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Review Article

Chlamydial Infection and Its Role in Male Infertility

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Introduction. *Chlamydia trachomatis* is an established cause of tubal factor infertility; however its role in male fertility is not as clear. We sought to determine the prevalence of Chlamydia in infertile men and evaluate its impact on male reproductive potential. *Materials and Methods*. We compared the incidence of Chlamydia in our infertile male population with that reported in the literature. We then reviewed the impact of Chlamydia infection on male fertility. *Results*. The incidence of Chlamydia infection in our population of infertile men was 0.3%. There is considerable variability in the reported incidence, likely due to variation in the population studied, and detection technique. The optimal testing method and sample are presently unclear. The effect of Chlamydia on male reproductive function is also variable in the literature, but appears to be relatively minimal and may be related primarily to sperm DNA fragmentation or female partner transmission. *Conclusions*. The prevalence of Chlamydia in the infertile male population is low and routine testing is not supported by the literature. For high-risk infertile men, nucleic acid testing of urine +/− semen is the most sensitive method to detect Chlamydia. A validated testing system for semen needs to be developed, so that a standardized methodology can be recommended. In this way the full implications of Chlamydia on male fertility can be elucidated.

1. Introduction

Chlamydia trachomatis (C. trachomatis) is the most prevalent sexually transmitted disease in the world and a common cause of pathology in both men and women, causing urethritis, epididymitis, prostatitis, cervicitis, pelvic inflammatory disease (PID), ectopic pregnancy, and tubal factor infertility [1]. While there are regional differences in the prevalence, it remains a common cause of genitourinary pathology in both men and women. In women *C. trachomatis* is a well-established cause of tubal factor infertility. In men it is a known common genitourinary pathogen, and electron microscopy has clearly demonstrated that *C. trachomatis* attach to spermatozoa [2–5], both on the surface and in the nucleus [6]; however its role in male fertility (sperm function, pregnancy rates, and live birth rates) is not clear.

As the etiology of approximately 55% of male factor infertility is unknown, it is possible that Chlamydia is contributory in some of these cases. In our study in a Canadian clinic, we identified a very low prevalence of Chlamydia in the infertile male population of only 0.3% [7]. This is the largest study of the prevalence of *C. trachomatis* ever published on infertile men with a total of 5588 men studied, and certainly seems to indicate that *C. trachomatis* infections are highly uncommon in men with infertility. However, there are significant differences in the reported prevalence of *C. trachomatis* infections in men with infertility, which vary by region and *C. trachomatis* detection techniques. In light of these findings, we sought to determine the overall prevalence of *C. trachomatis* in infertile men, and if the reported prevalence rates might be affected by the technique of *C. trachomatis*

detection or reflect a real difference in regional *C. trachomatis* rates.

2. Prevalence

C. trachomatis is the most prevalent sexually transmitted disease in the world [8]. Among infertile males, there is considerable variability in the rate of *C. trachomatis* infection, ranging from 0 to 90.3% (Table 1) [2, 3, 6, 9–39]. In our population of infertile Canadian males, we determined the prevalence of Chlamydia infection to be on the lower end of this spectrum, 0.304% [7]. Notably, in our population there was a reported history of treated Chlamydia of 2%, which still suggests a low prevalence in the infertile male population. The reported variability in Chlamydia prevalence is likely derived in part from the population studied, screening method, and type of sample used [2]. From 2007 to 2008, *C. trachomatis* prevalence in the United States among 14–39 year olds was 1.6%, with higher rates seen among females (2.2% versus 1.1% in males), African Americans (6.7%), and adolescents aged from 14 to 19 years (2.5%) [40]. This was slightly different than what we observed in our population, where the mean age of men that tested positive for Chlamydia was 35 years; however this represents a skewed population of men, who are presenting for fertility evaluation. In addition, there does seem to be some geographic variability in *C. trachomatis* prevalence. Rates are low in individuals throughout northern Europe, Iran, and Japan (mean of 2.7%) but higher in Tunisia (43%), South Africa (34%), and India (33%) [21, 41]. In addition, it is believed that the actual prevalence of Chlamydia is underestimated, in part, due to its (at times) asymptomatic nature [42].

