

Chlamydia-Caused Infertility in Women:
An Evolutionary Hypothesis

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ABSTRACT

Chlamydia trachomatis is a pathogen that has been linked to infertility in women. This paper will discuss the possible evolutionary reasoning for causing female sterility, by analyzing a highly conserved plasmid in *Chlamydia trachomatis*. I hypothesize selective forces encourage a sterility-virulent strain of *Chlamydia trachomatis* that can cause infertility through reinfection, which for biological reasons, women are more susceptible to. By my proposed mechanism of chlamydia-induced infertility in women, this highly conserved plasmid acts in two ways: (1) increasing pathogenicity in the upper genital tract, causing the infection to ascend and damage structures, causing infertility and (2) increasing infection of the GI tract for higher rates of reinfection via autoinfection, which women are more susceptible to because of the structure of their anatomy.

INTRODUCTION

Infections of the bacterium *Chlamydia trachomatis* are consequential for humans: when *Chlamydia trachomatis* infects the eyes, it can cause trachoma, the leading cause of blindness, and when it infects the genital tract, it is the most commonly identified sexually transmitted infection (STI) around the world (Linhares and Witkin 2010). Infections of the genital tract have been especially well-researched in women, because over 70% of cases are asymptomatic, and they can lead to more serious reproductive consequences if left untreated. These include pelvic inflammatory disease, which can progress to infertility, and extrauterine pregnancy (Linhares &

Witkin 2010, Menon et al. 2015). Additionally, it is possible for a genital infection of *Chlamydia trachomatis* in mothers to be passed on to their children as an ocular infection, during childbirth (CDC 2018). In contrast, there is little evidence that demonstrates Chlamydia infections negatively impact male fertility or sperm viability (Suarez et al. 2017). Thus, public health interventions have focused on early diagnostic testing of women to catch asymptomatic cases, because infections are treatable with antibiotics (CDC 2018). However, persistence is hard to detect and reinfection rates are high, which is especially problematic because reinfections are linked to higher risks of reproductive complications (Rank & Yeruva 2014, Schuchardt & Rupp 2016).

It is not immediately clear why a sexually transmitted infection like from *Chlamydia trachomatis* would cause infertility, given the existence of a reproduction-immunity tradeoff. An infertile host is able to divert its resources away from reproduction towards bulking up its immune system, rendering it more effective at attacking pathogens (Apari et al. 2014). Moreover, there are currently no comprehensive explanations for the sexual asymmetry in reproductive consequences: why does Chlamydia cause such drastically different fertility outcomes for women and men? Much of the literature surrounding genital Chlamydia infections discuss biological mechanisms causing infertility in women, and only mention this dichotomy, without further explanation.

I propose a novel hypothesis that considers what is evolutionarily advantageous for the *Chlamydia trachomatis* pathogen: The reason Chlamydia causes infertility more in women is because evolutionary forces favor a less mortality-virulent, but more persistent strain of *Chlamydia trachomatis* that can cause infertility through reinfection. Because of the proximity of

their lower genital tract to the end of the gastrointestinal tract, a putative reservoir of *Chlamydia trachomatis*, women are more susceptible to being infected again and becoming infertile.

More specifically, I will support my argument by exploring the evolutionary forces acting on an important piece of circular DNA — a plasmid — in the *Chlamydia trachomatis* bacterium. Despite the fact that this plasmid lacks both antibiotic properties and conjugative or integrative elements that would allow it to be easily laterally transferred within bacterial populations, this one piece of DNA is highly conserved. There are almost no isolates from humans of *Chlamydia trachomatis* that do not have copies of the plasmid (Nunes & Gomes 2014).

Therefore, in this paper, I will first discuss the evolutionary pressures for sexually-transmitted bacteria (like *Chlamydia trachomatis* and the copies of plasmid they contain) to cause infertility. Second, I will focus on the plasmid, and how it directly causes infertility: it increases pathogenicity in the upper genital tract, allowing the infection to ascend to structures important for reproduction and damage them. Third, I will address how the plasmid also acts indirectly to cause infertility: it increases the infection of other tissues. This helps to explain some of the sexual asymmetry we see in reproductive outcomes of Chlamydia infection. Infection of other tissues like the lower gastrointestinal (GI) tract means women can clear just their genital infection, but then easily reinfect themselves, something they are more susceptible to because of the structure of their anatomy. At the end, I will also contextualize my hypothesis in light of current research, diagnostic testing, and vaccine development.

BACKGROUND

This overview of the *Chlamydia trachomatis* bacterium explains the pathogen's method of transmission and reproduction. The bacterium's unique life cycle is essential to the explanations that follow, of two existing hypotheses regarding mechanisms of Chlamydia-

induced infertility in women. My proposed hypothesis of plasmid-caused infertility, the focus of this paper, is compatible with both existing hypotheses.

