infection thus determining the occurrence of *Chlamydia*-induced tubal factor infertility. A study by Morre and colleagues (2002) reported that almost 45% of women infected with genital chlamydial infections cleared the infection after one year with no interventional treatments. However, some authors have claimed that this study by Morre *et al.,*. (2002) was methodologically flawed (Risser and Risser, 2007; Simms and Horner, 2008). An increased risk of tubal pathology (as a result of aberrant immune responses) has been reported in 227 sub-fertile women following a *C. trachomatis* infection and carrying two or more single-nucleotide polymorphisms (SNPs) in genes (toll-like receptor (TLR)-9, TLR-4, CD14, and caspase recruitment domain protein 15 (CARD15)/nucleotide-binding oligomerization domain containing 2(NOD2) that encode pattern recognition receptors (PRRs) involved in sensing bacterial components (den Hartog *et al.,* 2006). In a more recent report that investigated 214 infertile women, 42 of whom had tubal pathology, it was found that polymorphisms in the major histocompatibility complex class I chain related A gene (specifically allele 008) correlated with *C. trachomatis* anti-IgG antibodies in infertilewomen (Mei *et al.,* 2009).

4.4.2 Chlamydia and PID

PID is caused by infection of the female genital tract with microorganisms including *C. trachomatis* (Bakken and Ghaderi, 2009) and testing for serum antibody to the chlamydial 60kDa Heat shock protein (i.e.CHSP60 antibody) is an accurate means for predicting *Chlamydia*-associated tubal factor infertility (Claman *et al.,* 1997)**.** A prospective study into serologic parameters of tubal disease reported that antibodies to CHSP60 were also predictive for lower spontaneous conception and pregnancy outcome after a first episode of ectopic pregnancy (Sziller *et al.,* 2008). A retrospective study of follicular fluid from 253 IVF patients for IgG antibodies to CHSP60 reported that antibodies to CHSP60 were found in

74.1% of women without embryo (s) and in 69.5% of women with tubal occlusion (Jakus *et al.,* 2008). In the PID evaluation and clinical health (PEACH) study 443 women with clinical signs of mild to moderate PID were followed for 84 months and assessed for long-term sequelae of chlamydial infections of the genital tract including PID recurrence and time to pregnancy (Ness *et al.,* 2008). It was found that IgG antibody responses to CHSP60 and elementary bodies (the extracellular form) of *C. trachomatis* serovar D were independently associated with reduced pregnancy rates and increased rates of recurrent PID (Ness *et al.,* 2008). In another study of 72 female patients a significant seropositivity to CHSP60 antibodies was detected in patients with secondary infertility from an infertile cohort clinically characterised primary and/or secondary infertility. This indicated that specific antibodies to CHSP60 may aid in the early prognosis of immunopathologic sequelae following genital tract infections with *C. trachomatis* (Dutta *et al.,* 2008). The 10kDa chlamydial heat shock protein 10 (CHSP10) has also been identified as a target of cell-mediated responses in human chlamydial infections. Women with tubal infertility have been shown to recognise CHSP10 more frequently than those women with current active chlamydial infections. Co-expression of both CHSP60 and CHSP10 were subsequently detected at higher levels in the infertile women compared to the fertile women (Jha *et al.,* 2009). CHSP10 and CHSP60 stimulation was reported to increase the cytokine responses of IFN-γ and IL-10 in *Chlamydia*-positive infertile women (Srivastava *et al.,* 2008). Srivastava *et al.,.* (2008) suggested that this could significantly affect the release of these cytokines from the cervical mononuclear cells, thus affecting the mucosal immune function against this pathogen and hence fertility outcomes in these women.

Linhares and Witkin (2010) recently reviewed the immunopathogenic consequences of CHSP60 expression in the female genital tract and they reported that scar formation and tubal occlusion resulted from the induction of pro-inflammatory immune responses following the release of CHSP60 from a *C. trachomatis* infection. They further reported that the production of CHSP60 cross-reacting antibodies and cell-mediated immunity to the human HSP60 was detrimental to subsequent pregnancy outcome in women infected in the upper reproductive tract with this microbial pathogen (Linhares and Witkin 2010).

4.4.3 Chlamydia screening

Screening for, and treatment of, chlamydial infection is aimed at reducing chlamydial transmission and preventing PID and the long-term sequelae of PID including infertility, chronic pain, recurrent episodes of PID and ectopic pregnancy.

