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The Role of *Chlamydia trachomatis* in Male Infertility

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1. Introduction

1.1 Cell biology of *Chlamydia trachomatis* (*C. trachomatis*)

Chlamydia spp. are associated with a broad clinical spectrum of human diseases, including cardiovascular disease, and pulmonary, ocular and urogenital tract infections [1]. *C. trachomatis* is an obligate intracellular pathogen. The infection cycle starts with the entry of an infectious particle (elementary body or EB) into an epithelial cell. The EB-laden cytoplasmic vacuole (inclusion) migrates to the peri-Golgi region as the EB differentiates into a noninfectious but metabolically active reticulate body (RB). After replication, progeny RBs differentiate back to EBs for exiting the infected cells to disseminate to adjacent cells [2].

Over 18 serological variants (serovars) of *C. trachomatis* have been identified based on monoclonal antibody typing of the major outer membrane protein (MOMP). Serovars A, B, Ba and C cause trachoma, the leading cause of infectious blindness worldwide. Serovars Ba and C are also rarely associated with urogenital infections. Serovars D to K, Da, Ia and Ja are responsible for sexual transmitted diseases (STD) worldwide. The lymphogranuloma venereum (LGV) serovars L1-L3 and L2a, along with serovars D and G, are prevalent in anorectal infections unlike other genital serovars [3].

The clinical course of *C. trachomatis* infection shows remarkable interindividual differences in transmission, symptomatic course, persistence or clearance of infection, and development of late complications. In general, the described differences in clinical course could be explained by interaction between the host (host factors), pathogen (virulence factors), and environmental factors (such as coinfections) [4].

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1.2 Urogenital infections with *C. trachomatis* in women and men

C. trachomatis is the most prevalent bacterial cause of sexually transmitted infections in the world and can result in severe genital disease. Over 90 million chlamydial infections are detected annually worldwide and various studies have estimated that there are four to five million new cases of chlamydial infection each year in the USA alone [5, 6]. However, the reported incidence rates of genital chlamydial infections in the population likely are an underestimate because of the highly asymptomatic nature of the pathogen. Approximately 75% of infected women and 50% of infected men have asymptomatic urogenital infections, which represents a huge population of untreated individuals who can transmit the organism [5].

In women, genital tract infections caused by *C. trachomatis* cause major complications as pelvic inflammatory disease (PID), ectopic pregnancy, infertility and infant pneumonia. The risks factors vary in different population groups. However, in most cases, higher prevalence rates in sexually active individuals have been associated with younger age, unmarried status, low socioeconomic conditions and the use of oral contraceptives. *C. trachomatis* is recovered more often from women who acquire gonorrhea than from similarly exposed women who do not acquire gonorrhea [7, 8].

Non-gonococcal urethritis (NGU) is the most common clinical genital syndrome seen in the male, and *C. trachomatis* is the most important etiological agent for NGU. According to the Centers for Disease Control and Prevention (CDC), reported cases of men infected with *C. trachomatis* in the USA raised from 210,955 in 2004 to 315,065 in 2008, and infection rate per 100,000 population in the same period raised from 144.0 to 209.1 [9].

Infection is primarily through penetrative sexual intercourse. In view of the increased practice of oral sex this has become a more important potential route of transmission for genital pathogens, including *C. trachomatis*, not only in homo/bisexual men, but also in heterosexual men [10, 11]. Cell-to-cell transmission, systemic dissemination, and autoinoculation of infectious fluids may contribute to chlamydial spread in the organism [12].

NGU may be complicated by epididymitis and orchitis. Thus, a role of *C. trachomatis* infection in the development of urethritis, epididymitis and orchitis is now well accepted, but a role for this pathogen in the development of prostatitis remains controversial [13, 14]. These disorders caused by *C. trachomatis* in men will be discussed in the next section.

2. Clinical manifestations of male urogenital infection with *C. trachomatis*

2.1 Urethritis

The symptoms of urethritis are variable. In acute urethritis, the patient notices a urethral discharge and dysuria. Others have no symptoms or are symptom-free throughout the day and only notice a drop of pus in the morning prior to the first voiding of urine. Sometimes the glans or meatus urethrae may present with some redness as a sign of inflammation [15].

Urethritis can be caused by several microorganisms. The most relevant are *Neisseria (N.) gonorrhoeae, C. trachomatis, Ureaplasma (U.) urealyticum, Mycoplasma (M.) genitalium* and *Trichomonas (T.) vaginalis* [15, 16]. *C. trachomatis* is the most common pathogen identified in NGU; up to 42% of NGU cases may be caused by this bacterium [17]. Furthermore, there are

three cases of chlamydia per case of gonorrhea each year in the USA [18]. Overall, the reported frequency of *C. trachomatis* in male urethritis ranges from 15 to 56% [15, 19].