Meet the bacterium: Chlamydia trachomatis

Chlamydia trachomatis is a spherical or oval-shaped bacterium with a cell wall lacking peptidoglycan (Malhotra et al. 2013). *Chlamydia trachomatis* is characterized by variation in the major outer membrane protein (MOMP). The variants that differ in MOMP or “serovars” are each best suited to different substrates: serovars A-C frequently infect the eyes, while serovars D-K infect the genital tract (Panzetta et al. 2018). These serovars demand specificity in treatment. For example, current vaccine development that will be discussed later, targets the serovars that infect the genital tract (Abraham et al. 2019).

Particularly of note is the fact that *Chlamydia trachomatis* is an obligate intracellular parasite: it cannot reproduce without entering a host cell and taking over its machinery. To accomplish this, the pathogen alternates between two forms, the infectious elementary body (EB) and the replicating reticulate body (RB) (Iliffe-Lee & McClarty 2003). Elementary bodies are 0.2 micrometers in diameter, and are meant for infection into the tissues. By design, they are more hardy, but they also cannot replicate (Panzetta et al. 2018). In contrast, reticulate bodies are much bigger, at 0.8 micrometers in diameter, and are meant only for replication inside of a host cell. If a reticulate body was in the extracellular matrix, it would be easily broken down (Panzetta et al. 2018).

Thus, the pathogen has a biphasic life cycle, transitioning between elementary and reticulate bodies depending on the environment. When *Chlamydia trachomatis* exists as the elementary body, its cell wall properties allow it to endure harsher environments outside of the cell and ultimately drive itself into a host cell (Elwell et al. 2016). Once inside, the pathogen can

then take on its larger reticulate body form, or its replicating form, where it co-opts the host cell's energy production to fuel its division by binary fission (Cappello et al. 2009).

It is when it exists as a reticulate body that *Chlamydia trachomatis* is able to counter host protection mechanisms. The bacterium prevents the infected host cell from undergoing apoptosis and committing suicide (Linhares & Witkin 2010). Additionally, if macrophage immune cells are infected by *Chlamydia trachomatis*, they will still secrete their tumor necrosis factor- α signal to recruit nearby immune cytotoxic T-cells. However, because the macrophages are infected, the signal instead induces the cytotoxic T-cells to undergo apoptosis as opposed to causing them to respond appropriately and target infected host cells (Jendro et al. 2004).

In the span of one to three days after existing as reticulate bodies, the *Chlamydia trachomatis* bacteria in a host cell eventually transition back to smaller elementary bodies in preparation for further infection of new host cells (Elwell et al. 2016).

Etiology: How is Infertility Caused in the Female Body?

It is important to note that the initial or "primary" infection alone is not sufficient to lead to female sterility. A remarkable feature of *Chlamydia trachomatis* infections of the female genital tract is its persistence. These persistent infections are what is associated with later pathologies in women, like inflammation of the reproductive organs (pelvic inflammatory disease) and sufficient damage and scar tissue to block the fallopian tubes, preventing sperm from being able to reach the egg (tubal factor infertility).

Persistence of *Chlamydia trachomatis* has been demonstrated in several ways. Studies conducted in the laboratory, or in vitro, have shown that when host cells containing *Chlamydia trachomatis* reticulate bodies (that normally divide) were placed in cell culture with antibiotics, the reticulate bodies stopped dividing, but were able to survive (Linhares & Witkin 2010). In

human subjects, or in vivo, experimental evidence has confirmed there are infections that persist in women for up to five years. This was accomplished using genotype sequencing to confirm the bacterial population in the host was the same the entire time. Alarming, long-term persistence was still observed even when antibiotics were repeatedly used — even though there are no antibiotic-resistance genes in *Chlamydia trachomatis* (Dean et al. 2000). This is because, under stressful conditions, the pathogen is able to induce multiple rounds of inflammatory responses from the host, which damage the genital tract, by a mechanism discussed below.

Turning to the dark side: how primary infections become persistent

While primary infections do not in themselves cause infertility, the host's response and antibodies meant to tag Chlamydial proteins can inadvertently trigger persistence, inducing a more insidious kind of infection that the host's immune system has a harder time detecting and ridding itself of.

During the primary infection, damaged host epithelial cells of the lower genital tract secrete chemokine and cytokine molecules to begin a cascade of signals that start inflammation (Malhotra et al. 2013). In response, host immune helper T cells secrete the signaling molecule interferon- γ , which activates an enzyme that breaks down the amino acid tryptophan in the host cells. Tryptophan is essential for reticulate body replication, but some *Chlamydia trachomatis* bacteria rely on host production because they cannot make it themselves (Linhares & Witkin 2010). Unfortunately, this only holds true for strains of bacteria that infect the eyes (ocular *Chlamydia trachomatis*): strains of *Chlamydia trachomatis* that attack the genital tract are often found with a functional tryptophan synthase, a likely byproduct of host-pathogen coevolution (Stephens 2003).

More dangerously, *Chlamydia trachomatis* populations in the genital tract can sense the attack from the host's immune system, and in response, they can take on a "persistent" form. While the entire biochemical mechanism of how this happens is not completely known, persistence has been observed in both in vitro and in vivo studies, as outlined above. Dividing reticulate bodies of *Chlamydia trachomatis* can transition to a persistent state in response to an assortment of stressors in the environment, like the cytokine signaling molecules of the immune system (particularly interferon- γ) or penicillin and other antibiotics. Once the unfavorable conditions are removed, the pathogen can return to its normal reticulate body form to resume reproduction and inflict further damage to its host (Malhotra et al. 2013).