For the host to successfully clear infections of the female genital tract caused by *C. trachomatis* an adequate immune response is required following recognition of the pathogen by pattern recognition receptors (PRRs) of the Toll-like receptor (TLR) and nucleotide binding oligomerization domain (NOD) families. If functioning correctly, the host immune response clears the infection but in some females the infection is not cleared and this allows for a persistent infection to manifest in these hosts. Since most infections with *Chlamydia* remain asymptomatic it is difficult to ascertain the risk of potential disease sequelae associated with previous chlamydial infections. There are several methods used to assess the risk of chlamydial infections in women that may lead to tubal factor sub-fertility and these have been reviewed (den Hartog et al., 2006). In particular it has been found that testing for anti-chlamydial IgG antibody in serum can indicate a previous infection but cannot predict a persistent infection. It has been noted that screening for serological markers of persistence

(including C-reactive protein) seems useful for identifying infected women at highest risk of tubal pathology. It has been proposed that three screening strategies would be useful for identifying tubal factor sub-fertility in women infected in the genital tract with *C. trachomatis*: (1) *C. trachomatis* IgG antibody testing, (2) high sensitivity CRP testing and (3) hysterosalpingography (den Hartog, 2008). A recent mathematical modelling study has analysed previously published data on the persistence of asymptomatic *C. trachomatis* infection in women, and has estimated the mean duration of the asymptomatic period to be longer (433 days) than previously anticipated. These authors conclude that their study shows that a longer duration of the asymptomatic period results in a more pronounced impact of a screening programme (Althaus *et al.,* 2010).

The incidence of PID in untreated women infected with *C. trachomatis* has been reviewed and widely discussed in the literature in terms of (1) its cost-effectiveness as a screening program and (2) as a predictor of tubal damage in infertile patients (Aghaizu *et al.,* 2008; Althaus et al., 2010; Bakken & Ghaderi 2009; den Hartog *et al.,* 2008; Dietrich *et al.,* 2010; Kalwij *et al.,* 2010; Land *et al.,* 2010; Low *et al.,* 2009; Low & Hocking 2010; Oakeshott *et al.,* 2010; Risser & Risser 2007; Simms & Horner 2008). In a comprehensive study that evaluated all available original research and assessed the incidence of PID following *C. trachomatis* infection, it was concluded that no study could adequately answer the question and that many studies either had inaccuracies, validition problems or only indirect evidence to support their reported incidences (Risser and Risser, 2007). A similar review of the literature was undertaken by Simms and Horner (2008) who stated that a reasonable estimate of PID incidence in untreated women after *C. trachomatis* infection was likely to be in the range of 10-20%. A Norwegian registry-linkage study of 24,947 women who were tested for *C. trachomatis* infection reported a correlation between diagnosed *Chlamydia* infection and subsequent PID. The incidence rate of PID in this study was found to be higher in women with prior *C. trachomatis* infection than among women with negative *C. trachomatis* tests although the rates were notably low in both groups (Bakken & Ghaderi 2009). It has therefore been suggested that the benefits of current *Chlamydia* screening programmes may have been overestimated (Low *et al.,* 2006).

A comprehensive review of seven electronic databases covering 17 years of register-based reports (until 2007) and opportunistic screening programmes for *Chlamydia* found that there was no evidence to support the most commonly recommended approach of opportunistic *Chlamydia* screening in a general population younger than 25 years. Furthermore, it was proposed by these authors that an effective approach when assessing biological outcomes of chlamydial infection currently reuqires multiple rounds of screening in randomized control trials (RCT) (Low *et al.,* 2009). A recent RCT (the POPI-prevention of pelvic infection-trial) was undertaken to determine whether a single screening test and treating a subset of 2529 women for chlamydial infection can in fact reduce the incidence of PID over a 12-month period (Oakeshott et al., 2010). The baseline prevalence of *Chlamydia* was 5.4% in the screened population of 2529 sexually active female students (mean age 20.9 years) and 5.9% in (deferred screening) controls with the incidences of PID found to be 1.3% and 1.9% respectively in these cohorts. It was reported that after 12 months, most episodes of PID occurred in women who tested negative for *Chlamydia* at baseline (9.5%) when compared to the intervention group (1.6%) and these authors concluded that the effectiveness of a single *Chlamydia* test in preventing PID over 12 months may also have been overestimated (Oakeshott et al., 2010).

A Danish randomised trial was conducted with 9-year follow-up testing of 4000 asymptomatic women for the presence of urogenital *Chlamydia trachomatis*. Data were collected on PID, ectopic

pregnancy EP, infertility diagnoses, IVF treatment and births in women. Results showed that no differences were found between the intervention group and the control groups of women for PID, ectopic pregnancy, infertility, IVF treatment and births. It was concluded that a population-based offer to be tested for urogenital *C. trachomatis* infection using non-invasive samples and DNA amplification testing did not reduce the long-term risk of reproductive complications such as PID and ectopic pregnancies in asymptomatic women (Anderson *et al.,* 2011). This finding agrees with the conclusions made by authors of an earlier review of 12 databases who reported (from the one study that satisfied inclusion criteria for their review) the absence of valid evidence on the risk of tubal factor infertility following an infection of the genital tract with *C. trachomatis* (Wallace *et al.,* 2008) and is also in agreement with the finding of Oakeshott and colleagues (Oakeshott et al., 2010).