C. trachomatis infection appears to be equally prevalent in symptomatic and asymptomatic urethral disease, again reiterating the highly asymptomatic nature of this pathogen [13, 20, 21]. When the infection is asymptomatic and undiagnosed, may have potentially serious consequences, like upper genital tract complications.

2.2 Epididymitis

The role of *C. trachomatis* as an etiological agent for the development of epididymitis also is widely accepted [13]. Untreated chlamydial infection of the urethra can spread to the epididymis. Patients usually have unilateral testicular pain with scrotal erythema, tenderness, or swelling over the epididymis [22]. The occurrence of chlamydial epididymitis is not always preceded by symptoms of urethritis and only in some cases they are accompanied by the increase of polymorphonuclear (PMN) leukocytes in urethral discharge. Chlamydial epididymitis is of milder course when compared to epididymitis of another etiology [23].

C. trachomatis is the causative agent in most cases of acute epididymitis in men younger than 35 years, whereas common urinary tract pathogens account for the etiology of the majority of acute and chronic epididymal inflammations in men above this age [24-26]. For example, Zdrodowska-Stefanow et al. found *C. trachomatis* infection in 45.8% of patients of epididymitis below 35 years, whereas in older men the presence of the bacterium was detected in 6.7% [26].

2.3 Epididymo-orchitis

Left untreated, the infection will progress from epididymitis to epididymo-orchitis. In the young patient it must be differentiated from torsion. In the older patient, subacute scrotal pain is most likely epididymo-orchitis. As in torsion, the testicle and/or epididymis is painful and tender; however, in this case, scrotal pain is more gradual in onset, there is no nausea or vomiting, the testicle is not high riding, and there is associated erythema and edema [27].

The causes of epididymo-orchitis reflect common causes of genitourinary infection in men based on particular age groups. In children and older men (>35 years), the most common cause of epididymitis is coliform organisms that result in bacteriuria. In contrast, the organisms that cause urethritis or STD are the common etiologies of epididymitis and orchitis in young adult men (<35 years) [28]. Chlamydial antigen has been detected in urethral or urine samples from 11 to 35% of men presenting with epididymo-orchitis [13].

2.4 Prostatitis

Prostatitis is one of the most common urological disorders and can affect men of any age. Approximately one-third of all men during their lifetime will experience symptoms consistent with prostatitis [29]. The early classification of prostatitis described four syndromes for which pelvic pain in the male was the common factor [30]. In 1995 and 1998, two consensus meetings of the National Institutes of Health (NIH) attempted to refine the

four traditional classes of prostatitis. The NIH classification system designates categories I and II for cases in which bacteria clearly cause acute or chronic prostatitis, respectively. NIH category III incorporates the third (nonbacterial) and fourth (prostadynia) traditional classes and designates the entire cohort as Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS), for which current clinical evidence does not indicate a clear bacterial cause. The fourth NIH category includes patients with prostatic inflammation in expressed prostatic secretions or on biopsy specimens performed for other clinical indications [31, 32].

In acute bacterial prostatitis, patients can present with voiding complaints, such as dysuria, frequency, urgency, or hesitancy. Other symptoms can include suprapubic pain, hematuria, or systemic symptoms such as fever, chills, nausea, vomiting, or malaise. Physical examination often reveals a warm, swollen, painful prostate. In chronic bacterial prostatitis a hallmark of a small minority of men is recurrent episodes of urinary tract infection. There may be irritative or lees often obstructive lower urinary tract symptoms and patients often complain of pain in perineum, lower abdomen, genitalia, back and lower rectum. These men also present with exacerbations of acute urinary infection with worsening of symptoms of bladder irritation, occasionally pyrexia, abdominal and loin pain. Pain is the most severe and commonly reported symptom in patients with CP/CPPS. The "classic" presentation is that of a patient who presents with pelvic, perineal, or genital pain associated with voiding and/or sexual dysfunction, characterized by a relapsing, remitting course. Finally, patients included in the fourth NIH category of prostatitis are asymptomatic, but there is evidence of inflammation, infection or both in prostate-specific specimens after massage and/or in cytological or histological investigations of prostatic biopsy specimens which have been obtained on account of elevation of the serum prostate-specific antigen (PSA). [29, 33- 35].

The most important agents of acute and chronic bacterial prostatitis are *Escherichia coli* (accounting for up to 87% of cases), *Pseudomonas* spp., *Enterobacter* spp., *Proteus* spp., *Klebsiella* spp. and *Enterococcus* spp. [35-37]. It is still under debate if and to what extent *C. trachomatis* can cause prostatitis. The definitive association between isolation of the bacterium and a prostatic origin is limited by the fact that diagnostic material from the prostate may reflect only urethral contamination. The prevalence of *C. trachomatis* infection in patients with prostatitis has ranged from 3 to 40% approximately [for reviews see 13, 23].