A body in shock: How persistent infections lead to infertility

It is multiple rounds of a host inflammatory response, triggered by a specific heat shock protein produced by persistent *Chlamydia trachomatis*, that cause the fallopian tubes to scar so greatly that they become completely obstructed (Spandorfer et al. 1999). The exact mechanisms that cause infertility are also not fully understood, but there are several lines of evidence pointing to several plausible explanations. Two main hypotheses will be explained here, both focusing on the heat shock protein *Chlamydia trachomatis* produces under stress: (1) focusing on the direct effect of an autoinflammatory response and (2) on toll-like receptors that indirectly lead to more inflammation.

In the presence of antibiotics or in the face of an attack from host immune cells, *Chlamydia trachomatis* populations will pause activity. Production of new reticulate (replicating) bodies, secretory molecules, and major outer membrane protein (MOMP) all halt, making the pathogen less detectable (Panzetta et al. 2018, Dean et al. 2000). However, *Chlamydia trachomatis* is not completely metabolically inactive in its persistent state. In fact, the one protein

that the bacteria produce and secrete has been shown to be a likely etiological agent of downstream infertility in female hosts: a singular 60 kiloDalton (unit of size) protein called heat shock protein-60 (hsp60) (Linhares & Witkin 2010).

The family of heat shock proteins is highly conserved across many species in the evolutionary tree, including humans, because these proteins play an important role as “protein chaperones,” or as proteins that help other proteins fold correctly. They are often expressed under stressful conditions, when misfolded proteins may be more common and a protein chaperone like heat shock protein-60 is needed to help (Cappello et al. 2009). In humans, as a mechanism of cell-to-cell communication, heat shock protein-60 is often brought to the cell membrane, because a cell producing too many misfolded proteins may be damaged beyond repair. If this is the case, for example in a tumor cell, self-produced antibodies can recognize the heat shock protein-60 presented on the surface of a cell and tag that cell for apoptosis (Zügel & Kaufman 1999). If the cell is necrotic (unprogrammed, uncontrolled cell death), heat shock protein-60 released into the extracellular milieu and bloodstream becomes a signal to other cells of tissue death. This initiates the proinflammatory cascade in response (Pockley 2003).

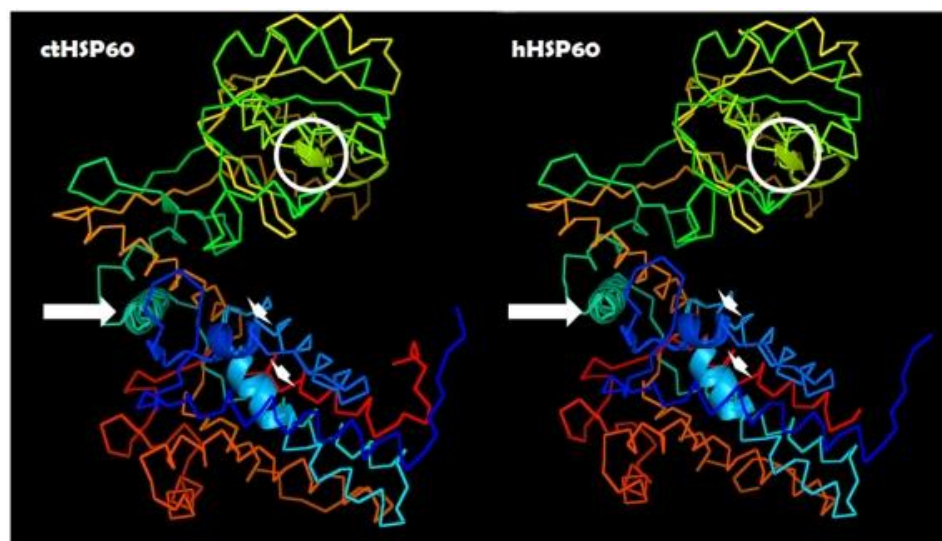


Figure 1: Comparison of *Chlamydia trachomatis* heat shock protein and Human heat shock protein (Source: Cappello 2009)

One hypothesis for why hosts exhibit multiple inflammatory responses against the pathogen draws on this inherent susceptibility in humans, because of our own use of a heat shock protein to create inflammatory responses. This is because the heat shock protein is so highly conserved: the heat shock protein-60 protein (hHsp60) possessed by humans is homologous to the *Chlamydia trachomatis*-produced heat shock protein-60 (ctHsp60), as demonstrated by **Figure 1**. When large amounts of antibodies meant to be specific for *Chlamydia trachomatis* heat-shock-protein-60 are produced, they increase the risk of mistakenly tagging the endogenous host's self-produced protein, causing downstream inflammatory responses and host tissue destruction (Witkin et al. 1996).