3. Biology

C. trachomatis is intracellular bacteria that produces a wide variety of clinical pathologies. The organism has a unique developmental cycle in which it exists in two forms: the inactive elementary body and the infectious reticulate body, providing a mechanism for continued transmission among sexual partners. Elementary bodies, reticulate bodies, or the lipopolysaccharide that they release, may be encountered by any gametes that are present in the reproductive tract at that time. The full clinical consequences of exposure are still being uncovered but are likely multifaceted.

Infection is characterized by a range of clinical manifestations, from a subclinical infection to a robust inflammatory response [1]. Up to 75% of cases in women and 50% in men are asymptomatic, which may lead to repeated transmission [9]. We found this to be true in our study of Canadian infertile men, where none of the men that tested positive for Chlamydia reported any symptoms of infection on history or physical exam [7]. The level of immune response to *C. trachomatis* may be affected by the size of the initial inoculum and the number of repeated infections, as well as genetic variation. Such genetic variation has been shown to correlate with *C. trachomatis* infection with the severity of tubal damage in women [43]. These responses have been studied primarily in women but likely can be extrapolated to men.

Chlamydia can persist in the host even after the development of an immune response, leading to chronic infection [1]. Surprisingly, only 15% of the men in our cohort who tested positive for Chlamydia reported a prior history of sexually transmitted disease [7], suggesting that most of these men cleared their initial infection. It is unclear why some individuals clear infections, while others do not; however some evidence has shown that the cytokine IFN-γ may be involved [44]. Persistence is serologically characterized by elevated *C. trachomatis* heat shock protein 60 (CHSP60) [45], which in women has been associated with PID, ectopic pregnancy, scarring trachoma, tubal infertility, and spontaneous pregnancy loss [45, 46].

4. Testing

Some of the variability that we observed in the prevalence of Chlamydia infection is likely due to a variety of testing methods, which have variable sensitivities and specificities, and also different implications for fertility in both men and women.

Current screening recommendations for *C. trachomatis* in the infertile couple are vague, and at the present time it is not clear how to best detect *C. trachomatis* in the male. This was part of why in our initial study we tested both urine and semen samples [7]. Review of the male fertility literature reveals that in the past many different methods, as well as a variety of specimens, have been used (serum, urethral swab, urine, and semen). First catch urine has become the most widely accepted testing method for *C. trachomatis* detection [47]. Testing of semen for *C. trachomatis* is a relatively recent concept and currently there is no approved methodology for Chlamydia testing in semen [47–49]. Most commercially available methods have rarely been applied to the detection of *C. trachomatis* in semen [48], and in our study we used nucleic acid testing of both urine and semen in order to assess if there would be some cases which were captured by semen testing alone [7].

The role of seminal testing is not known. It is thought that the presence of Chlamydia in semen may indicate an infection of the upper genital tract [20, 34, 37, 50]; however this may also reflect a contaminated urethra, through which the ejaculate passes. A recent review by Eley and Pacey [41] found that semen testing does uniquely capture some cases of Chlamydia infection, a finding which was corroborated by our study [7]. The presence of *C. trachomatis* in urethral samples and its absence in semen specimens may indicate an asymptomatic lower tract infection that may have less fertility-related relevance. However, patients testing positive for *C. trachomatis* DNA in semen specimens and negative for *C. trachomatis* DNA in urethral samples may indicate that *C. trachomatis* resides in the male secretory glands and as such may serve as a marker for an invasive Chlamydial infection [51].

Table 1: Frequency of *C. trachomatis* detection among infertile males.