Of note from the Oakeshott study was that a screening intervention at 12 months would not have prevented the 10 reported cases of chlamydia-positive PID among women who were *Chlamydia*-negative at baseline and serves to highlight the ongoing transmission of *Chlamydia* as the elemental problem. The results of the POPI trial suggested that current levels of chlamydial screening are unlikely to have much impact on the overall incidence of PID (Low & Hocking 2010). A recent modelling study based on a comprehensive literature survey on the epidemiology of chlamydial infection and risk-estimates of its late complications has concluded that the risk of developing tubal infertility after a *Chlamydia* lower genital tract is low (at around 4.6%), and these authors stated that high quality RCTs investigating the transition from cervicitis to tubal infertility are needed (Land et al., 2010). It has also been reported in a prospective study evaluating the sensitivity of multiple-site swab testing (cervix, urethra, vagina and Fallopian tubes) in 2,020 fertility patients over 12 months that multiple site sampling does not increase the detection rate of *C. trachomatis* among infertile women and in fact that routine DNA testing for *C. trachomatis* should be confined to cervical sampling (Dietrich et al., 2010). A review from the National *Chlamydia* screening programme in London has highlighted the need for *Chlamydia* testing to be offered routinely to young people (under 25 years) as part of an overall approach to sexual health in the community (Kalwij et al., 2010).

It has been noted recently that no studies have yet published results of the effects of greater than one round of screening or indeed screening for repeat *Chlamydia* infections on reproductive sequelae in women following asymptomatic *C. trachomatis* genital infection (Gottlieb *et al.,* 2010). However two new trials the *Chlamydia* Screening Implementation (CSI) Project (van den Broek *et al.,* 2010) and the Australian *Chlamydia* Control Effectiveness Pilot (ACCEPt) trial (Hocking *et al.,* 2008) are currently underway investigating multiple screening rounds and using *Chlamydia* prevalence (and not PID) as the end point. These trials should provide more conclusive information regarding the effectiveness of chlamydial screening to control morbidity associated with genital chlamydial infection.

4.4.4 Chlamydia and vaccines

Fertility in women is overwhelmingly affected by unresolved or untreated infection of the female reproductive tract with *C. trachomatis*. Since greater than 70% of chlamydial genital infections in women are asymptomatic and sequelae of infection manifest as diseases resulting from severe pathological consequences such as tubal occlusion, a vaccine is likely to be imperative to control infections caused by this sexually transmitted mucosal pathogen. Many animal models of infection-induced immunity including murine and guinea pig

models continue to be essential in providing knowledge of the infection processes and immune responses to a variety species found within the Chlamydiaceae. These have recently been reviewed by several groups (Cochrane *et al.,* 2010; Farris & Morrison 2011; Hafner *et al.,* 2008; Hafner 2007; Hafner & McNeilly 2008; Lyons et al., 2006; Miyairi *et al.,* 2010; Rank & Whittum-Hudson 2010). These animal models have proved invaluable in providing knowledge of many novel candidate antigens for a vaccine (Barker *et al.,* 2008; McNeilly *et al.,* 2007; Murthy AK *et al.,* 2011; Murthy et al., 2009) as well as novel delivery vehicles (Xu *et al.,* 2011) and delivery routes such as oral and transcutaneous immunization for protection of genital infections (Hickey *et al.,* 2010; Hickey et al., 2009) and have investigated a myriad of potential adjuvants (reviewed in Cochrane *et al.,* 2010; Farris and Morrison, 2011; Hafner *et al.,* 2008) and immune responses elicited following animal immunization trials (Cunningham *et al.,* 2011; McNeilly et al., 2007; Patton *et al.,* 1983). For example it has recently been reported that a *Vibrio cholerae* ghost (VCG) multisubunit chlamydial vaccine delivered to mice by the intramuscular route stimulated immune memory in these animals (Eko *et al.,* 2011). Protection correlates that have been assessed in these models to determine vaccine efficiency have included reduced shedding of viable chlamydial infectious bodies, reduced duration of infection and reduced tissue pathologies such as hydrosalpinx; a vaccine that can achieve one and/or any of these outcomes will greatly aid in diminishing the pathological sequelae of chlamydial genital infections.