Another proposed hypothesis for *Chlamydia trachomatis*'s mechanism of causing infertility draws on the closely related species, *Chlamydia pneumoniae*, that infects the lungs and can cause pneumonia. The *Chlamydia pneumoniae*-produced heat shock protein 60 also reacts specifically with toll-like receptors on host immune dendritic cells, or antigen-presenting cells. Toll-like receptors are transmembrane proteins that can recognize general characteristic domains common to many antigens on the extracellular side and transmit that information to induce innate immune responses and inflammation. Research has demonstrated similarities in the responses of toll-like receptors during an infection of *Chlamydia pneumoniae* and during an infection of *Chlamydia trachomatis* (Stephens 2003). This suggests an alternate mechanism of inflammation for *Chlamydia trachomatis* infections, where *Chlamydia trachomatis*-produced heat shock protein-60 is recognized by toll-like receptors on dendritic cells in the female genital tract.

These two hypotheses, both surrounding host responses to the heat shock protein-60 produced by *Chlamydia trachomatis*, are not the only ones proposed. Indeed, it is likely there are other pathways that further contribute to inflammation — some still being discovered. For example, there have been several studies regarding causes of infertility conducted in mouse models as well, that demonstrate antibodies against *Chlamydia trachomatis* heat shock proteins can also directly induce trophoblast apoptosis or impair embryonic development at various stages, although this has never been actually observed in human embryos (Neuer et al. 1998, Equils et al. 2006). Regardless of the specific mechanism, the proinflammatory responses of each can cause damage to the tissue of the genital tract over time. Specifically in the fallopian tubes, resultant scarring can cause tubal occlusion, or blockage of the fallopian tubes, preventing sperm from reaching the egg or a fertilized egg from descending to the uterus (Tang et al. 2020).

EVOLUTIONARY ADVANTAGE OF STERILITY IN STDS:

There's Something About Infertility

However, for all the discussion that has been had about inflammation causing infertility, biochemical analyses offer no answers as to if the expressed goal of *Chlamydia trachomatis* is sterilizing the host. Considering just the *Chlamydia* heat shock protein, it is still possible that the triggered host immune responses only inadvertently cause infertility later on. However, taken in the context of evolutionary perspectives of tradeoffs in pathogen spread, it is more likely that *Chlamydia trachomatis* has evolved to purposefully induce female sterility.

We can understand why by drawing comparisons to other types of pathogens. Non-sexually transmitted infections that are also spread horizontally (across a population, not passed on vertically from a parent to offspring) face a transmission-virulence tradeoff. In this tradeoff, competition amongst a pathogen population within a single host favors the survival of the more

virulent strains that extract more resources from that host. However, if the population is too virulent, the pathogen risks killing the host and never being transmitted (Stearns & Medzhitov 2018). This tradeoff becomes complicated when considering sexually transmitted pathogens, like *Chlamydia trachomatis*. Sexually transmitted pathogens have *two* types of virulence that may prevent transmission if too great: they suffer from risk of killing the host and the risk of diminishing host sexual activity, the mechanism by which it is passed on (Wardlaw & Agrawal 2019). There are many examples of sexually transmitted pathogens specific to humans that avoid both host mortality or diminished sexual activity and have disproportionately escalated their sterility virulence in comparison to their mortality virulence. Beyond *Chlamydia trachomatis*, other bacterial infections like *Neisseria gonorrhoeae*, *Treponema pallidum*, *Gardnerella vaginalis*, or even eukaryotic sexually transmitted infections like *Trichomonas vaginalis* all negatively impact host reproduction without killing the host (Apari et al. 2014). Evidently, there is something favorable about a sexually transmitted pathogen causing infertility in the host. This section will not only address (1) the mode of transmission that promotes sterility virulence in *Chlamydia trachomatis* and its plasmid, but also (2) a discussion of three evolutionary hypotheses that help justify the reasoning for this.

Frequency-Dependent Transmission: Why it Matters

Whether or not an infection like *Chlamydia trachomatis* should cause infertility depends on its mode of transmission. It makes a difference whether or not a pathogen depends on the density of infected hosts or frequency of infected hosts to spread. For most nonsexually-transmitted infections, for example like in the case of the currently relevant SARS-CoV-2, the more people that are infected, the greater the likelihood of new transmissions from those hosts to the susceptible population. If there are more infected people in a given area (density of infected

hosts), there will be a greater number of novel infection events just by chance. In the case of SARS-CoV-2, susceptible hosts can become infected by talking to or spending enough time with an infected host: this is an example of density-dependent transmission. However, if we consider *Chlamydia trachomatis*, an increase in the number of infected hosts (density of infected hosts) does not necessarily increase the number of sexual encounters a host will choose to have.

Instead, what matters is the proportion of population with *Chlamydia trachomatis*, something we call frequency-dependent transmission. Say one out of every ten people in a given population has a *Chlamydia trachomatis* infection. If the population grows proportionally, so the one out of ten ratio is still the same, even if technically there are more people that have *Chlamydia trachomatis* in the population, the risk to a susceptible host is still the same, so long as they choose to not increase their number of sexual encounters (Thrall et al. 1993; O’Keefe 2005). This assumption that the number of sexual encounters doesn’t change is not completely perfect — there may be weak associations between density and transmission events — but generally, sexually transmitted infections can be described as frequency-dependent (McCallum et al. 2001).

