MINI-REVIEW

Male infertility: the intracellular bacterial hypothesis

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Abstract

Infertility is a disease that affects one in seven couples. As male infertility affects approximately 30% of these couples with an unknown cause in half the cases, it represents a major public health concern. The classic treatment of male infertility involves intrauterine insemination, with modest outcome, and *in vitro* fertilization with intracytoplasmic sperm injection, which is known to be invasive and expensive, without treating the specific cause of infertility. Male fertility is mainly evaluated through a semen assessment where abnormal parameters such as concentration and motility can be associated with a decreased chance of conception. Infectious processes represent plausible candidates for male infertility. *Chlamydia trachomatis* is well known to cause female infertility through tubal damage but its role in male infertility remains controversial. The link between ureaplasmas/mycoplasmas and male infertility is also debatable. The potential negative impact of these bacteria on male fertility might not only involve semen parameters but also, as with *C. trachomatis*, include important physiological mechanisms such as fertilization processes that are not routinely assessed during infertility investigation. Basic research is important to help determine the exact effect of these bacteria on male fertility to develop targeted treatment and go beyond *in vitro* fertilization with intracytoplasmic sperm injection.

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Introduction

The World Health Organization (WHO) describes clinical infertility as a disease of the reproductive system defined by 'the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse' [1]. This disease, which affects one in seven couples, may be due to female factors, male factors, or both. A male infertility factor with abnormal semen parameters is found in approximately 50% of couples seeking infertility treatment [2]. The known causes of male infertility include endocrine diseases, malignancies and genetic anomalies, but the cause remains unknown in 50% of cases. First-line evaluation of male fertility comprises a semen assessment. The criteria include volume, total sperm number, sperm concentration, vitality, progressive motility, total motility and morphology. In couples with infertility, the interpretation of a so-called abnormal semen assessment result (a parameter below the 5th centile compared with WHO reference values) remains difficult. For example, the clinical significance of a sperm concentration slightly under 15 million/mL versus markedly decreased (for example below 1 million/mL) implicates a very different prognosis for the couple regarding chances of conception.

Mild and severe male infertility are frequently used in the literature to describe an abnormal semen assessment but are purely descriptive and without clear definition and relationship to the aetiology, which is only found in approximately 50% of cases. Even when a diagnosis is confirmed, the treatment administered rarely targets the exact cause. Therefore, the vast

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majority of men with male infertility are offered generic treatment based on the severity of the anomaly observed on the semen assessment, for example intrauterine insemination in mild situations and *in vitro* fertilization (IVF) with intracytoplasmic sperm injection for severe semen anomalies or when intrauterine insemination has failed. In vitro fertilization with intracytoplasmic sperm injection has revolutionized management of severe male infertility, but it does not treat the exact cause of infertility. It not only mainly involves the female partner, but is also invasive and expensive with increased risks of multiple births and their related complications. It is time to better understand the causes of male infertility so as to tailor treatments that target the aetiology and so increase the chance of natural pregnancy and decrease the need for invasive procedures.

The infectious hypothesis

Adequate sperm production and function require a healthy urogenital tract to allow normal fertility. Inflammatory processes and bacterial infections have been associated with male infertility, but the exact mechanisms remain poorly understood [3]. The effect of an acute infection might not be as deleterious as a chronic infection where a silent/asymptomatic inflammatory process might have long-lasting negative impact on sperm function, on spermatogenesis, and on permeability of the vas deferens and/or ejaculatory duct. Pathogens that chronically colonize the male urogenital tract could have a negative impact on fertility by affecting the parameters of the semen assessment (count or motility) or even by inducing apoptosis [4]. In that regard, microorganisms such as *Chlamydia trachomatis*, ureaplasmas and mycoplasmas represent interesting candidates to understand a link between infection and male infertility.

Chlamydia trachomatis

Chlamydia trachomatis is the most common sexually transmitted disease, affecting millions of men and women annually, but the true prevalence of *Chlamydia* is difficult to determine because the infection is asymptomatic in up to 85%–90% of infected individuals [5].