Results of recent studies using animal models have revealed many promising novel candidate antigens for eliciting protection against chlamydial genital tract infections in humans. The fusion protein CTH1 is composed of chlamydial proteins from two highly conserved (>97% homology) immune-dominant antigens CT443 (*omc*B) and CT521 (r116) that are targets both for cell-mediated and for humoral immunity and thus can be expected to allow for cross-protection amongst the various chlamydial serotypes (Olsen AW *et al.,* 2010). In addition, CT 521 has also been found to be a strong and frequent target for T cells during a natural *C. trachomatis* infection in humans (Olsen *et al.,* 2006). In 2009 a study investigating 55 chlamydial ORFs covering all putative type III secretion components and control molecules were expressed as fusion proteins. This study measured the reactivity of these fusion proteins with antibodies from sera collected from patient infected with *C. trachomatis* in the urogenital tract (24 antisera) (Wang *et al.,* 2009). It was reported that immunization of mice with the translocated actin recruiting phosphoprotein (Tarp) induced Th1-dominant immunity that significantly reduced the shedding of live bacteria from the lower genital tract and attenuated inflammatory pathologies in the Fallopian tube tissues (Wang *et al.,* 2009). Using the C3H/HeN murine model the subunit vaccine CtH1 delivered subcutaneously with a Th1-inducing adjuvant (CAF01) induced a protective CD4+T cell response and high levels of CTH1-specific antibodies in both the sera and genital tracts of immunised mice however it failed to provide a CD4 independent protective response needed for complete protection (Olsen et al., 2010). A second promising vaccine candidate that has induced CD4+Th1 cells both in *Chlamydia*-infected mice and in humans diagnosed with chlamydial genital tract infections is CT043 a highly conserved hypothetical protein that could potentially provide cross-serotype protection. DNA priming/protein boost immunization with this protein the bacterial load was also significantly reduced in the murine lung infection model (Meoni *et al.,* 2009). The fact that CTD43 has been shown to reduce (1) chlamydial infectivity in the murine model and (2) to prime a CD4+Th1 response in over 60% of patients infected with genital serovars of *C. trachomatis*, means that this antigen could be a promising vaccine candidate for chlamydial genital tract infections. A

third candidate antigen showing great promise particularly for prevention of infertility resulting from repeated infections with *C. trachomatis* is the recombinant chlamydial protease-like activity factor (rCPAF) (Murthy et al., 2011).This antigen has been reviewed as a potential vaccine candidate (Murthy *et al.*, 2009) and has successfully induced a combination of neutralising antibodies and cell-mediated responses against genital chlamydial challenge in a murine model of genital chlamydial infection (Li *et al.*, 2010). More recent studies have reported that mice vaccinated intranasally with rCPAF and the adjuvant CpG were significantly protected against infertility as seen by a reduction in hydrosaplinx in rCPAF+CpG vaccinated mice following a primary genital challenge with *C. muridarum* (Murthy *et al.,*, 2011). This latest finding augurs well for the inclusion of this candidate antigen in a vaccine for use in humans to protect against female infertility.

Recently, a proteomics approach has been used to identify potential vaccine candidates for chlamydial infections. In one study three strains of mice, BALB/c, C3H/HeN and C57BL/6, were inoculated with live and inactivated *C. muridarum* by different routes of immunization. Using a protein microarray, serum samples collected from the mice after immunization were tested for the presence of antibodies against specific chlamydial antigens. This has identified a panel of seven *C. muridarum* dominant antigens (TC0052, TC0189, TC0582, TC0660, TC0726, TC0816 and, TC0828) (Molina, 2010). In a second study by the same group antigen identification was done by constructing a protein chip array by expressing the open reading frames (ORFs) from *C.muridarum* genomic and plasmid DNA and testing it with serum samples from *C.muridarum* immunized mice. This second approach has resulted in the identification of several new immunogens, including 75 hypothetical proteins thus identifying a new group of immunodominant chlamydial proteins that can be tested for their ability to induce protection (Cruz-Fisher *et al.*, 2011).

4.5 *Neisseria gonorrhoeae*

N. gonorrhoeae has been implicated in tubal infections. Both the bacteria themselves or components of the bacterial cell wall, lipopolysaccharide or peptidoglycan reportedly cause cessation of the ciliary activity (Mardh 1979) however, infection of the Fallopian tubes does not always result in ultrastructural damage to the mucosal surface (Woods & McGee 1986). Gonococci only invade the non-ciliated cells of the Fallopian tube mucosa, whereby the neighbouring ciliated cells become sloughy and detached (McGee 1981). *Neisseria* spp. infection of the Fallopian tubes results in a dose-dependent response to bacterial cells. Low numbers of bacterial cells induce secretion of TNF-α and subsequently apoptosis of infected cells, however, when bacterial cell numbers increase, the apoptosis appears to be inhibited, favouring bacterial survival (Dean and Powers, 2001). Studies have suggested that ectopic pregnancy is now more likely to be associated with non-gonococcal rather than *N. gonorrhoeae* upper genital tract infection (Kamwendo *et al.*, 1996).

4.6 Mycoplasma species

M. hominis reportedly causes ciliostasis and swelling of Fallopian tube cilia (Mardh & Westrom 1970). *Mycoplasma* spp. have been isolated from the female upper genital tract and Fallopian tubes (Cohen 2005; Heinonen & Miettinen 1994; Stagey *et al* 1992). Serological testing of women has confirmed an association between mycoplasmas and cases of PID (Moller *et al.*, 1985)and mycoplasma, PID and ectopic pregnancy (Jurstrand *et al.*, 2007). A prospective study of 212 infertile couples was undertaken to investigate the presence of

M.genitalium in women with tubal factor infertility and it was found that antibodies to *M.genitalium* were shown to be independently and significantly associated with tubal factor infertility (Svenstrup *et al.,* 2008).