Mathematical modelling has been done that accounts for the timescale of evolution, the density of infected and susceptible hosts, and the existence of a reproduction-survival tradeoff. It was found that if mortality is density-dependent, then as the transmission parameter approaches a frequency-dependent mode of transmission, strains of a sexually transmitted infection that sterilize the host will be favored (McLeod & Day 2019; see **Supplementary Information** for analysis of McLeod & Day 2019 mathematical proof). This helps to explain why the pathologies of *Chlamydia trachomatis* and its plasmid all ultimately sterilize the host. Thus, epidemiological studies have demonstrated sexually transmitted infections (STIs) have frequency-dependent

transmission, and would thus be evolutionarily favored to cause sterility over nonsexually-transmitted infections, which have density-dependent transmission.

Evolutionary Perspectives: Why is *Chlamydia trachomatis* sterilizing?

There are a few plausible arguments that attempt to explain why this trend of STIs causing sterility exists. The first argument suggests that hosts drove the evolution of sterilizing STIs like *Chlamydia trachomatis* to encourage monogamy in host partners. The basis of this argument is that fewer partners lowers the risk of contracting an STI, and hosts may use STIs to their advantage to encourage monogamous behavior in their partner. Comparative biology already sows seeds of doubt against this hypothesis: STIs exist in animal species across the phylogenetic tree, in several mating systems that are not monogamous (Lockhart et al. 1996). However, even when dealing with reliably monogamous systems, it is not entirely clear if STIs would always encourage monogamy. While it is true that fewer partners may reduce the risk of STIs, the host still must reproduce and having fewer partners may be a higher fitness cost (McLeod & Day 2014). Mathematical modelling has demonstrated that only STIs that impact mortality will favor monogamous relationships. *Chlamydia trachomatis*, which impacts fecundity without killing the host, will not encourage monogamy (McLeod & Day 2014). It is thus unlikely that STIs evolved to be sterilizing because hosts wanted to encourage monogamy in their partner.

Instead of considering mating systems, the two remaining evolutionary hypotheses each take on the perspective of a different player in the host-pathogen relationship: the host or the pathogen.

From the perspective of hosts that are infected with *Chlamydia trachomatis*, their aim is to maximize reproductive success with the lowest risk of infection (Lockhart et al. 1996). This

further drives asymptomatic infections, as mate choice of prospective partners or lower libido of the host in symptomatic infections may hinder the host from passing on its gametes as successfully (Ashby 2020). The dynamics of host evolution in response to pathogens is still a topic of current research.

From the perspective of the sexually-transmitted pathogen *Chlamydia trachomatis*, it aims to infect new hosts when possible. Sterilizing a host can destabilize a couple that would be otherwise monogamous and encourage promiscuity, because of the host's interests in passing on their gametes. For *Chlamydia trachomatis*, this is favorable because it now involves the introduction of new susceptible hosts (Apari et al. 2014). Even further, the infected host may be agnostic regarding their own infertility: *Chlamydia trachomatis* infections are often asymptomatic and there may be multiple attempts to reproduce, that would not have occurred had the host been fertile (Lockhart et al. 1996). While, in the presence of a reproduction-immunity tradeoff, it is unfavorable for a pathogen to induce sterility because the host can use those resources for pathogen defense systems, all hosts must mate (Apari et al. 2014). This ensures that at the very least there exists a low level of transmission at a population level (Lockhart et al. 1996).

It is clear that there are evolutionary advantages to infertility that are not straightforward, but exist at a delicate balance of trade-offs: hosts want to reproduce but do not want *Chlamydia trachomatis* infections, the *Chlamydia trachomatis* bacterium wants to be transmitted to as many hosts as possible but also not so virulent it is detectable or deadly to the host.

THE HIGHLY CONSERVED PLASMID:

What's so special?

In the search for the specific cause of infertility in *Chlamydia trachomatis*-infected females, more recent research has turned to an interesting 7.5 kilobase piece of circular DNA with eight sites for translation (open reading frames) that is highly conserved across several species of Chlamydia. Evidence that this plasmid is involved in causing infertility in infected women is not in conflict with the discussion of heat shock protein mechanisms mentioned above, but rather, adds to it.

Several lines of research demonstrate particular properties of the Chlamydia plasmid that make it interesting to study. This plasmid, which accounts for less than 1% of the *Chlamydia trachomatis* bacterium's total genetic material, is universal to Chlamydia, and yet it contains no elements that allow it to be easily passed across bacteria or that confer antibiotic resistance (Nunes & Gomes 2014). Even further, *Chlamydia trachomatis* without a plasmid is able to grow and proliferate normally when plated under laboratory conditions (in vitro), but isolated strains living in organisms (in vivo) almost always have the plasmid (Zhong 2017). Here, I will argue that evolutionary pressures have favored maintaining this plasmid and suggests host-parasite interactions are a factor in its maintenance in humans. The plasmid's continued existence is evolutionarily favored for two reasons: (1) it increases the pathogenicity of *Chlamydia trachomatis* in the genital tract and (2) it increases the pathogen's transmissibility to other tissues of the same host.

