

Chlamydia trachomatis and male fertility

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Abstract

There is increasing evidence that the function of human spermatozoa can be significantly affected by direct exposure to the bacterium *Chlamydia trachomatis*. This may contribute to sub-fertility in infected individuals by a route that is independent of any damage to the reproductive epithelium. In addition, if a *C. trachomatis* infection is undiagnosed it could contribute to poor outcomes in assisted conception techniques such as *in vitro* fertilization. The antibiotics routinely used in IVF culture systems are largely ineffective against chlamydia, emphasizing the importance of screening patients prior to treatment. Moreover, given the many thousands of semen samples provided for analysis by men in primary care (many of which will never undergo assisted conception treatment), it is suggested that this may represent a wasted opportunity to provide screening (and treatment) for the infection using an appropriate test specimen and without the need for additional hospital visits.

Keywords: *Chlamydia trachomatis*, male fertility, spermatozoa, semen.

Introduction

Chlamydia trachomatis is currently the major bacterial cause of sexually transmitted infection in the UK as well as many other countries throughout the world. According to figures released by the Health Protection Agency, there were more than 82,000 chlamydia diagnoses at Genito-Urinary Medicine Clinics in the UK in 2002. This represents an increase of 14% since 2001 and 141% since 1996 and this has alarming implications for public health. Although some of this increase may be due to more widespread screening and improved diagnostic methods (for review see Johnson et al., 2002), it is alarming that the majority of infections are in young men and women under the age of 25. As such, there are significant implications for the future fertility of women who can suffer permanent impairment of their reproductive tract as a consequence of infection (see below). Moreover, it has been concluded (Chief Medical Officer's Expert Advisory Group, 1998) that there was now sufficient evidence to support opportunistic screening programmes of *C. trachomatis* in sexually active women aged under 25 years.

The results of such screening programmes have recently been published (Pimenta et al., 2003 a & b) and have revealed that in General Practice the prevalence of *C. trachomatis* infections in young women between the ages of 16 and 24 was approximately 9%. Such figures are presently unavailable for the prevalence of *C. trachomatis* in the UK male population.

Chlamydia trachomatis in men and women

The focus of concentrating screening effort on women is understandable given that the female reproductive tract appears to be more seriously affected by chlamydial infections in comparison to the male reproductive system. In men, chlamydial infection is associated with epididymitis, non-gonococcal urethritis, and/or prostatitis that rarely leads to complications (Purvis & Christiansen, 1995) and can be relatively easily treated once a diagnosis is made. However, up to 50% of men remain asymptomatic (Gonzalez et al., 2004) and therefore do not seek treatment. In women, however, a chlamydial infection can lead to an acute inflammatory reaction

which if untreated ultimately leads to permanent scarring and functional impairment of the infected mucous membranes (Schachter, 1990). This increases the risk of infertility, and ectopic pregnancy (Barlow et al., 2001) and in 1994 was estimated to cost the NHS up to £50 Million per year to manage (Taylor-Robinson, 1994).

As a consequence, there has been less emphasis by healthcare professionals on the possible effects of *C. trachomatis* on the reproductive biology of males. It has been controversial as to whether semen quality may be compromised by a current infection in the male (Gonzalez et al., 2004) and more emphasis has been placed on the fact that infections in the male provide a reservoir of infection for transmission of the bacteria to women (Krause & Bohring, 2003). Indeed, it has been suggested that the male gamete may act as a vehicle of infection through the female reproductive tract by virtue of the bacterium “piggy-backing” on motile spermatozoa (Purvis & Christiansen, 1995; Keck, Gerber-Schafer, Clad, Wilhelm, & Breckwoldt, 1998). However, there is very little by way of direct evidence to support this hypothesis and our understanding of the nature of any interaction between spermatozoa and *C. trachomatis* has been very limited.

Background to *Chlamydia trachomatis* and its developmental cycle

The possibility that spermatozoa may be directly affected by *C. trachomatis* may come about as a result of the unique developmental cycle of the bacterium. Briefly, *C. trachomatis* exists in two forms: alternating between an extracellular but metabolically inactive infectious form called the elementary body and an intracellular metabolically active reproducing form called the reticulate body (Schachter, 1990). This is necessary because chlamydia needs to utilize the intracellular processes of a host cell in order to reproduce. As such, the reproductive epithelium of an infected individual of either sex will periodically release elementary bodies (EBs) into the reproductive tract that may then be encountered by any gametes within the reproductive tract at that time. This could be in the epididymis or penile urethra of males or in the cervix, uterus or uterine (Fallopian) tubes of women.