Author	Year	Method of *C. trachomatis* testing	Specimen tested	Men testing positive for Chlamydia
Nagy et al. [10]	1989	Culture	Semen	14.1% (26/184)
Bjercke and Purvis [14]	1992	ELISA for Chlamydia IgA	Semen	24% (24/100)
Eggert-Kruse et al. [15]	1992	ELISA for Chlamydia IgG	Serum	0
		Culture	Urethral swab	0
Samra et al. [16]	1994	Culture	Urethral swab	9.6% (13/135)
		ELISA for Chlamydia IgG	Serum	4.4% (6/135)
		ELISA for Chlamydia IgA	Serum	1.5% (2/135)
		ELISA for Chlamydia IgG	Semen	0
		ELISA for Chlamydia IgA	Semen	8.9% (12/135)
Munoz and Witkin [12]	1995	ELISA for Chlamydia IgG	Serum	25% (12/48)
		ELISA for Chlamydia IgA	Serum	16.7% (8/48)
		ELISA for Chlamydia IgG	Semen	8.3% (4/48)
		ELISA for Chlamydia IgA	Semen	29.1% (14/48)
Dieterle et al. [17]	1995	PCR	Semen	8% (4/50)
		ELISA for Chlamydia IgG	Serum	46% (23/50)
		ELISA for Chlamydia IgA	Serum	12% (6/50)
		ELISA for Chlamydia IgG	Semen	12% (6/50)
		ELISA for Chlamydia IgA	Semen	16% (8/50)
Eggert-Kruse et al. [18]	1996	Immunofluorescence testing	Semen	0
		ELISA for Chlamydia IgG	Semen	8.1% (16/197)
		ELISA for Chlamydia IgA	Semen	18.8% (37/197)
		ELISA for Chlamydia IgG and IgA	Serum	16.2% (32/197)
Cengiz et al. [19]	1997	Monoclonal antibodies to Chlamydia	Urethral swab	12.6% (36/284)
Eggert-Kruse et al. [20]	1997	Indirect immunofluorescence assay	Serum	12.6% (164/1303)
		Tissue culture	Urethral swab	0
		ELISA for Chlamydia IgG	Semen	1.5% (50/1303)
		ELISA for Chlamydia IgA	Semen	3.5% (46/1303)
Bornman et al. [21]	1998	Tissue culture	Urethral swab	18.3% (24/131)
		EIA	Urethral swab	25.2% (33/131)
		DFA	Urethral swab	26% (34/131)
		EIA	First void urine	25.2% (33/131)
		EIA	Semen	26.7% (35/131)
Levy et al. [22]	1999	ELISA for Chlamydia IgG	Serum	22.8% (21/92)
		ELISA for Chlamydia IgA	Serum	8.6% (8/92)
		PCR	Semen	10.9% (10/92)
Ochsendorf et al. [23]	1999	ELISA for Chlamydia IgG and IgA	First void urine	0.8% (1/125)
		ELISA for Chlamydia IgG and IgA	Ejaculate	0
		ELISA for Chlamydia IgG and IgA	Seminal plasma	11.2% (14/125)
		ELISA for Chlamydia IgG and IgA	Serum	31.2% (39/125)
		PCR	First void urine	1.6% (2/125)
		PCR	Ejaculate	1.6% (2/125)
Habermann and Krause [24]	1999	ELISA for Chlamydia IgG and IgA	Semen	90.3% (187/207)
Videau et al. [25]	2001	ELISA for Chlamydia IgA	Semen	23% (23/102)
Mania-Pramanik et al. [26]	2001	Direct antigen detection	Urine	33.3% (5/15)
		Direct antigen detection	Semen	13.3% (2/15)
		Direct antigen detection	Serum	0
		ELISA for Chlamydia IgG	Urine	0

Table 1: Continued.