Recently it has been reported that a genetic polymorphism in one of the components of the inflammasome - a cytoplasmic structure producing interleukin-1 - increases the likelihood of mycoplasma infection-associated female infertility (Witkin *et al.,* 2010).

4.7 *Ureaplasma spp.*

Ureaplasma spp. have been implicated in infections of the lower (bacterial vaginosis) and of the upper genital tracts (PID, endometritis) of women (Kanakas *et al* 1999). Further, tubal infertility has been associated with ureaplasma PID in a small number of cases (Henry-Suchet *et al* 1980. Inoculation of *Ureaplasma* spp. into *in vivo* Fallopian tube organ cultures resulted in replication of the pathogen, suggesting that this genital mycoplasma may also play a role in tubal damage.

4.8 Anaerobic bacteria

Anaerobic species are frequently isolated from the female genital tract and in cases of acute PID (Saini *et al* 2003). The polymicrobial nature of anaerobic infections appears to enhance the pathogenicity of the implicated species implicated (Eschenbach et al 1975). Previous upper genital tract pathology caused by upper genital tract infections, pelvic adhesions, endometriosis or prolonged or continuous menstruation, have been associated with the reactivation of PID. It is likely that the compromised areas of the pelvic cavity promote the establishment of a niche for bacterial survival (El-Shawarby *et al* 2004). Ness *et al.* (2005) reported that the presence of anaerobic bacterial vaginosis-associated microorganisms in the vagina was a significant risk factor for infection of the upper genital tract leading to long-term sequelae and possible PID.

Mobiluncus spp. are frequently isolated from women with bacterial vaginosis. Members of the *Mobiluncus* genera have been shown to produce cytotoxins, resulting in the loss of cilia, and bloating and detachment of the ciliated cells of the Fallopian tube mucosa (Taylor-Robinson *et al* 1993). Another of the genera associated with bacterial vaginosis, the gram-negative *Bacteroides* spp. also releases lipopolysaccharide, resulting in the sloughing of Fallopian tube epithelial cells and loss of ciliary activity within the Fallopian tubes (Fontaine *et al* 1986).

4.9 Aerobic/Microaerophilic bacteria

Gram-positive species, *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. have been isolated from Pouch of Douglas aspirates of a polymicrobial microflora in women with symptoms of genital tract infection (Saini et al 2003). Saini *et al.* (2003) proposed that the microflora of the Pouch of Douglas was likely to be more representative of Fallopian tube microorganisms in women with salpingitis than the vaginal flora of those same women.

Members of the Enterbactereaceae have been detected in upper genital tract infections. The Gram-negative bacillus, *Klebsiella* spp. are frequently isolated from women with PID (Saini et al., 2003). Laufer *et al.,*. ((Laufer *et al.,* 1984)) reported that inoculation of the Fallopian tubes with *Escherichia coli* resulted in complete de-ciliation or damage to the cilia. When damage occurred, the cilia were swollen and short. In addition, microvilli were lost from the non-ciliated cells. The oxidative species, *Pseudomonas aeruginosa* is also a causal agent of PID in females (King *et al.,* 2002).

Based on previous studies, it has been concluded that genital tract infections including salpingitis, which causes tubal factor infertility are polymicrobial in nature. Furthermore, a diverse range of microorganisms are capable of colonising and possibly infecting the genital tract tissues. Opportunistic pathogens identified in genital tract infections are frequently members of the normal regional flora and should be further investigated given that 60% of PID is non-gonococcal and non-chlamydial. Infectious causes of salpingitis and tubal factor infertility require further investigation to better establish prevalence and causality. The identification of microorganisms that are particularly detrimental to the Fallopian tubes may result in effective treatment and a reduction in the overall frequency of tubal factor infertility and tubal ectopic pregnancy.

5. Ectopic pregnancy and chlamydia

Infectious agents cause damage to the Fallopian tube mucosa either directly or because of the host inflammatory response aimed at clearing the infection. Alteration to the mucosa can result in poor transport of the embryo and subsequent implantation of the blastocyst outside the endometrial lining of the uterine cavity – an ectopic pregnancy. Tubal ectopic pregnancy is a result not only of impaired transport of the embryo causing the embryo to be maintained in the Fallopian tube but also a result from alterations in the tubal environment that allows early implantation to occur (reviewed in Shaw *et al.,* 2010). A recent review also summarises the results of investigations of the Fallopian tube with respect to the roles of of caspase 1, cannabinoid receptor and Dicer 1 knockout mice and how these contribute to tubal dysfunction and contribute to ectopic pregnancies (Shao 2010).