The bacterium *C. trachomatis* is one of three species that belongs to the taxonomic genus chlamydia. There are 19 human serovars and numerous variants of *C. trachomatis* that differ in their cell biology and also in their clinical presentation (Ngandjio et al., 2004). Serovars A – C are largely restricted to causing infections of the eye, whereas serovars D – K cause both ocular and genitourinary infections. Serovars LI – III are the cause of

lymphogranuloma venereum (LGV) which causes genitourinary infections in addition to a more significant involvement of the lymphatic system.

In our experiments to investigate the interaction between human spermatozoa and *C. trachomatis* (Hossein-zadeh, Brewis, Pacey, Moore, & Eley, 2000; Hossein-zadeh, Brewis, Eley, & Pacey, 2001; Hossein-zadeh, Pacey, & Eley, 2003) we have chosen to use two different serovars: E and LGV. In addition to the above differences in their clinical presentation, these two serovars also differ in their prevalence in different populations: serovar E is more common in Europe and the USA, whereas serovar LGV is rarely seen outside the tropics (Moulder, 1991; Morr e et al., 2000).

Chlamydia trachomatis and sperm function

Early experiments to investigate the nature of any interaction between spermatozoa and *C. trachomatis* relied upon electron microscopy to examine the possibility of interaction between them (Wolner-Hanssen & Mardh, 1984; Erben-gi, 1993; Mavrov, 1995). However, although these experiments generated useful micrographs of bacteria closely associated with spermatozoa, the observations provide no information about the functional status of the spermatozoa, nor did they provide support for the argument that “piggy-backing” on spermatozoa was a mechanism by which *C. trachomatis* was spread through the female reproductive tract (see above).

To investigate the nature and effect of any interaction, Hossein-zadeh et al. (2000) investigated the tyrosine phosphorylation of sperm proteins in response to co-incubation with elementary bodies from *C. trachomatis*. This approach was interesting because in other cell types studied (Bliska, Galan, & Falkow, 1993; Birkelund Johnsen, & Christiansen, 1994; Fawaz, Van Ooij, Homola, Mutka, & Engel, 1997), increased protein phosphorylation was associated with chlamydial attachment and infection. Therefore, to observe protein phosphorylation would provide evidence of a receptor mediated chlamydial attachment to spermatozoa. In addition, since the process of human sperm capacitation is regulated by the phosphorylation of sperm proteins, any chlamydial mediated phosphorylation could suggest that the interaction might also be affecting sperm function directly. The results obtained by this approach (Hossein-zadeh et al., 2000) indicated that co-incubation with serovars E and LGV of *C. trachomatis* lead to the increased phosphorylation of an 80 kDa sperm protein. Moreover, incubation with serovar E led to the additional phosphorylation of a 95 kDa sperm protein. This was the first molecular evidence of a direct effect of *C. trachomatis* on human sperm function and suggested that different serovars

of *C. trachomatis* may exert different effects on reproductive tissues.

In a subsequent series of experiments, sperm motility, viability and acrosomal status was assessed following coinubation with serovars E and LGV (Hosseinzadeh et al., 2001). These were chosen as obvious yet relatively straightforward aspects of sperm function that could be measured in the laboratory. The results of these experiments indicated that during co-incubation with serovar E, but not LGV, there was a statistically significant decline in the percentage of motile sperm that was paralleled by a concomitant increase in the proportion of non-viable (dead) spermatozoa. In addition, the effect was dose dependent, with an increasing proportion of sperm death observed with increasing numbers of elementary bodies in the incubation. Interestingly, however, there was no effect of co-incubation on sperm acrosomal status, even at the highest concentration of bacterium. Further experiments (Hosseinzadeh et al., 2003) suggested that the active component of *C. trachomatis* responsible for the sperm death was lipopolysaccharide (LPS). This is illustrated in Figure 1. Although the spermicidal properties of LPS have been described previously (Dennis, 1962), it is interesting that chlamydial derived LPS is over 500 times more potent than that observed by other Gram negative bacteria, such as *E. coli* (Hosseinzadeh et al., 2003).