3. Which is the role of *C. trachomatis* in male infertility?

3.1 Role of the inflammation of the urogenital tract

Both acute and chronic infection and/or inflammation can cause partial or complete obstruction of sperm transport with, respectively, oligozoospermia or azoospermia. Bilateral obstruction of the epididymis is common after recurrent infection with *C. trachomatis* [38, 39]. On the other hand, chronic inflammatory changes in the seminiferous tubules observed in orchitis, would be expected to disrupt the normal process of spermatogenesis and cause alterations both in sperm number and quality [40].

Besides of the anatomical consequences of obstruction, inflammation may act as a co-factor in the etiopathogenesis of infertility. Pressure-induced rupture of the epididymal duct or ductuli efferentes will disrupt the blood-testis barrier, activating an immunological defense reaction and inducing the production of anti-sperm antibodies (ASA) [38]. The presence of ASA can lead to the immobilization and/or agglutination of spermatozoa, which may

significantly impair sperm motility affecting acrosome reaction, cervical mucus penetration, zona pellucida binding, and sperm-oocyte function. Also, ASA can prevent implantation and/or arrest embryo development. ASAs were observed in the serum and/or in the seminal plasma or on the sperm surface in approximately 10% of infertile male partners [41, 42].

An increased prevalence of an autoimmune response to sperm in men with *C. trachomatis* in semen suggests that a subclinical chlamydial infection may activate an immune response to sperm [8]. Witkin et al. demonstrated that the presence of a humoral immune response to *C. trachomatis* is correlated with the development of an autoimmune response to spermatozoa [43]. Also, other authors found that chronic male genital infection with *C. trachomatis* could be associated with the development of ASA [44]. However, other groups found no association between chlamydial antibodies in semen and the presence of ASA [45], or between chronic inflammatory or infectious disease of the male reproductive tract with *C. trachomatis* and presence of ASA in semen [46].

On the other hand, heat shock proteins (HSP) are essential mammalian and bacterial stress proteins. At the cellular level, they act as chaperones, have important regulatory functions, and are considered to be an essential factor for reproduction. Members of the 60 kDa HSP family (HSP-60) in particular have been recognized as immunodominant antigens of many microbial pathogens, including *C. trachomatis*. Since the amino acid homology between many microbial HSP (e.g. chlamydial HSP-60) and the human 60 kDa HSP is high, the development of an autoimmune response to the human HSP-60 in susceptible individuals with chronic infections has been suggested [47-49]. According to one study, HSP-60 was present in a soluble form in semen primarily in men with evidence of immune system activation within their genital tract, and this presence correlated with the occurrence of antichlamydial immunoglobulin-A (IgA) [50]. However, Karinen et al. found that in male partners of subfertile couples serum antibody levels to HSP chlamydial antigens were lower than in controls [51]. Future research is necessary to assess the possible role of immunoresponse to bacterial HSP in reproductive function.

Cytokines are regulatory proteins produced by leukocytes and other cells that control inflammation. Controversy exists as to whether elevated cytokine levels are related to semen quality. Kokab et al. found that men infected with *C. trachomatis* had lower percent progressive sperm motility, a higher leukocyte count, and a raised concentration of IL-8 in semen compared with men without infection. However, when median IL-6 concentrations in *C. trachomatis*-infected and –noninfected groups were compared, there was no statistical significance between them [52]. Eggert-Kruse et al. found only one subfertile male patient (1/137) who was positive for *C. trachomatis* and had high IL-8 concentration in seminal plasma [53]. Thus, more studies are necessary in this area.

3.2 Direct interaction of *C. trachomatis* with sperm function and its impact on semen parameters

Attachment of *C. trachomatis* to human spermatozoa was first observed in *in vitro* experiments using immunofluorescence tests with monoclonal antibodies to the bacterium, and transmission electron microscopy. Furthermore, sperm penetration tests revealed that spermatozoa, when progressing forward, can carry chlamydiae attached to them [54]. Also, electron microscope observations on male ejaculates revealed the presence of EBs and RBs of *C. trachomatis* in spermatozoa. After the passage of the infectious EB into the nucleus, all

stages of RB formation in the head of the spermatozoon were detected. Thus, was postulated that *C. trachomatis* can infect and be transmitted by spermatozoa to the female partner and cause infertility [55].

Friberg et al. observed that *C. trachomatis* was attached to spermatozoa recovered from the peritoneal cavity of patients with salpingitis, and suggested that spermatozoa may serve as vectors for *C. trachomatis* and spread this pathogen to the peritoneal surfaces of the uterus and fallopian tubes [56]. Also, Vigil et al. suggested that the possible effect of *C. trachomatis* on male fertility is not due to alterations in sperm quality or function, but rather to the transmission of the disease to female partners, causing inflammatory processes and promoting the generation of ASA [57].