A recent review highlighted the many risk factors for ectopic pregnancy and these are summarised in Table 1.

Risk Factors for ectopic pregnancy
High risk
Tubal surgery
Tubal Ligation
Previous ectopic pregnancy
In utero exposure to diethylstilbestrol
Use of intrauterine devices
Tubal pathology
Assisted reproduction
Moderate risk
Infertility
Previous genital infections
Multiple sexual partners
Salpingitis isthmica nodosa
Low risk
Previous pelvic infection
Cigarette smoking
Vaginal douching
First intercourse (<18 years)

Table 1. Risk Factors for ectopic pregancy (adapted from Kulp and Barnhart, 2008)

Ectopic pregnancy accounts for up to 11% of all pregnancies and there is serological evidence that links ectopic pregnancies with *C. trachomatis* infection in women (Chow *et al.*, 1990; Swenson & Schachter 1984) It was noted in one study that 19/21 *C. trachomatis* seropositive women with ectopic pregnancies had antibodies to the chlamydial 57kDa antigen and it was suggested that perhaps immune responses to this antigen may be involved in the immunopathogenesis of ectopic pregnancy associated with *C. trachomatis* infections (Brunham *et al.*, 1992). A recent review has reported that one third of ectopic pregnancies could be attributable to chlamydial infection (Bebear and de Barbeyrac, 2009).

The finding of chlamydial RNA in Fallopian tube biopsy samples collected from women with ectopic pregnancies suggested that viable, metabolically active bacteria were present in the Fallopian tubes of these women (Gerard *et al.*, 1998). Chlamydial DNA has also been detected in the Fallopian tube tissue collected from women at the time of ectopic pregnancy (Barlow *et al.*, 2001; Noguchi *et al.*, 2002). A report to the contrary regarding the detection of chlamydial DNA in fresh tissue from the Fallopian tubes of women with ectopic pregnancy has, however, also been published and suggested that persistent chlamydial infection of Fallopian tubes was rare in ectopic pregnancy (Bjartling *et al.*, 2007). A retrospective study looking at births and ectopic pregnancy in 20, 762 women in Norway has reported that the risk of ectopic pregnancy increased in a dose-dependent manner with the increasing number of prior chlamydial infections (Bakken *et al.*, 2007). In a comprehensive review by the same author it has been reported that relatively low risks of ectopic pregnancy are recorded after a positive *C. trachomatis* diagnosis with, in Sweden for example, a cumulative incidence before the age of 35 years of rates of 2.7% *C. trachomatis*-positive and 2% *C. trachomatis*-negative (Bakken 2008).

In a small study of 14 ectopic pregnancy patients who were serologically-positive for *C. trachomatis*, subjective immunohistochemistry techniques were used to show an increase in the expression of inducible nitric oxide synthase (iNOS) (which is related to inflammation and infection and which can generate nitric oxide) and activin A (a member of the transforming growth factor beta family that has been reported to increase inflammation and repair) in Fallopian tubes from these women (Refaat *et al.*, 2009). It was proposed by these authors that tubal activin A and nitric oxide (NO) could perhaps be involved in microbial–mediated damaging immune response within the Fallopian tubes of *Chlamydia*-infected women and that their pathological expression may lead to ectopic pregnancy development (Refaat *et al.*, 2009). Nitric oxide is similarly proposed as the damaging agent of cells in the uterine tubes of female mice in the murine model of chlamydial genital infection. It is proposed that nitric oxide expressed in macrophages in response to a *C. muridarum* infection in mice could perhaps be the cause of damage to oviduct interstitial cells of Cajal (ICC-OVI) that have been identified as oviduct pacemaker cells critical for egg transport along the Fallopian tubes (Dixon *et al.*, 2010). The expression of iNOS by human Fallopian tubes has recently been reported as being cyclical during different stages of the menstrual cycle and the intensity of expression of iNOS was found to be higher in the Fallopian tubes of 15 women bearing an ectopic pregnancy when compared with pseudo-pregnant women (Al-Azemi *et al.*, 2010). These results suggested that increased iNOS levels in response to a microbial infection could lead to an increased expression of nitric oxide which may in turn affect the contraction of muscles and/or the ciliary beat in Fallopian tubes, ultimately leading to retention of the embryo at this site.

A recent report from The Netherlands has presented the finding that a peak incidence of admissions for PID in 1983 preceded a peak incidence of ectopic pregnancy in 1988 mainly

due to a decrease in ectopic pregnancy in women over 35 years of age. The report also states that women born between 1985 and 1990 and less than 25 years of age are now at an increased risk of ectopic pregnancy and this rise has not been preceded by a peak incidence of PID (Mol *et al.*, 2010). These authors further conclude that the significant rise in ectopic pregnancies may in fact be related to an increase in positive tests for chlamydial infection of the genital tracts (Mol *et al.*, 2010).