Plasmid-Induced Infertility: Sugar, Travel, and More Inflammation

The plasmid seems to have multiple mechanisms that contribute to its pathogenicity in the female genital tract. It can exacerbate the existing infection and host heat-shock protein responses in the following ways: (1) helping the bacterium accumulate glycogen stores for itself within the host cell, (2) aiding the pathogen's travel up the fallopian tube, and (3) inducing

inflammation so consequential that the end portion of the fallopian tube near the ovary becomes blocked with fluid (a condition called hydrosalpinx) (Zhong 2017). Many of the following cited studies have been conducted on mouse models, but the mechanisms of *Chlamydia trachomatis* (the strain that infects humans) and *Chlamydia muridarum* (the strain that infects mice) are highly similar.

Initial research first discovered that the plasmid helps the pathogen sequester sugar from the host for its own use (O'Connell & Nicks 2006). A major limitation of any obligate intracellular pathogen, which needs the host's resources to survive, is its ability to take sufficient important nutrients for reproduction. Studies using mice infected with plasmid-free strains of *Chlamydia muridarum* demonstrated that without the plasmid, the pathogen was unable to accumulate glycogen (O'Connell & Nicks 2006). Studies conducted later confirmed that part of the plasmid's role in chlamydia is greater glycogen accumulation. After the plasmid was reintroduced into plasmid-free strain of *Chlamydia trachomatis*, more glycogen granules in the vacuoles of the host cytoplasm were found. These vacuoles, created by the pathogen, are also known as inclusions. Increased glycogen synthesis was associated in another study with the upregulation of the gene (*glgA*), involved in the *Chlamydia trachomatis* metabolic pathway (Carlson et al. 2008; Wang et al. 2011). Thus, it is likely the plasmid plays a regulatory role on this gene which is essential for the pathogen to synthesize glycogen.

The plasmid has also since been shown to contribute to the ability of the *Chlamydia trachomatis* to ascend the genital tract. This is important because, for women, the source of an infection is via intravaginal inoculation, or from the lower genital tract, and it must travel upwards to the upper genital tract to eventually cause infertility (Chen et al. 2015). Being able to infect the cervix, which opens up into the upper genital tract structures like uterus and the

fallopian tubes, is essential. While plasmid-free *Chlamydia muridarum* was still able to cause inflammation and damage to upper reproductive structures after an intrauterine inoculation (near the upper genital tract), intravaginal inoculation of the plasmid-free *Chlamydia muridarum* was not able to travel up the genital tract and cause the same damage (Lei et al. 2014). Thus, crossing the cervical barrier is essential for an infection to cause infertility, demonstrating how the plasmid plays a direct role in the pathogen's ascension.

Finally, perhaps most relevant to the sterility consequences in women, the plasmid also induces greater inflammation of the upper reproductive tract, often inducing fluid blockage of the fallopian tubes, a pathology called hydrosalpinx. This condition is often a detectable marker of tubal factor infertility, which is caused when the sperm is prevented from reaching the egg because of the blockage (Lei et al. 2014). The mechanism of this induction of inflammation can be traced back to one of the gene products of the plasmid. While the functions of all eight plasmid glycoproteins (pGP1-pGP8) encoded by the plasmid are not yet completely mapped out, it is understood that the third plasmid glycoprotein (pGP3) is a key virulence factor that contributes to downstream infertility sequelae. Specifically, a slew of host signaling and inflammatory pathways are activated by pGP3: interleukin-1 receptors, tumor necrosis factor receptors, CXC-chemokine receptors, and more (Zhong 2017). Interestingly, in studies where some of these inflammatory signaling molecules have been removed from the host, it has been found that they do not affect infection progression (Cheng et al. 2008). These studies suggest the plasmid may have evolved to stimulate inflammatory responses that are not only unhelpful to the host, but also detrimental to host fertility.

THE PLASMID AND OTHER TISSUES

A Puzzling Observation: Plasmid-Mediated Infectiousness

Recent research on the *Chlamydia* plasmid has revealed that it does more than increase inflammation in the genital tract to cause infertility. The plasmid also aids the *Chlamydia trachomatis* in infecting other tissues. In studies with nonhuman primates, plasmid-deficient strains of *Chlamydia trachomatis* that infect the eye (ocular serovars) were less infectious and the same was shown in studies with mouse models using *Chlamydia muridarum* in the gastrointestinal (GI) tract (Kari et al. 2011, Ma et al. 2020). However, this is not universally true. The initial infectiousness, or “shedding” of *Chlamydia trachomatis* as a method of bacterial spread from the lower genital tract, was shown to be comparable in plasmid-deficient strains to strains with the plasmid (Zhong 2017). In other words, even without the plasmid, *Chlamydia trachomatis* had other sufficient mechanisms of infectiousness in the genital tract. This seems to suggest the evolutionary reason for the incorporation of the plasmid was not only to increase infertility outcomes directly but also to increase the infection of *other* tissues, like in the eye and GI tract.