However, other studies suggested that *C. trachomatis* may cause a direct damage to spermatozoon. Diquelou et al. observed abnormal sperm movements in men with positive culture for *C. trachomatis* [58]. Hosseinzadeh et al. showed that following incubation with EBs of *C. trachomatis* serovar E, the tyrosine phosphorylation of two major sperm epitopes of 80 and 95 kDa is significantly increased. As a result, these authors hypothesized that *C. trachomatis* serovar E may compromise sperm function by accelerating sperm capacitation, since tyrosine phosphorylation of sperm proteins is closely associated with capacitation *in vitro* [59]. However, a further series of experiments demonstrated that serovar E was causing sperm death, suggesting that the chlamydial-induced increase in tyrosine phosphorylation was associated more with cell death than with capacitation [60]. Finally, in another work Hosseinzadeh et al. demonstrated that *C. trachomatis*-induced death of human spermatozoa is caused primarily by lipopolysaccharide (LPS) [61].

On the other hand, the impact of *C. trachomatis* on semen quality is controversial. *In vitro* studies (see above) show that co-incubation of spermatozoa with chlamydia causes a significant decline in numbers of motile sperm and results in premature sperm death. By, contrast, *in vivo* studies of *C. trachomatis* in men have provided conflicting evidence as to whether it is associated with reduced fertility [62]. Idahl et al. demonstrated that the presence of IgA and IgG antibodies to *C. trachomatis* in serum of male partners of infertile couples was significantly correlated with reduced motility of the spermatozoa, increased number of dead spermatozoa, higher prevalence of leukocytes in semen, decrease in sperm concentration, decrease in the number of progressive spermatozoa and a rise in the teratozoospermia index [63]. Veznik et al. examined 627 sperm samples. Sperm analysis showed significant differences between Chlamydia-positive and –negative samples. The Chlamydia-contaminated group showed lower values of normal sperm morphology, volume, concentration, motility and velocity, than the Chlamydia-negative samples [64]. However, Vigil et al. did not found significant differences between *C. trachomatis*-infected and –noninfected male partners of infertile couples in any of the sperm parameters assessed (sperm concentration, motility and morphology) [57]. Gdoura et al. reported that the mean values of seminal volume, sperm concentration, sperm viability, sperm motility, sperm morphology, and leukocyte count were not significantly related to the detection of *C. trachomatis* DNA in semen specimens of male partners of infertile couples [65]. Finally, another example of the controversial role of *C. trachomatis* in semen parameters is observed in prostatitis patients. Whereas Mazzoli et al. found that *C. trachomatis* affects sperm concentration, percentage of motile sperm and normal morphological forms in prostatitis patients [66], Motrich et al. found that chlamydial infection has no detrimental effects on

sperm quality [67]. Thus, for a number of reasons, mostly based on methodological aspects, the impact of *C. trachomatis* on semen parameters is controversial and further investigations should be performed to understand the disparities seen among the available studies.

3.3 The controversial role of leukocytospermia on fertility

Leukocytes are present throughout the male reproductive tract, are found in most ejaculates, and are thought to play an important role in immunosurveillance and phagocytic clearance of abnormal sperm [68]. PMN leukocytes are the most prevalent type of leukocyte in semen (50 to 60%), followed by macrophages/monocytes (20 to 30%), and T-lymphocytes (2 to 5%) [69]. Leukocytospermia is defined by the World Health Organization (WHO) as the presence of peroxidase-positive leukocytes in concentrations greater than $1x10^6$/mL of semen [70].

Leukocytospermia may be due to either a genital tract infection or an inflammatory immunologic response. Other possible etiologies of leukocytospermia besides infection need to be considered. Environmental factors, such as smoking, alcohol consumption, and marijuana use, increase leukocytes in semen. Prolonged abstinence and certain sexual practices (use of vaginal products or anal intercourse) can produce leukocytospermia. Increased leukocytes in semen may be seen in men with abnormal spermatogenesis as a mechanism for the removal of defective sperm from the ejaculate. Finally, varicocele or vasovasostomy can result in a high number of leukocytes in semen [71, 72].

Neofytou et al. found a statistical significance between leukocytospermia and the presence of DNA of the Epstein-Barr virus (EBV) but not with the detection of other members of the Herpesviridae family [73]. Most studies show no correlation between the presence of leukocytes and bacteria in semen [74-76]. However, Punab et al. observed a positive correlation between the leukocyte count and the number of different bacteria detected in semen, and also between the leukocyte count and the total count of microorganisms in semen samples [77]. In fact, these authors along with Gdoura et al. [78] demonstrated that the WHO-defined cut-off point ($1x10^6$ leukocytes per mL) has very low sensitivity for discriminating between patients with and without significant bacteriospermia, as a more optimal sensitivity/specificity ratio appears at $0.2x10^6$ leukocytes per mL of semen.