Acute chlamydial infection in men causes urethritis, epididymitis (-orchitis) and prostatitis [6]. Inflammation of the epididymis can induce infertility through sperm tract obstruction, especially when both testes are infected [7]. The evidence of a potential deleterious effect of *Chlamydia* on male fertility comes from animal models of infection and *in vitro* experiments. Destruction of male germ cells and Sertoli cells was observed in a murine model of *Chlamydia* infection [8]. Electron microscopy studies have demonstrated that *Chlamydia* can interact with sperm cells and can induce apoptosis through lipopolysaccharide [9,10]. The mechanism involves interaction of lipopolysaccharide with sperm cell CD14 receptor and consequently release of reactive oxygen species that can induce apoptosis through caspases [11]. Motility, an important parameter of male fertility, has also been shown to be altered by *Chlamydia* [4]. Interestingly, another *Chlamydia*-related bacterium, *Waddlia chondrophila*, which was strongly associated with miscarriage in humans, was able to adhere and penetrate inside human spermatozoa and decrease viability, as well as mitochondrial membrane potential [12].

An alternative impact of *Chlamydia* on fertility might involve a state of subclinical chronic infection. Upon exposition to stressors, such as increased interferon-γ levels or antibiotics, bacteria might form aberrant bodies that represent a persistent, non-replicative and non-infectious form of *Chlamydia* [13]. When the stressors are removed, *Chlamydia* can re-enter a normal life cycle and recover its infectious potential.

Retrospective epidemiological data have failed to demonstrate that past *Chlamydia* infection is associated with altered sperm characteristics [14]. In a study where 284 male partners of infertile couples were screened for *C. trachomatis* infection, there was no statistical difference between infected and non-infected male partners in terms of semen parameters and function [15]. In another study including 104 asymptomatic infertile men attending a Tunisian infertility clinic, detection of *C. trachomatis* in semen was not associated with abnormal classic semen parameters [16]. These results are in contrast with a study involving 627 sperm donors, in which the *Chlamydia*-positive group had significantly reduced morphology (14.4%), volume (6.4%), concentration (8.3%), motility (7.8%) and velocity (9.3%) compared with the control group [17]. Moreover, an association between *C. trachomatis* IgG antibodies and subfertility (defined as time to conceive $\geq$12 months) was observed in Finland [18]. Similarly, in couples referred to a Swedish infertility clinic, decreased pregnancy rates were correlated with the presence of *C. trachomatis* IgG antibodies in the male partner independently of tubal infertility factor [19]. However, presence of *C. trachomatis* IgG in the male partner was not associated with an abnormal parameter of the semen assessment. These data suggest that the potential detrimental effect of *C. trachomatis* on male fertility might not involve the semen parameters assessed routinely, but may potentially affect attachment of the sperm to the oocyte or later steps of cell division or implantation. Therefore other mechanisms involved in sperm function might give clues on how *C. trachomatis* might affect male fertility. Acrosome reaction, which represents an

essential step of fertilization, was compared between three groups (*Chlamydia*-infected infertile men, *Chlamydia* non-infected infertile men and healthy controls) [20]. A significant lower acrosome reaction was observed in the infected infertile group compared with the non-infected infertile group. There was no difference in the semen parameters between these two groups (count, motility and morphology) reinforcing the concept that the *in vivo* effect of *C. trachomatis* on male fertility might not affect the classic semen parameters. Alternatively, chronic *C. trachomatis* inflammation might negatively impact the vas deferens and ejaculatory duct in a similar pathogenic mechanism at play in tubal infertility.

Mycoplasmas and ureaplasmas

The *Mycoplasmataceae* represent a family of bacteria with two genera, *Mycoplasma* and *Ureaplasma*, which are among the smallest self-replicating known organisms. They are considered as facultative intracellular pathogens, having the ability to replicate outside and inside host cells.

Genital mycoplasmas (*Mycoplasma genitalium* and *Mycoplasma hominis*) are known to colonize the female and male genital reproductive systems, contaminating the semen during ejaculation and also causing pathologies [21,22]. In females, *M. genitalium* has been shown to be associated with endometritis, cervicitis, pelvic inflammatory disease and infertility as well as perinatal morbidity and mortality [23,24]. *Mycoplasma hominis* has been linked to chorioamnionitis [25]. In men, *M. genitalium* is recognized as a cause of non-gonococcal urethritis, as it was first isolated from men with urethral discharge [26]. Genital ureaplasmas (*Ureaplasma urealyticum* and *Ureaplasma parvum*), like mycoplasmas, are commensals of both the female and male reproductive tract, also contaminating sperm during ejaculation. They have been associated with chorioamnionitis, pelvic inflammatory disease, urethritis, prostatitis, epididymitis, and infertility [27–29]. There is a scarce knowledge on the exact impact of ureaplasmas on the male genital system, as the majority of the studies do not differentiate between *U. urealyticum* and *U. parvum*. Of note, treatment of genital mycoplasmas in colonized pregnant women is associated with a lower rate of premature labour and neonatal complications [25].