The management of ectopic pregnancy has recently been addressed (Kulp & Barnhart 2008; Mol *et al.*, 2011).

6. Endometriosis and Fallopian tube function

Women with endometriosis have an increased incidence of tubal ectopic pregnancy, suggesting that endometriosis results in impairment of tubal transport of gametes and embryos. Previous *in vitro* studies have revealed that peritoneal fluid collected from women with endometriosis caused a decrease in the Fallopian tube ciliary beat frequency (Lyons *et al.*, 2006), which supports the increased incidence of implantation of the embryo within the Fallopian tubes of these women. The pelvic inflammation associated with endometriosis can also cause adhesion and scar tissue formation within the Fallopian tubes creating a physical obstruction to embryo transport (Halis & Arici 2004). The pathogenesis of endometriosis involves changes both in cellular and in humoral immunity. Impaired natural killer cell activity results in inadequate removal of debris following retrograde menstruation. Elevated primary inflammatory mediators, which are characterised by increased numbers of macrophages, result in the production of secondary inflammatory mediators such as cytokines, chemokines and growth factors (Harada *et al.*, 2001) However, the secretion of primary inflammatory mediators can also be induced by microbial stimuli (Wira *et al.*, 2005). This highlights the relevance of a non-sterile endometrium in this aetiology. It may be that the retrograde menstruation of colonised or contaminated (by microorganisms) menstrual blood enhances the pathology of endometriosis by recruiting macrophages, which then secrete elevated levels of pro-inflammatory cytokines.

A small study investigating eutopic and ectopic endometrium, identified DNA with a 96% homology to the Gram-negative bacterium *Shigella* spp. in ectopic but not in eutopic endometrium. Therefore, an infection hypothesis was proposed for the pathogenesis of endometriosis (Kodati *et al.*, 2008). Recently, Khan *et al.,*. (Khan *et al.*, 2010) reported a significant increase in the number of colony forming units of *E. coli* recovered from the menstrual blood of women with endometriosis when compared to women without the disease. In their study, the bacterial endotoxin concentration was also higher both in the menstrual blood and in the peritoneal fluid samples from women with endometriosis. The relative level of *E. coli* within the peritoneal fluid of women with endometriosis was likely due to retrograde menstruation through the Fallopian tubes and into the pelvic cavity. The 'open' nature of the female genital tract makes it unlikely that secretions from the uterus, Fallopian tubes and peritoneal cavity remain compartmentalised. Transport of microorganisms within the upper genital tract may well be an area requiring further investigation in the pathogenesis of endometriosis and its increased association with tubal ectopic pregnancy.

Interestingly, *E. coli* has also been cultured from tubo-ovarian abscesses in women with ovarian endometriomas and pelvic endometriosis (Kavoussi *et al.*, 2006; Lin *et al.*, 2010).The fluid-filled ovarian endometrioma may provide an excellent growth medium for

microorganisms, and endometriomas have been identified as a risk factor for tubo-ovarian abscess formation (Kubota *et al.*, 1997; Lin *et al.*, 2010). Women with endometriosis appear to represent a population at increased risk of infection and subsequent tubo-ovarian abscess due to an altered local immune environment (Chen *et al.*, 2004; Lebovic *et al.*, 2001). The risk of endometriosis is also increased in women with shorter menstrual cycles and an increased menstrual flow (Halme *et al.*, 1984). Again, the retrograde menstruation of non-sterile menstrual blood into the peritoneal cavity provides a route for microbial transport. The menstrual debris may also promote continued survival and persistence of these microorganisms in the upper genital tract. Microorganisms have been detected in the endometrium of 83% of women during the post-partum period (Andrews *et al.*, 2005). However, both vaginal and endocervical cultures demonstrated low concordance with endometrial cultures (Cicinelli *et al.*, 2009). This may suggest that tropisms exist in the genital tract that can modulate microbial survival. In studies investigating women with chronic endometritis, Cicinelli *et al.*,. (Cicinelli *et al.*, 2008) reported isolation aerobic bacteria in over 73% of cases in symptomatic women but in only 5% of women without clinical evidence of endometritis. A shortcoming if their study was that they did not screen endometrial samples for the presence of anaerobes, which dominate the genital microflora. Together, these studies suggest that the endometrial cavity is not sterile, and that the presence of microorganisms does not necessarily result on overt inflammation. The possibility exists that in women with endometriosis, who have an impaired genital tract immune response, that (1) these microorganisms may replicate causing increased pathology, including tubal damage and (2) the microflora represent a stimulus for the enhanced chemotaxis of macrophages and the subsequent secretion of secondary inflammatory mediators identified in this condition.