EBs of *C. trachomatis* incubated in the presence of complement or specific antibody or both caused chemotaxis of human PMN leukocytes *in vitro* [79]. Four types of *C. trachomatis*-leukocyte interaction were observed *in vitro*: (i) minimal to no bacterial binding, (ii) bacterial binding, followed by ingestion and high-level multiplication, (iii) bacterial binding, followed by ingestion but minimal multiplication, and (iv) bacterial binding, but minimal entrance or replication [80]. *In vivo*, was observed that men whose ejaculates were positive for chlamydial DNA had a significantly higher mean concentration of leukocytes than in those whose ejaculates were negative for the presence of DNA of the pathogen. Leukocytospermia was twice as common in men that were positive for chlamydial DNA, but it was not always associated with the presence of chlamydial DNA in semen [81]. Also, Idahl et al. showed that *C. trachomatis* serum IgA was significantly correlated with a higher prevalence of leukocytes in semen [63]. However, Bezold et al. found no difference in prevalence of *C. trachomatis* and other pathogens between asymptomatic male infertile patients with and without leukocytospermia [82]. Also, other authors found no relation between infection with *C. trachomatis* and leukocyte count in semen [65, 83, 84].

The effect of leukocytospermia on male fertility is controversial: whereas some studies found an adverse effect of leukocytospermia on semen quality, others found no effect on semen parameters or even an improvement. This is probably due to different detection methods, different populations studied and to the fact that leukocyte subtypes in semen may have different functions [69, 85-88].

Thus, according to the reviewed literature, the impact of *C. trachomatis* on leukocyte count and male fertility is controversial. In previous works, we presented ultrastructural findings encountered in semen samples of infertile men infected with *C. trachomatis* and/or mycoplasmas: a) structural damage of spermatozoa, b) phagocytosis of damaged spermatozoa by leukocytes, c) destruction of bacteria by leukocytes, d) persistence of bacteria in leukocytes, and e) phagocytosis of damaged spermatozoa and leukocytes by epithelial cells of the genital tract. However, less than 1% of the samples analyzed presented leukocytospermia. Taken together, these data suggest that leukocytes might have a biological impact in fertility in some patients regardless of their count in semen [89-91].

Henkel et al. found that leukocyte counts $<1x10^6$/mL caused a significant decrease of motility and DNA integrity of spermatozoa [92]. Sharma et al. were unable to identify a safe lower limit for leukocyte count in semen because the presence of any leukocytes, no matter how few, was associated with elevated oxidative stress [93]. Aitken et al. concluded that low concentrations of leukocytes in the human ejaculate caused reactive oxygen species (ROS) generation and sperm damage [94]. Thus, the WHO threshold value for leukocytospermia has seriously been questioned by our data and those of others and should be re-evaluated.

3.4 Sperm DNA damage

3.4.1 Oxidative stress

ROS are short-lived chemical intermediates which contain one or more electrons with unpaired spin. In order to overcome this state of unpaired electrons, they are highly and unspecifically reactive molecules able to oxidize proteins, lipids and nucleic acids. In many cases this oxidation causes irreversible damage to biological systems [95].

ROS leads to oxidative damage of the sperm membrane, which reduces fluidity of the membrane and disrupts the fusion events of the acrosome reaction. Sperm damaged by ROS lose motility, especially progressive motility, and have decreased viability and diminished fertilizing ability in the hamster egg sperm penetration assay and in *in vitro* fertilization [71]. Strong evidence suggests that high levels of ROS mediate the occurrence of high frequencies of single- and double-strand DNA breaks commonly observed in the spermatozoa of infertile men. Furthermore, studies in which the sperm was exposed to artificially produced ROS resulted in a significant increase in DNA damage in the form of modification of all bases, production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal rearrangements [96].

ROS are associated with both increased apoptosis leading to spermatozoa DNA damage and decreased sperm variables (motility, concentration, and morphology) in semen of infertile men [97]. Also, several studies demonstrated an increased ROS generation in the ejaculates of infertile men [98, 99].

In the context of infections, either the pathogen itself may induce an increased generation of ROS or the invading inflammatory cells could generate ROS during the respiratory burst,

consuming the antioxidants present. In the male genital tract, ROS are generated by the pathogen, leukocytes (PMNs and macrophages) and defective spermatozoa [100, 101].

Studies with cell cultures infected with *C. trachomatis* demonstrated the production of ROS, which was associated with formation of lipid peroxides in host cell membranes [102]. More recently, other authors also have demonstrated that infection with *C. trachomatis* elicits the production of ROS [103, 104]. These data and the fact that *C. trachomatis* infection has been associated with a raised leukocyte count and the presence of damaged spermatozoa in semen (see precedent sections), indicate that *C. trachomatis* might have a role in male infertility trough the production of ROS.

3.4.2 Apoptosis

The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions [105]. Apoptosis controls the overproduction of male gametes and restricts the normal proliferation levels during conditions unsuitable for sperm development [106]. Also, apoptosis has been involved in the removal of abnormal cells from the testicles of patients with spermatogenetic failures [107].