Author	Year	Method of *C. trachomatis* testing	Specimen tested	Men testing positive for Chlamydia
			Semen	0
			Serum	46.7% (7/15)
Bollmann et al. [27]	2001	ELISA for Chlamydia IgA	Semen	38.1% (317/834)
		ELISA for Chlamydia IgA	Serum	19.1% (62/324)
		ELISA for Chlamydia IgG	Semen	15.9% (133/834)
		ELISA for Chlamydia IgG	Serum	67.9% (220/324)
Gdoura et al. [28]	2001	Direct fluorescence antibody	Semen	1.1% (1/92)
			Urethral swab	4.3% (4/92)
		Cell culture	Urethral swab	1.1% (1/92)
		PCR	Semen	16.3% (15/92)
		PCR	Urethral swab	18.5% (17/92)
		ELISA for Chlamydia IgA	Semen	21.7% (20/92)
		ELISA for Chlamydia IgA	Urethral swab	8.7% (8/92)
Vigil et al. [6]	2002	Direct immunofluorescence	Semen or urethral swab	38.6% (110/284)
Eggert-Kruse et al. [29]	2003	LCR	Urine	1.6% (11/707)
		LCR	Semen	0.7% (5/707)
Idahl et al. [30]	2004	Microimmunofluorescence for Chlamydia IgG	Serum	20.1% (49/243)
		PCR	Urine	7.1% (5/70)
Hamdad-Daoudi et al. [31]	2004	PCR	Urine	3.6% (4/111)
		PCR	Prostatic massage	0.9% (1/111)
		PCR	Semen	2.7% (3/111)
		ELISA for Chlamydia IgG	Serum	0.9% (1/111)
		ELISA for Chlamydia IgA	Serum	0
Hosseinzadeh et al. [32]	2004	PCR	Semen	4.9% (31/642)
		LCR	Semen	4.3% (28/642)
de Barbeyrac et al. [33]	2006	PCR	Semen	0.3% (1/260)
		PCR	Urine	0.7% (2/260)
		ELISA for Chlamydia IgG and IgA	Serum	9.5% (22/231)
		ELISA for Chlamydia IgG and IgA	Semen	4.3% (10/231)
Gdoura et al. [34]	2008	PCR	Semen	42.3% (44/104)
		PCR	Urine	39.4% (41/104)
Joki-Korpela et al. [35]	2009	EIA to Chlamydia IgG	Serum	27.8% (25/90)
		EIA to Chlamydia IgA	Serum	22.2% (20/90)
Ouzounova-Raykova et al. [36]	2009	Culture	Urethral swab	6.6% (4/60)
		PCR	Urethral swab	8.3% (5/60)
Kokab et al. [37]	2010	Strand displacement amplification	Urine	3.5% (9/255)
		Strand displacement amplification	Semen	7.0% (18/255)
		PCR	Urine	2.4% (6/255)
		PCR	Semen	6.3% (16/255)
Eggert-Kruse et al. [38]	2011	ELISA for Chlamydia IgG	Semen	22.5% (39/173)
		ELISA for Chlamydia IgA		20.8% (36/173)
		ELISA for Chlamydia IgM		5.8% (10/173)
Rybar et al. [39]	2011	Sperm chromatin structure assay	Semen	13% (38/293)
Domes et al. [7]	2012	Strand displacement amplification assay	Semen or urine	0.3% (17/5588)

Unfortunately, there is no approved methodology for the testing of semen for *C. trachomatis* [49, 52]. In fact, the question as to whether semen is a suitable sample for detection of *C. trachomatis* in infertile men is not even completely clear [52], as components of seminal fluid have been shown to be toxic to cell culture growth [53]. Attempts to dilute semen to decrease the toxicity have resulted in a decreased sensitivity to detect *C. trachomatis* [50, 54]. Recommendations have been made for developing standardizing semen testing for *C. trachomatis* [41]; however at this point these have not led to a standardized protocol.

Historically, Chlamydia was grown in culture; however this was replaced in many centers by antigen detection [52]. This indirect approach is inherently flawed but nonetheless has been extensively utilized. The most commonly studied antibodies include those directed against *C. trachomatis* IgG and CHSP60. Most individuals with urogenital Chlamydial infection develop serum IgG and IgA antibodies, which persist for years and have been considered a marker of past infection [55]. Heat shock proteins are stress response proteins, and CHSP60 (a marker of Chlamydia persistence) stimulates the inflammatory response [56, 57]. In male serum, *C. trachomatis* IgA and IgG, but not CHSP60 antibodies, have been shown to correlate with lower female partner pregnancy rates [58]. However, the role of *C. trachomatis* antibodies in men remains unclear.