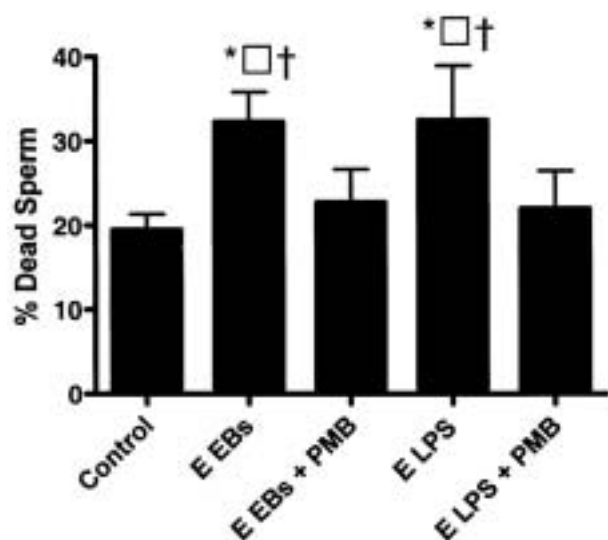


Figure 1. The effect of 0.54×10^6 elementary bodies (EBs) per ml and $0.1 \mu\text{g ml}^{-1}$ lipopolysaccharide (LPS) from chlamydia serovar E on human sperm viability after 6 h incubation. Also shown is the effect of $100 \mu\text{g ml}^{-1}$ of polymyxin B (PMB) to counteract the spermicidal effect of both EBs and LPS. The data shown are redrawn from Table 3 of Hosseinzadeh et al. (2003) and is the mean \pm SEM for six experiments. The symbol (*) shows a significant difference of $P < .001$ from the control whereas the symbol (†) shows a significant difference of $P < .01$ from the same treatment without the addition of PMB.

An obvious conclusion of this work is that chlamydial infection might compromise fertility by a mechanism that is independent of any damage to the reproductive epithelium. As such, there are two possible scenarios where this may happen. First, if a man without an infection ejaculates spermatozoa into the reproductive tract of his partner who has an infection, then sperm may die before they can take part in fertilisation if they are exposed to elementary bodies. Second, if a man has an active infection then it is possible that his spermatozoa, if exposed to elementary bodies (of an appropriate serovar) in the male reproductive tract, may die long before they are ejaculated. Clearly, if the latter hypothesis were true then the ejaculates of such men would be predicted to contain greater numbers of dead sperm when compared to their uninfected counterparts.

Chlamydia trachomatis and semen quality

The question as to whether *C. trachomatis* infection leads to a reduction in semen quality has been difficult to answer until recently because the many studies conducted have often provided conflicting and confusing results (reviewed by Gonzalez et al., 2004). Many studies are difficult to interpret because they have used inappropriate laboratory methods to either diagnose chlamydial infection and/or undertake semen analysis. Moreover, the populations studied are often from men attending infertility clinics and it could be argued that these are more likely to have reduced semen quality anyway. Finally, infection by *C. trachomatis* is often associated with organisms such as *Mycoplasma genitalium* (Taylor-Robinson, 2002) or *Ureaplasma urealyticum* (Levy et al., 1999) that may independently influence semen quality if not diagnosed and treated at the same time.

To address some of these shortcomings, Hosseinzadeh, Eley, and Pacey (2004) recruited men referred from primary care for diagnostic semen and correlated their semen analysis results with the presence or absence of chlamydial DNA. Surprisingly, although the semen samples of men who were positive for chlamydial DNA had significantly higher ejaculate volumes and leukocyte numbers, they did not contain greater numbers of dead sperm. This would suggest that infection does not influence semen quality. This is further supported by a recent study by Idahi, Boman, Kumlin, and Olofsson (2004), which found that semen quality was unaffected by either past or current infection of chlamydia, although interestingly the probability of conception (either through assisted conception or not) was independently (and negatively) correlated to past infection in the male partner. This reinforces the conclusion by Gonzalez et al. (2004) that past rather than current infections are more important to

male fertility. Clearly, the mechanism by which this occurs is very subtle and may not be evident in traditional measures of semen quality examined at semen analysis (e.g. sperm count, motility and morphology). One possible explanation is that a previous infection could have permanently affected the function of accessory glands rather than had any influence on spermatogenesis. It has been suggested previously (Bollmann et al., 2001) that if the secretion of antioxidants by the accessory glands was reduced post-infection that this might leave sperm more susceptible to attack from free radicals.