Oosterhuis et al. found that about 20% of ejaculated spermatozoa are apoptotic, and that the concentration of spermatozoa is lower in men with more apoptotic spermatozoa [108]. Apoptosis has been observed in ejaculated spermatozoa from infertile men. Baccetti et al. observed that apoptosis is abnormally frequent in the sperm cells of the ejaculate of sterile men, and that it shows the classical biochemical and ultrastructural pattern in spermatozoa, spermatids, and apoptotic bodies [109]. Barroso et al. found that spermatozoa from infertile men display hallmarks of apoptosis, such as translocation of membrane phosphatidylserine (PS) and DNA strand breaks [110]. McVicar et al. found that the apoptotic marker Fas was expressed in sperm of infertile men, although DNA fragmentation was observed in all sperm of fertile and infertile men [111].

On the other hand, Shen et al. found that the detection of apoptotic cells in sperm of subfertile patients was inversely correlated to sperm motility and vitality, and positively correlated to total sperm counts, sperm concentration and abnormal sperm morphology [112]. These results support the abortive apoptosis theory, which establishes that apoptosis in mature sperm is initiated during spermatogenesis, after which some cells earmarked for elimination via apoptosis may escape the removal mechanism and contribute to poor sperm quality [113, 114].

Several studies show that *C. trachomatis* causes apoptosis of spermatozoon. Eley et al. observed that co-incubation of sperm with *C. trachomatis* LPS results in cellular death which is in part due to apoptosis [115]. Satta et al. observed that the experimental *C. trachomatis* infection causes sperm PS externalization and DNA fragmentation [116]. Gallegos et al. determined that patients with genitourinary infection by *C. trachomatis* and mycoplasmas have increased sperm DNA fragmentation in comparison with fertile controls [117]. We identified spermatozoa showing ultrastructural features resembling apoptosis in semen samples of infertile men infected with *C. trachomatis* and mycoplasmas. These features included: loose fibrillar-microgranular chromatin network, presence of vacuoles in the

chromatin, partially disrupted nuclear membranes and membranous bodies within the vacuoles of the chromatin [90, 91]. Lastly, Sellami et al. showed that inoculation of fertile male Swiss mice in the meatus urethra with *C. trachomatis* could lead to alteration of semen parameters, induction of apoptosis in spermatozoa, and decrease of the reproductive performance of male mice [118]. Taken together, these data support a role of *C. trachomatis* on sperm apoptosis. However, more research is needed to assess the real effect of this process in male fertility.

3.4.3 Other phenomena associated with sperm DNA damage

Besides of oxidative stress and apoptosis, defective sperm chromatin packaging may be another mechanism by which DNA damage arise in human spermatozoa. A variety of causes have been correlated with increased levels of sperm DNA damage, such as cigarette smoking, iatrogenic processes, malignancies (mainly testicular cancer, Hodgkin's disease and leukemia), environmental toxicants, physical agents as radiation and heat, drugs, increasing male age, elevated body mass index and medical conditions as insulin dependent diabetes (for reviews see 96, 119, 120].

4. *C. trachomatis* coinfections and male infertility

Urethritis has traditionally been classified as gonoccocal or NGU. However, the replacement of Gram stain testing of urethral discharge with combined laboratory testing for both gonococcal and chlamydial infections makes this distinction less important [16]. In addition, many studies have shown that some men with gonococcal urethritis are coinfected with *C. trachomatis*.

The frequency of *C. trachomatis* and *N. gonorrhoeae* coinfection in men can vary dramatically depending on several factors, as the individual incidence and prevalence of each of these microorganisms in the studied population, the type of sample analyzed, the method used to detect the bacteria, etcetera. For example, whereas Barbosa et al. reported a coinfection prevalence of 4.4% in Brazil [121], Papadogeorgakis et al. found gonococcal coinfection in 30% of the *C. trachomatis*-infected patients in Greece [122]. Kahn et al. found that 51% of males with gonorrhea were coinfected with chlamydia in US juvenile detention centers [123]. Thus, most of the studies are geographically limited and targeted to a certain portion of the population. However, the fact that some men with gonococcal urethritis are coinfected with *C. trachomatis* has led to the recommendation that men treated for gonoccocal urethritis should be treated routinely with a regimen that is effective against chlamydial infection [124].

Besides of *C. trachomatis* and *N. gonorrhoeae*, obligate pathogenic microorganisms in the male urogenital tract are *Mycobacterium tuberculosis, Treponema pallidum, Haemophilus ducreyi, Klebsiella (Calymmatobacterium) granulomatis*, herpes simplex virus (HSV) 2, human papilloma viruses (HPVs), and *T. vaginalis*. In systemic disease human immunodeficiency viruses, hepatitis B virus (HBV), hepatitis C virus, hepatitis D virus, and cytomegalovirus (CMV) may be excreted with semen, often in high concentrations [37]. Mixed infections are common and failure to diagnose and treat may lead to serious complications and continued transmission.