As ureaplasmas and mycoplasmas can colonize the male reproductive tract, their involvement in male infertility can be suspected, but remains controversial. In infertile males, Gdoura et al. failed to demonstrate a relationship between altered semen parameters and the presence in semen of these bacteria [16]. In a meta-analysis comparing positive sample (semen, urethra, first-void urine) between infertile men and controls, an association with the risk of infertility could be demonstrated with presence of *M. hominis* and *U. urealyticum* whereas no association was present with *M. genitalium* and *U. parvum* [30]. Nevertheless, the observation of *M. genitalium* attachment to human spermatozoa and the demonstration that the bacteria can be carried by motile sperm suggest a potential role in infertility [31]. The role played by *U. urealyticum* and *M. hominis* infections in semen quality was further investigated [32]. A significant difference in prevalence was observed for *U. urealyticum* (10.22% versus 3.65%) and *M. hominis* (3.16% versus 0.89%) between infertile individuals and controls. Moreover, a significant difference in progressive motility, total motility and normal forms was demonstrated when comparing the infertile individuals with or without *U. urealyticum* infection. These results, which suggest a negative impact of *M. hominis* and *U. urealyticum* on male fertility, are somewhat reminiscent of *C. trachomatis* where a deleterious effect on fertility might not (only) be associated with abnormal semen parameters but also with negative impact on other reproductive mechanisms. Presence of *U. urealyticum* was associated with a significantly higher production of semen reactive oxygen species and malondialdehyde [33]. Oxidative stress through production of reactive oxygen species and malondialdehyde is known to cause DNA damage [34]. The pathophysiological mechanisms involved in the deleterious effect of *U. urealyticum* on male fertility were further investigated [35]. The expression of P34H (a protein necessary for sperm–zona pellucida interaction incorporated on the spermatozoa as it crosses the epididymis) and the activity of hyaluronase (an important enzyme for the acrosome reaction) were significantly lower in the *Ureaplasma*-positive group, in addition to an increased higher DNA fragmentation. These data might help us to understand how bacterial infection might impair reproduction by interfering with subtle mechanisms that are not part of routine male infertility investigation.

There are very limited and contradictory data on the effect of mycoplasmas and ureaplasmas on assisted reproductive technology success rate. Two early studies have suggested that *Ureaplasma* might have a detrimental effect on pregnancy rates during IVF as a reduction in pregnancy rate was demonstrated in the infected group [36] and treatment of *Ureaplasma* infection improved pregnancy rates [37]. The effect of *U. urealyticum* on IVF outcome was further investigated in 191 asymptomatic male partners of women undergoing an IVF cycle [38]. There was no difference, however, in fertilization rates and pregnancy rates between the infected and non-infected groups. Nevertheless, a higher risk of miscarriage was present in the infected group, potentially related to maternal infection. More data are certainly needed to better characterize the effect of ureaplasmas on IVF outcome.

Conclusion

The negative effect of bacterial infections caused by *Chlamydia,* ureaplasmas *and* mycoplasmas on male fertility remains controversial. Their potential deleterious effect might not be apparent on classic male infertility investigation, such as semen assessment, as they might affect fertility through subtle effects on essential reproductive mechanisms. It is time to go back to basic research, to go beyond IVF with intracytosplasmic sperm injection and to offer targeted treatments to infertile couples with male infertility. Moreover, it is also time to investigate other intracellular bacteria including *Coxiella burnetii*, *Listeria monocytogenes* and *Waddlia chondrophila*, which are intracellular bacteria already known to negatively impact pregnancy [13,39]. Bacterial infections or colonization might represent good candidates to explain some causes of male infertility, as other aetiologies still need to be unravelled.

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Authors' contributions

NV was responsible for the writing of the manuscript. All other authors corrected and improved the manuscript and agreed with the content of the final submitted version.

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