The method that we used in our assessment of the incidence of Chlamydia infection in the infertile male population was nucleic acid amplification testing (NAAT) [7], which has become the method of choice for *C. trachomatis* detection [59]. NAAT has been shown to have better sensitivity than nonmolecular methods; however, an important consideration for using NAAT testing in semen is that there are more NAAT inhibitors in semen than in urine [31, 33]. The presence of these inhibitors has been corroborated by the finding of a lowered sensitivity of NAAT in the detection of *C. trachomatis* from semen when compared with urine [41, 60]. Extracting DNA from semen prior to polymerase chain reaction (PCR) or ligase chain reaction (LCR) testing has been shown to greatly improve the detection rate. Thus, if NAAT is to be used for *C. trachomatis* detection in semen, DNA extraction will likely be essential in a commercial testing system.

The relationship between *C. trachomatis* antibodies and DNA is still under investigation. Weidner et al. [61] found that there was some (but not all) overlap between men testing positive for Chlamydial antibodies and genetic material, among both seminal and serum samples. Clearly, each fluid and testing method will identify slightly different groups, but the clinical implications of this are unknown. The inconsistent link between Chlamydial antibodies and presence of Chlamydial DNA has been corroborated by other groups [11, 17, 28, 51]. It is the authors' opinion after reviewing this literature that the assessment of Chlamydial IgG and IgA antibodies in serum or semen is likely of limited use in the male infertility workup. One benefit of detecting *C. trachomatis* in the infertile male is that it serves as a marker for infection in the female partner. Sperm may serve as vectors for *C. trachomatis* [62, 63], spreading the pathogen to the female reproductive tract [64] and also inducing an immune response to sperm in women [11].

Also noteworthy is the role of seminal leukocytes with Chlamydial infection. It is well known that seminal leukocytes negatively impact male fertility, and studies have demonstrated an association between seminal antibodies to *C. trachomatis* and seminal inflammation [23, 65, 66]. Bollmann et al. [67] sought to determine if any negative effects are due to the Chlamydia or the leukocytes and found that this was more likely due to the seminal leukocytes and not the presence of *C. trachomatis*.

5. Male Issues

The primary site of male Chlamydia infection is the penile urethra [68], with subsequent retrograde infection of the epididymis and testis [69]. The role of Chlamydia infection of the male accessory glands, including the prostate and seminal vesicles, is unclear. Prostatic infection is particularly interesting, as mouse models have demonstrated that Chlamydia may persist in the prostate after treatment, establishing an immune-privileged niche and avoiding the host immune response, which may result in serving as a reservoir for continuous infection [70]. Recent evidence suggests that prostatitis caused by Chlamydia, as opposed to more common pathogens, had lowered sperm concentration, motility, and morphology and that strong correlations between anti-*C. trachomatis* IgA and sperm concentration and normal forms were noted [71]. Coinfection in these patients may further impair semen parameters.

Chlamydia infection is responsible for 40–80% of epididymitis [72]. These men can subsequently develop orchitis and prostatitis, which can lead to canalicular system damage, testicular atrophy, and obstructive azoospermia [73, 74]. In addition to the obstructive component, the epididymis plays a crucial role in sperm functional maturation, and *C. trachomatis* infection may negatively impact sperm function [72].

6. Sperm Parameters

While a large number of studies have demonstrated that Chlamydia infection has no effect on semen parameters [6, 11, 17, 19, 20, 23, 24, 29, 32–34, 61, 75, 76], both Gdoura and Witkin et al. have identified a relationship between the detection of *C. trachomatis* DNA in semen and poor sperm motility [11]. There is also some evidence to suggest that infection with *C. trachomatis* may lead to a defective acrosome reaction [77]. Studies identifying no relationship between Chlamydia infection and poor semen parameters may be difficult to interpret given the unclear relationship between Chlamydial antibodies and current/prior infection. While the data are not conclusive, overall, it seems that the link between Chlamydia infection and sperm parameters is relatively weak. Interestingly though, evidence suggests that Chlamydia infected men will have an improvement in semen parameters [78] and DNA fragmentation rates [74] after treatment with antimicrobials.