About 200 established species have already been described within the class Mollicutes, and this number continues to rise [125]. Several Mollicutes species have been isolated from

humans. For six of them: *U. urealyticum*, *M. hominis*, *M. genitalium*, *M. primatum*, *M. spermatophilum* and *M. penetrans*, the genital tract is the main site of colonization [126]. These microorganisms can be found commensal in lower genitourinary tracts of sexually active men and women. Moreover, they cause many disorders such as nonchlamydial NGU [127, 128]. The role of mycoplasmas and ureaplasmas in male infertility has been discussed controversially. Dieterle found no evidence that *U. urealyticum* has a significant impact on male infertility [129]. However, Zeighami et al. found that *U. urealyticum* was more common in semen of infertile men that in semen of healthy controls. Also, in infertile patients infected with ureaplasmas, the volume, count and morphology of semen samples were lower than in infertile patients negatives for the detection of the microorganisms [130]. Gdoura et al. reported that the comparison of the semen parameters of infertile men with and without genital mycoplasmas and ureaplasmas showed no significant differences, apart from the sperm concentration in the infection of *M. hominis* and *M. genitalium* and sperm morphology in the infection of *M. hominis*. Mixed species of mycoplasmas and ureaplasmas were detected in 6.7% of semen samples [131].

The identification of *C. trachomatis* in men infected with other sexually transmitted pathogens and their impact on male fertility have been reported in the literature. Gunyeli et al. determined the prevalence of *C. trachomatis*, *M. hominis* and *U. urealyticum* infections among infertile couples and effects of these infections on infertility. No difference was found between fertile and infertile couples in terms of the effects of these infections on sperm parameters and infertility. Moreover, the prevalence of these infections was found to be the same in fertile and infertile groups [132]. Gdoura et al. found that the mean values of seminal volume, sperm concentration, sperm viability, sperm motility, sperm morphology, and leukocyte count were not significantly related either to the detection of *C. trachomatis* DNA or to that of genital ureaplasma or mycoplasma DNA in semen specimens of asymptomatic male partners of infertile couples [65]. Bezold et al. detected the presence of DNA of CMV, HPV, human herpesvirus type 6, HSV, HBV, EBV, and *C. trachomatis* in semen from asymptomatic infertile men. The presence of DNA of the pathogens was associated with a decrease in sperm concentration, motile sperm concentration, total sperm count, and neutral alpha-glucosidase concentration [82]. Lastly, we found *C. trachomatis* and mycoplasmas coinfection in semen samples of 25% of the infertile men analyzed. Ultrastructural data suggested that leukocytes might have a role in fertility of some individuals infected with these bacteria [90, 91].

5. Diagnosis and treatment of *C. trachomatis* infections

Laboratory testing of *C. trachomatis* has traditionally consisted of cell culture of inocula prepared from urogenital specimens and later, the antigen and nucleic acid detection technologies were developed [23]. Antibodies to *Chlamydia* spp. are best detected with a microimmunofluorescent (MIF) assay, but these assays are not widely available [133]. The MIF test is valuable in the diagnosis of urogenital infections but is expensive and labor-intensive while the complement fixation (CF) test yields reliable results only for LGV. The value of *Chlamydia*-specific antibodies (IgM, IgG, IgA) in the diagnosis of urogenital infections using indirect immunofluorescence (IF) and immunoperoxidase (IPO) methods is limited since these antibodies are genus-specific and thus will also be elevated in infections with *Chlamydophila pneumoniae* [37].

With regard to its high specificity, the bacteriological culture has been the method of choice for diagnosis. Another advantage is that these cultures maintain the viability of the microorganisms for additional studies such as genotyping or antimicrobial susceptibility tests. One disadvantage of the culture is the low sensitivity of 70-85%. Furthermore, the costs, the high level of technical expertise necessary and the time required to obtain results, are significant disadvantages of this method [8, 134]. Cycloheximide-treated McCoy or HeLa cell lines are used most frequently to isolate *C. trachomatis*. Centrifugation techniques appear to enhance absorption of chlamydiae to cells. Intracytoplasmic inclusions can be detected at 48 to 72 hours with species-specific immunofluorescent monoclonal antibodies for *C. trachomatis* and Giemsa or iodine stains [133].

Rapid methods for diagnosis of a *C. trachomatis* infection include a direct fluorescent-antibody stain (DFA) and enzyme immunoassays (EIA) to detect antigens of the bacterium. Both types of assay have low sensitivities compared to culture and DNA amplification methods. Compared to culture, the sensitivity of these assays, depending on many variables including the population examined and the culture technique used for comparison has been reported to vary from 50 to 90% [135]. However, the use of the DFA technique enables to detect *C. trachomatis in situ* in tissue biopsies, which is not possible with serological or DNA amplification methods (Gallegos et al., personal communication), and its specificity ranges from 98 to 99% [136].