Other chemical mediators have also been investigated in women with endometriosis. Inducible nitric oxide synthase, activated by cytokines and growth factors (Morris and Billiard, 1994; Nussler and Billiar, 1993), regulates embryo transport within the Fallopian tube. What is interesting is that in women with endometriosis and PID, nitric oxide levels are increased (Alpay *et al.*, 2006; Bouyer *et al.*, 2002; Sioutas *et al.*, 2008). Lipopolysaccharide was capable of *in vitro* activation of macrophages in the peritoneal fluid of women with endometriosis, increasing inducible nitric oxide synthase and nitric oxide production in these women but not in women without disease (Osborn *et al.*, 2002). If endometriosis is part of an infectious condition, then these results may be evidence of the activity f polarised macrophages in this population. A macromolecular ovum capture inhibitor, causing formation of a membrane over the fimbrial cilia, has been also detected in the peritoneal fluid from women with endometriosis (Suginami & Yano 1988).

Studies investigating ectopic pregnancy following IVF are limited however, it has been suggested that the hormonal stimulation protocol and the infertility history may also be mechanisms predisposing women to ectopic pregnancy (Chang & Suh 2010).

7. IVF

Hydrosalpinx is associated with decreased IVF success (Wainer *et al.*, 1997). Approximately 30% of women undergoing IVF for tubal factor infertility have hydrosalpinges (Blazar *et al.*, 1997; Murray 1997). The incidence of ectopic pregnancy was significantly higher in patients having IVF treatment for tubal factor infertility than in those diagnosed with infertility die to endometriosis or idiopathic infertility (Dubuisson *et al.*, 1991). It has been reported that

there was no relationship between the ectopic pregnancy rate and the ovarian hyperstimulation protocol used for ovulation induction (Dubuisson *et al.*, 1991). This is despite some studies suggesting that the hormonal protocols used in IVF contribute to ectopic pregnancy possibly by alterations in tubal muscle contractions or ciliary beat.

The ectopic pregnancy rate for women having IVF with natural and stimulated cycles was around 11% (Dubuisson et al., 1991), this is consistent with previous reports (Yovich *et al.*, 1985) indicating that in infertile women undergoing IVF and embryo transfer, the rate of tubal ectopic pregnancy is significantly higher compared to women conceiving naturally.

8. Conclusions

Infectious agents can damage biological functions of the female reproductive tract with devastating consequences. Among the most common microorganisms involved in sexually transmitted infections and interfering with female fertility are *C. trachomatis* and *N. gonorrhoeae*. These two pathogens are involved in damage to the cervix, Fallopian tubes and tubal luminal architecture in infected women. Tubo-peritoneal damage seems to be the leading cause of microbial interference with human fertility. *C. trachomatis* is considered the most important cause of tubal obstruction and PID. Screening for repeat chlamydial infections using randomised control trials and prevalence of *Chlamydia* rather than PID as an end point should provide information useful for controlling morbidity associated with these infections. Infection of the female genital tract with other bacterial organisms including *M. genitalium,* Ureaplasma spp., anaerobes and aerobes/microaerophiles can also affect the precise functioning of components of this site resulting in tubal occlusion and infertility. Bacterial vaginosis is strongly linked to tubal infertility as causative agents can produce ascending infections of the female upper genital tract. The role of bacterial infections particularly those caused by *C. trachomatis* and immune responses to these infections as causes of damage to sperm function require further investigation. Finally, continued efforts in vaccine development to control *C. trachomatis* genital infections would seem prudent to prevent sequelae of unresolved or untreated infections of the female genital tract that can have profound effects on fertility in women.

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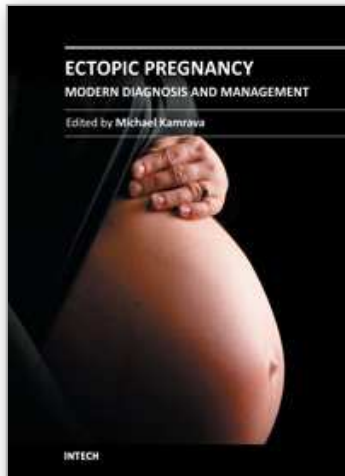
Ectopic Pregnancy - Modern Diagnosis and Management

Edited by Dr. Michael Kamrava

Ectopic pregnancy is the second major cause of maternal mortality in the United States and a leading cause of maternal morbidity and mortality in the world. This book contains the practical methods to early diagnosis of various forms of ectopic pregnancies and their modern management. Ectopic Pregnancy - Modern Diagnosis and Management is a comprehensive book which guides the reader through all features of ectopic pregnancy, both practical and academic, covering all aspects of diagnosis and management of ectopic pregnancy in a clear, concise, and practical fashion. The book is organized so that it can either be read cover to cover for a comprehensive tutorial or be kept desk side as a reference to the ectopic pregnancies. Each chapter introduces a number of related ectopic pregnancy and its diagnosis, treatment and co-morbidities supported by examples. Included chapters bring together valuable materials in the form of extended clinical knowledge from practice to clinic features.

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