7. Antibodies

Any negative effect that Chlamydia actually has on sperm may also be due to the formation of anti-sperm or anti-Chlamydial antibodies [12, 79, 80]. Detection of anti-Chlamydial IgA and IgG antibodies in male serum has been associated with poor semen characteristics [19, 35] and reduced pregnancy rates regardless of female partner antibody status [30, 58, 81, 82]. In addition, asymptomatic infection with Chlamydia may be the reason for unexplained infertility in some men [72], as infertile men are more likely to be seropositive for antibodies to *C. trachomatis* at a titer of 1 : 64 or higher, and men with higher titers have a higher probability of being infertile [72].

Finally, the relationship to CHSP60 antibodies is unclear. Antibodies to CHSP60 are known to be related to tubal factor infertility in women, the result of an autoimmune cross-reaction to human HSP60 expressed in the female reproductive tract [83]. In men, Idahl et al. [58] found a decrease in sperm motility but no change in pregnancy rates in men who were CHSP60 IgG positive. Likewise, Karinen et al. found no relationship between CHSP60 IgG expression in men and subfertility [84]. Together these results suggest that the role of CHSP60 in male fertility is quite minimal.

As mentioned the mechanism of any effect is unclear. Is there a direct effect on male fertility or is the effect a result of sexual transmission to the female partner leading to tubal damage?

8. Cytotoxicity

While the exact mechanism of Chlamydia induced damage is not known, incubation of sperm with the elementary bodies of *C. trachomatis* leads to decreased sperm motility, stimulates tyrosine phosphorylation [85], and results in cytotoxicity [85]. In addition, the presence of IgA against *C. trachomatis* has been shown to correlate with lipid peroxidation of the sperm membrane [86]. *C. trachomatis*-induced cell death may also be caused by lipopolysaccharide (as generated by the elementary body), as lipopolysaccharide negating agents have been shown to inhibit the spermatocidal properties of elementary bodies [87]. In addition, it has been shown that lipopolysaccharide can induce sperm to generate reactive oxygen species which may be a component of the toxicity [88].

9. DNA Fragmentation

Recent studies have shown that Chlamydia infection may result in sperm DNA fragmentation, which has been associated with a low potential for natural male fecundity [89, 90], reduced fertility potential in vivo and in vitro [91], decreased embryo quality, and lower implantation rates [92]. Satta et al. [93] found that sperm from normospermic men had an increase in DNA fragmentation when incubated with *C. trachomatis*, an effect that occurred quickly and at a low bacterial concentration, suggesting that sperm may become damaged during transit in an infected female genital tract. Likewise, Gallegos et al. [74] found that, in men with Chlamydia, the mean percentage of sperm with fragmented DNA was 35.2%, 3.2 times higher than in fertile controls (10.8%). Finally, sperm infected by *C. trachomatis* may be sufficiently damaged to prevent the successful development of embryos should fertilization be successful [48].

10. Chlamydia and Pregnancy Outcomes

Similar to its effect on sperm, the effect of male *C. trachomatis* on pregnancy rates is not completely clear. Serologic studies have shown mixed results, some studies demonstrating lower pregnancy rates [58], and others demonstrating no difference [33]. Chlamydia can be transmitted by donor insemination [94, 95], likely by direct transmission adherent to sperm [63], and is not always removed by centrifugation prior to insemination or intracytoplasmic sperm injection [96]. Sperm-Chlamydia interaction may be an unrecognized cause of fertilization failure during in vitro fertilization (IVF) [41, 97], and if this is the case, then routine sperm testing prior to advanced reproductive procedures is warranted.

In 1999, Witkin [98] reported that women with cervical anti-Chlamydial and anti-CHSP60 IgA antibodies were three times more likely to have an early pregnancy loss after IVF than women without these antibodies. Further, incubation of embryos in media containing human sera positive for anti-human HSP60 antibodies inhibited embryo development [98]. These results have been corroborated by others [99]. Women without antibodies to CHSP60 have been shown to be five times more likely to have intrauterine conceptions and term deliveries when compared to those with positive serologies [100]. Together, these studies suggest that prior Chlamydial infection has an impact on intrauterine conception and delivery rate, and these outcomes appear to be related to antibodies to CHSP60 or Chlamydial IgG or IgA. This data suggests a role for semen testing in couples undergoing advanced reproductive technologies.