Because of their high sensitivity and specificity, and their possible use for a large range of sample types, nucleic acid amplification tests (NAATs) are the tests of choice for the diagnosis of *C. trachomatis* genital infections. Several commercial NAATs are available and make use of different technologies: polymerase chain reaction (PCR) and real-time PCR, strand displacement amplification, transcription-mediated amplification, and nucleic acid sequence-based amplification. The major targets for amplification-based tests are generally multiple-copy genes, e.g. those carried by the cryptic plasmid of *C. trachomatis*, or gene products such as rRNAs [137].

A new genetic variant of *C. trachomatis* was discovered in Sweden in 2006. The variant has a 377 base pair deletion in the plasmid which was the target for the commonly used NAATs. Therefore this new variant was initially not detected by the NAATs. So far the variant has been only occasionally detected outside Sweden or other Scandinavian countries. Many recent studies have focused on the new variant and disease syndromes associated with this variant. The clinical manifestations seem not to differ from the clinical manifestations caused by the wild types. Subsequently, NAATs have been modified so that this variant can now be detected by all NAATs commonly used in microbiology laboratories [138].

New guidelines for the treatment of patients with sexually transmitted chlamydial infection have been recently published. Recommended regimens are azithromycin 1 g orally in a single dose or doxycycline 100 mg orally twice a day for 7 days. Alternative regimens include erythromycin base 500 mg orally four times a day for 7 days, or erythromycin ethylsuccinate 800 mg orally four times a day for 7 days, or levofloxacin 500 mg orally once daily for 7 days, or ofloxacin 300 mg orally twice a day for 7 days [139].

Clinical trials demonstrate equivalent efficacy and tolerability of azithromycin versus doxycycline regimens for chlamydial infections. Each antibiotic regimen has advantages or disadvantages in terms of cost, convenience, and compliance, yet both regimens will remain

recommended as first-line therapy for uncomplicated chlamydial infection in nonpregnant individuals [140]. However, in patients who have erratic health-care-seeking behavior, poor treatment compliance, or unpredictable follow-up, azithromycin might be more cost-effective in treating chlamydia because it enables the provision of a single-dose of directly observed therapy [139, 141].

6. Conclusions

Infection with *C. trachomatis* accounts for the most common bacterial sexually transmitted infection in the world. In men, *C. trachomatis* can cause urethritis, epididymitis, epididymo-orchitis and prostatitis, although asymptomatic infections are quite common. Both acute and chronic infection and/or inflammation can cause partial or complete obstruction of sperm transport with, respectively, oligozoospermia or azoospermia. A subclinical chlamydial infection may activate an immune response to sperm. Infection of the testis and prostate is implicated in a deterioration of sperm, possibly affecting fertility. Also, there is increasing evidence that the function of human spermatozoa can be significantly affected by direct exposure to the bacterium or by the host immune response induced by it. The role of leukocytospermia in the pathogenesis of male infertility remains controversial. The mechanisms by which leukocytes and *C. trachomatis* may lead to sperm damage include ROS generation and the induction of sperm apoptosis. On the other hand, the co-infection with *C. trachomatis* and other microorganisms may be a cause of the impairment of sperm quality, motility and function. Because of their high sensitivity, NAATs are the more reliable methods for the diagnosis of *C. trachomatis* infection. However, in tissue biopsies a DFA assay may be more useful to detect the bacterium. Treatments options for uncomplicated urogenital infections include therapy with azithromycin or doxycycline.

7. References

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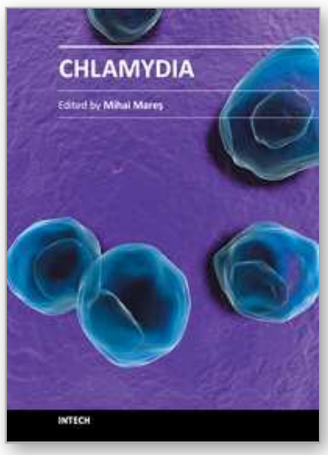

Chlamydia

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Nowadays, Chlamydia still represents a redoubtable pathogen. Among its consequences, the blindness in children and severe impairment of reproductive health in adults are the most mutilating. Worldwide, it is estimated that six million of people suffer from post-trachoma blindness and almost 90 million become sexually infected each year. Due to its silent evolution and sexually transmission, the chlamydial infection can occur in anyone. The book “Chlamydia - A Multifaceted Pathogen” contains an updated review of all-important issues concerning the chlamydial infection. It comprises 18 chapters grouped in four major parts dealing with etiology and pathogenicity, clinical aspects, diagnosis and prevention. The new molecular data about the pathogenicity and the exhaustive presentation of clinical findings bring novelty to the book and improve our knowledge about Chlamydia induced diseases.

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