This may seem like an inadvertent result. One might argue that perhaps the plasmid’s prevalence in the wild was due to random chance, and the increased ability to infect other tissues is not evolutionarily favored. This may be a valid argument in cases regarding transmission from the eye. While there have been cases of neonates with conjunctivitis (infections of the eye) because during birth the mother’s genital infection of *Chlamydia trachomatis* was passed on, infection from the eyes back to the genital tract has not been recorded (CDC 2018). Research has cited strong substrate differentiation between ocular serovars (strains of *Chlamydia trachomatis* meant to be specific to the eye) and genital serovars as the reason (Faris et al. 2019). Genital serovars make tryptophan using a tryptophan synthase, evading immune mechanisms that restrict

the essential amino acid's availability in the genital tract, as discussed earlier, while strains specific to the eye have lost their functional tryptophan synthase (Hu et al. 2013).

In contrast, serovars that infect that genital tract are no different from the serovars that can infect the gastrointestinal (GI) tract (Rank & Yerova 2014). Infections from the genital tract to the GI tract and back again are not only possible, but have also been observed. Those arguing that the plasmid's infectivity of other tissues is inadvertent may point out that in the GI tract, the pathogen is unable to induce inflammatory responses to cause host sterility. It is true that the immune responses are significantly attenuated in the GI tract, but I argue below that the GI tract is used as a reservoir for infection instead of a direct contributor to sterility through inflammation.

PLASMID-MECHANISM OF PERSISTENCE:

Self-Infection from the GI Tract

Therefore, my hypothesis is that because the goal of *Chlamydia trachomatis* is to induce infertility to increase its transmission, the plasmid increases the infectivity of other tissues, specifically the GI tract. The advantage of infecting other tissues of the same host would be that it increases self reinfection rates, increasing the likelihood of host infertility.

The idea of the GI tract as a reservoir for infection is not new. Although animals are infected with different species within the genus *Chlamydia*, numerous animal studies have provided support for *Chlamydia* infection of the gastrointestinal tract, particularly the cecum, or beginning of the large intestine, in parrots, sheep, pigs, and cows (Meyer & Eddie 1993; Kawakami et al. 1958; Köbl 1969; York & Baker 1951). Further recent research has demonstrated that when mouse models were infected both orally and intravaginally with *Chlamydia muridarum*, there were immune reactions in both, but marked persistence in the

cecum when compared with the genital tract (Rank & Yerova 2014). Worryingly, it has also been demonstrated that the antibiotic azithromycin is much less effective on the *Chlamydia muridarum* infection of the GI tract than the genital infection (Hocking et al. 2014; Rank & Yerova 2014). It is possible immune downregulation in the cecum prevented the adaptive immune response that would have aided with clearing the infection in the GI tract.

Having the GI tract available as an infection reservoir would make it evolutionarily favorable for the plasmid to carry regulatory components that allow for increased infectivity of other tissues. The mechanism of the plasmid's contribution to Chlamydial infectivity was illustrated in a recent study that differentially inoculated different parts of the gastrointestinal tract of mice with *Chlamydia muridarum* (Ma et al. 2020). While strains containing the plasmid were able to infect tissues in all areas of the gastrointestinal tract, plasmid-deficient bacteria were shown to be killed in the stomach (Ma et al. 2020). A direct inoculation of the jejunum (part of the small intestine) demonstrated *Chlamydia muridarum* without the plasmid was 530 times less infectious, an evaluation based on the amount of infectious elementary bodies in the lumen (Ma et al. 2020). Finally, intracolonic infection of the plasmid deficient bacteria was also much less effective than strains with the plasmid, as the plasmid appeared to be important for quicker adjustment to the colon environment (Ma et al. 2020).

This study supports the argument that the plasmid allows *Chlamydia trachomatis* to purposefully infect the GI tract, which can act as a safe haven for a subset of the *Chlamydia trachomatis* population in the host. If self-reinfection is possible, host immune responses launched in the genital tract become Pyrrhic victories, as they inflict increasing damage to the host's reproductive structures while the pathogen can continue to reinoculate the genital tract from the GI tract. This has been confirmed in clinical studies, where more of these reinfection

episodes have been correlated with greater risk of tubal factor infertility (Schuchardt & Rupp 2016). It is true that it is difficult to determine the existing rates of self-reinfection in women because of other factors that may lead to two positive results of the same strain (e.g. partner reinfection or from the same infection). However, from data collected on positive tests 8-10 months apart, it is estimated reinfections range from about 12-20 percent of all infections (Schuchardt & Rupp 2016). It is feasible that because evolution favors sterilizing STIs to be less mortally virulent and more persistent, this plasmid has enhanced *Chlamydia trachomatis*'s infection of other tissues. The pathogen sets itself up for future reinfections, ultimately increasing its success in causing infertility in a female host.