A further important finding from the study by Hosseinzadeh et al. (2004) is that almost 5% of men undergoing infertility investigations with their partner, and presumably in monogamous relationships, were found being positive for chlamydial DNA in their semen. Given the high concordance of chlamydial infection between sexual partners when DNA amplification diagnostic techniques are used (Quinn et al., 1996), then it is reasonable to conclude that the partners of these men were similarly infected.

Issues for assisted conception

Although our understandings of the effect of *C. trachomatis* on sperm function are incomplete, it is clear that there are some important considerations for assisted conception, particularly when gametes are being manipulated *in vitro*. Whilst it is suggested that prior to any uterine instrumentation of the female reproductive tract that consideration be given to screening of, or providing appropriate antibiotic prophylaxis to, all women considered to be high risk of *C. trachomatis* infection (Royal College of Obstetricians and Gynaecologists, 1998), it is not clear how widespread this practice is. Therefore, in undiagnosed/treated patients undergoing egg collection, for example, not only is there a risk of spreading or reactivating an existing infection, there is also a risk that along with each egg, elementary bodies could have also been collected and subsequently introduced into the culture system (De Punzio et al., 1991). As such, sperm function may become compromised before fertilization has taken place and as a consequence be an unrecognized contributor to fertilization failure in some individuals. It is unclear whether elementary bodies could be introduced into the culture system from the spermatozoa, although we know that sperm washing techniques can remove some microbes (Wong, Balmaceda, Blanco, Gibbs, & Asch, 1986). Moreover, whilst some IVF culture media do contain antibiotics, this is not always the case. In addition, of the antibiotics generally used in IVF systems only penicillin has some antimicrobial activity against *C. trachomatis* (Storey & Chopra, 2001) with streptomycin or gentamicin being largely ineffective.

It is not known whether there is also the possibility of any elementary bodies within the female reproductive tract of an infected woman affecting sperm function during simpler insemination procedures, such as Intra Uterine Insemination. Although the insemination with partner's sperm may be no less likely to be affected than sperm deposited within the female tract following intercourse, during donor insemination - where screened (British Andrology Society, 1999) cryopreserved sperm are used - there is the possibility of significant sperm death because frozen/thawed sperm are generally more fragile (Keel & Webster, 1993).

Screening and management of *Chlamydia trachomatis* in assisted conception

The screening and treatment of patients prior to infertility treatments (Royal College of Obstetricians and Gynaecologists, 1998) or sperm (British Andrology Society, 1999) or egg (British Fertility Society, 2000) donation has already been identified as an important aspect of good clinical practice. However the arguments to support the screening of patients and donors is primarily underpinned by a desire to either eliminate the risk of cross infection from donor to patient or prevent the flare up of an existing infection within the reproductive tract of a woman being provided with treatment. Little attention has been paid to the possibility that infertility treatment may be compromised as a result of infection by a direct effect on gamete function, although from the data reviewed in this paper this should now be considered as a realistic possibility. Whilst the majority of studies from our own laboratory (described here) and others (e.g. Vigil, Straberger, Esterbauer, Fink, & Schmeller, 2002; Jungwirth et al., 2003) have concentrated exclusively on the effects of *C. trachomatis* on sperm function, the possibility that the function of the unfertilised egg or the developing embryo may be similarly compromised (see also Levy et al., 1999) should also be considered and requires further research.

Finally, whilst national screening programmes within the UK (Pimenta et al., 2003 a & b) have concentrated on the screening of women, the detection and treatment of chlamydial infections in men have to date relied upon contact tracing methods. However, it has been argued that screening of men is necessary because partner notification methods are currently not sufficiently effective (Catchpole, Robinson, & Temple, 2003). Perhaps an additional approach would be to undertake chlamydia diagnoses on the remaining portion of the semen samples provided for diagnostic purposes and that is normally discarded as clinical waste once the semen analysis is finished. Of the tens of thousands of men providing semen samples for

analysis within the UK each year, of which only a small proportion would eventually receive assisted conception treatment with their partner, this would contribute in a small and potentially cost effective way (without the need for additional hospital appointments to obtain the test sample) to potentially help reduce the burden of *C. trachomatis* infections within the UK.

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