The mechanism for decreased fertility potential in females from prior Chlamydia exposure may be related to the pelvic inflammatory response provoked either by *C. trachomatis* or directly by the organism itself. Interestingly, the human trophoblast has been shown to express the 60 kDa Chlamydial antigen of human heat shock proteins, although this does not normally trigger an immune response [45, 80, 99], and cross-reactivity between heat shock protein previously sensitized to Chlamydia may compromise fetal or maternal cell viability. In addition, the presence of infectious forms of Chlamydia in the spermatozoon nucleus may result in an infected or damaged embryo.

11. Screening

One of the conclusions that we drew from our 2012 study [7] is that routing testing for Chlamydia in the infertile male population cannot be recommended given its low prevalence in this population. While recommendations exist for female testing, and indications for testing in fertile men exist (leukocytospermia and symptoms of infection), there are no recommendations that specifically address the indications

for, and methodology of, Chlamydia testing in infertile men. At this point infertile men are treated in the same manner as fertile men, but should this be the case?

In the United Kingdom, the Royal College of Obstetricians and Gynaecologists Evidence-based Clinical Guidelines [101] recommend that, prior to intrauterine instrumentation, women be offered screening for *C. trachomatis*. In the United States, the 2012 Institute for Clinical System Improvement recommends Chlamydia screening for all sexually active women of age 25 years and younger, although it makes no recommendations on screening for men. The 2010 American Urological Association Best Practice Statement "The Optimal Evaluation of the Infertile Male" states that "those patients with true leukocytospermia ($>1 \times 10^6$/mL) be evaluated for a genital tract infection or inflammation" but there are no specific recommendations on the sample type or testing methodology. Although screening for *C. trachomatis* among infertile men is practiced widely, it is not addressed in the Male Infertility Best Practice Guidelines published by the American Society for Reproductive Medicine and the American Urological Association [102].

At our center we found a rate of *C. trachomatis* of 0.304% in asymptomatic Canadian infertile men, significantly lower than that of the general population [7]. If cost is analyzed, we found that the reagent cost alone to diagnose one case of Chlamydia was $38,669, not inconsequential [7]. Given that the direct fertility consequences of Chlamydia infection in males are not completely clear, the utility of Chlamydia screening in this low-risk population may have little benefit apart from preventing infection to the female partner. In this respect, it may serve as a form of secondary disease prevention employed by some infertility centers, as it may reduce health care cost by decreasing long-term reproductive complications [65]. However, as noted above, the rates of *C. trachomatis* infection are regionally variable, and screening should correlate with local prevalence rates.

Finally, there are no studies demonstrating the effectiveness of screening for asymptomatic *C. trachomatis* in men with infertility for either reducing transmission of Chlamydia to female partners or preventing infections or complications in males [103, 104]. Large population-based studies in women have demonstrated reductions in PID with increased Chlamydia screening efforts [105]. However, other Chlamydia screening studies have not demonstrated a reduction in adverse female reproductive outcomes or epididymitis [106]. In men, the literature focuses on risk-group (primarily age directed) screening [107–109], as it has been shown to be cost-effective in these populations. In one proposed algorithm, if the female partner has a history of ectopic pregnancy, testing for anti-CHSP60 antibodies could be performed. Those with positive antibodies may be counseled to consider immediate IVF to optimize their chance for a live birth [73].

12. Conclusions

Our prior research has shown that the prevalence of Chlamydia in our infertile male population is low (0.304%), although regional prevalence is variable. The reasons for this are likely multifactorial, related to actual differences in infection rates, testing methodology, and bodily fluid tested. While the impact of *C. trachomatis* infection on female fertility is well established, its role in male infertility is less clear. In all probability, a direct connection between disturbed male fertility, impaired function of the male accessory glands, and *C. trachomatis* infection only exists in isolated cases. Furthermore, the low detection rate in infertile men of about 0.3% means that at best only a small proportion of cases of male infertility are due to *C. trachomatis* infection. The optimal testing method for Chlamydia is unclear at this point; infections will be missed if urine is the only test specimen and semen is not tested as well. A validated, commercially available testing system for semen needs to be developed so that a standardized methodology can be recommended for universal use.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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