SEXUAL DIFFERENCES IN INFERTILITY EXPLAINED

Why only infertility in females?

If it is true that *Chlamydia trachomatis* has evolved to be more persistent, through plasmid-mediated mechanisms of infection of the GI tract, this would explain why it might seem that *Chlamydia trachomatis* targets female hosts. In reality, it is not so much *targeting* females as it is more successful when using females as the primary host, because of anatomical differences. In female hosts, especially those younger than 20 (the age group at highest risk for Chlamydia), the squamocolumnar junction of the cervix, located in the lower genital tract, turns outwards more and is more exposed to infection (Zhong 2017). Moreover, it is possible that the anus (of the GI tract) and the vagina are simply proximal when compared to the distance between the anus and male ureter.

Previous researchers, even those not expressly exploring the contributions of the plasmid-mediated reinfection to *Chlamydia trachomatis* infection pathology, have also made suggestions that the accessory glands of males act more as possible reservoirs for female infection (Krause &

Bohring 2003; Purvis & Christiansen 1993). If females are a ‘better’ host for *Chlamydia trachomatis* because their susceptibility to reinfection makes them more likely to become sterile, there would be an advantage for the pathogen to particularly specialize to seriously affect female hosts (Schuchardt & Rupp 2016). Evolution in *Chlamydia trachomatis* for this level of sexual preference or infection specificity has not been observed, and this much is still conjecture. What remains clear is the proximity of the anus to the vagina in female hosts allows for a greater chance of reinfection and by extension, a greater chance of sterility. This would explain the more serious fertility consequences of a *Chlamydia trachomatis* infection in females when compared to males.

CURRENT RESEARCH:

What does the future look like?

While research of *Chlamydia trachomatis* has been robust and expanding, additional evolutionary perspectives are vital if we want to limit infections and remove its place as the most commonly diagnosed STI (Linhares and Witkin 2010). This is important across many levels, from diagnostic testing to vaccine development.

The use of diagnostic testing is our best way of early treatment to prevent the inflammatory responses that lead to infertility, and its importance is underscored by our understanding of *Chlamydia* persistence. As bacterial populations are known to accumulate mutations, it may be a good idea to search for the deeply conserved sequences within the *Chlamydia* plasmid that are vital to its survival when diagnostic testing. Being able to properly diagnose infections is a good first step towards eliminating the bacteria: we can prevent errors like the false negative diagnostic test in Sweden, where a mutant strain there contained a deletion of the plasmid (Jones et al. 2020; Zhong 2017).

Additionally, so far, most epidemiology has focused on sufficient testing of women. However, if men are reservoirs for infection of women, it may be beneficial to place equal pressure on males to take diagnostic tests. While the infection may not cause infertility in males, increasing their testing may indirectly help with diagnosis or prevention of infection in female hosts.

As of October 2019, phase I human trials are underway for a Chlamydia vaccine. It had been previously reported in mouse models that protective immunity was significantly stronger when live bacteria were delivered (via nose or muscle injection) as opposed to inactivated *Chlamydia muridarum* (Lu et al. 2011). The current trial vaccine uses an antigen that is a recombinant version of the major outer membrane protein (MOMP) with epitopes of all genital serovars of *Chlamydia trachomatis* (Abraham et al. 2019). It has not yet been determined how successful the vaccine is, but for now, it gives us hope that there have been no recorded instances of antibiotic resistance in *Chlamydia trachomatis*. It is only important to realize that confirming the clearance of the genital tract with antibiotics may not be sufficient, as the gastrointestinal tract should also be separately tested to lower existing rates of reinfection.

CONCLUSION

Evolutionary perspectives are exciting because they help to answer questions that are not necessarily intuitive: why would a sexually transmitted infection like *Chlamydia trachomatis* cause infertility? As it is spread via frequency-dependent transmission, it is beneficial for the pathogen to manipulate host behavior to increase its transmission. If hosts are asymptomatic, repeatedly attempt to breed, and are driven to be promiscuous by an infertile female, this is all beneficial to the pathogen. Later questions, like why an asymmetry in fertility consequences across sex exists, requires focusing on the evolutionary pressures faced by *Chlamydia*

trachomatis that specifically manifested in the functions of its cryptic plasmid. How does the plasmid contribute to infertility directly? As part of a sexually transmitted infection, the plasmid aids in causing infertility, by helping the infection ascend the female genital tract and by inducing inflammatory responses unnecessary to the host but with higher fertility consequences. But why does it not contribute to colonization of the genital tract? While genital tract infectiousness is already regulated by the *Chlamydia trachomatis* genome, the survival of the plasmid in *Chlamydia trachomatis* is enhanced because it gives the pathogen properties that increases its colonization of *other* tissues. Particularly of consequence is the greater likelihood for infections of the GI tract, which is an immune suppressed organ that can act as a reservoir for infection. Thus, because of the proximity of the female genital tract opening to the anus of the GI tract, women are more susceptible to reinfection, even after initial clearance of their genital infection. This helps to explain why infertility consequences from a *Chlamydia trachomatis* genital tract infection are much more common in females than in males.

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