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REVIEW ARTICLE

The value of testing semen for *Chlamydia trachomatis* in men of infertile couples

A. Eley* and A. A. Pacey†

*Department of Infection and Immunity, The University of Sheffield Medical School, and †Academic Unit of Reproductive and Developmental Medicine, Department of Human Metabolism, The University of Sheffield, Sheffield, UK

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Correspondence:
Adrian Eley, Department of Infection and Immunity, The University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK.
E-mail: a.r.eley@sheffield.ac.uk

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Summary

Chlamydia trachomatis is an important bacterial cause of infertility. In men, the recommended specimen for diagnosing chlamydial infection is urine, with little or no emphasis placed on testing semen. A systematic review of the literature was conducted to search for studies in which men undergoing investigations for infertility produced both samples of urine and semen that were tested concurrently for *C. trachomatis*. An analysis of these studies was then performed. From 322 papers identified from the US National Library of Medicine PubMed database, 14 were selected for a detailed review and 11 were analysed further. Overall, the size of the study groups was only modest and differences between the studies included variation in geography and test methodology, especially whether commercial testing systems had been used. There was also a lack of consistency with regard to including men with azoospermia. Patients were typically 30–35 years old and the median duration of infertility was about 4 years. Of those patients positive for *C. trachomatis* in the 11 studies, 56% could be detected in both semen and urine, 20% only in urine and 23% only in semen. Deficiencies in existing studies would not allow for a meta-analysis, emphasizing the need for further research in this area for which a number of recommendations are made.

Introduction

Chlamydia trachomatis is an unusual bacterium having a unique developmental cycle comprising infectious, non-metabolically active elementary bodies and non-infectious, metabolically active reticulate bodies. It is responsible for the most common sexually transmitted bacterial infection worldwide, affecting more than 90 million people [World Health Organisation (WHO), 2001] and has been known for some time to have a significant impact on human reproduction (Paavonen & Eggert-Kruse, 1999). However, the role of *C. trachomatis* in male infertility is still controversial (Ochsendorf, 2008).

To try and shed further insight into its effects, a number of studies have specifically looked at the relationship between chlamydial infection and semen quality. Some studies have shown that *C. trachomatis* infection is associated with poorer semen quality (Custo *et al.*, 1989; Wolff *et al.*, 1991; Witkin *et al.*, 1995; Cengiz *et al.*, 1997; Al-Mously *et al.*, 2009), whereas others have claimed that it is not (Gregoriou *et al.*, 1989; Nagy *et al.*, 1989; Eggert-Kruse *et al.*, 1990, 1996, 1997; Soffer *et al.*, 1990; Dieterle *et al.*, 1995; Weidner *et al.*, 1996; Habermann & Krause, 1999; Hosseinzadeh *et al.*, 2004). More recently, mostly in vitro investigations have shown that elementary bodies of *C. trachomatis* can adversely effect sperm function and stimulate the tyrosine phosphorylation of sperm proteins (Hosseinzadeh *et al.*, 2000), lead to premature sperm death (Hosseinzadeh *et al.*, 2001), stimulate an apoptosis-like response in sperm (Eley *et al.*, 2005a; Satta *et al.*, 2006), which lead to increased levels of sperm DNA fragmentation (Satta *et al.*, 2006; Gallegos *et al.*, 2008). Many of these experiments have contributed to the concept that exposure to elementary bodies of *C. trachomatis* can damage sperm and that this can provide a possible explanation of the pathogenesis of the organism in male infertility (Pacey & Eley, 2004; Eley *et al.*, 2005b).

Current screening recommendations for *C. trachomatis* in an infertile couple are vague and unhelpful. In the United Kingdom, the Royal College of Obstetricians and Gynaecologists Evidence-based Clinical Guidelines (RCOG, 1999) and the National Institute for Health and Clinical Excellence (NICE, 2004) recommend that before undergoing uterine instrumentation, women (presumably in an attempt to prevent spread of infection) should be offered screening for *C. trachomatis* using an appropriately sensitive technique. If the result of a test for *C. trachomatis* is positive, women and their sexual partners should be referred for appropriate management with treatment and contact tracing. In the United States, the Institute for Clinical System Improvement or ICSI (2004) describes symptoms of a possible genital tract infection in men, although no further information is provided to help investigate the cause. Both the European Association of Andrology (Dohle *et al.*, 2010) and the Male Infertility Best Practice Policy Committee of the American Urological Association and the Practice Committee of the American Society for Reproductive Medicine (2006) comment on the finding of significant numbers of leucocytes in a urethral smear, first void urine (FVU) or semen as indicative of a possible genital tract infection, but give no information as to how it should be investigated further. The only more specific advice is found in Dohle *et al.* (2010) and is from an older reference of Taylor-Robinson (1997) who states that despite modern DNA detection techniques, the ideal diagnostic test for *C. trachomatis* in semen has not yet been established.

Interestingly, in spite of the aforementioned UK guidelines, Sowerby & Parsons (2004) found that 53% of UK in vitro fertilization (IVF) clinics neither screen the woman nor give antibiotic prophylaxis and only 4% screen the man. In the screening of sperm, egg and embryo donors, recent guidance (Association of Biomedical Andrologists, Association of Clinical Embryologists, British Andrology Society, British Fertility Society, RCOG, 2008) has suggested that all donors be screened for *C. trachomatis* before and after donation, according to the strategy developed by the British Association for Sexual Health and HIV (2006). However, what is not clear is how best to detect *C. trachomatis* in men as many different methods have been employed over the years. The aim of this review was to attempt to assess the value of testing semen for *C. trachomatis* in men of infertile couples.

Laboratory methods for detecting *Chlamydia trachomatis*

A comparison of the different methodologies for detection of *C. trachomatis* is shown in Table 1 and the strengths and weaknesses of each are discussed next.

Screening by cell culture

In the early days of *C. trachomatis* screening, the bacterium was grown on cell monolayers inoculated with the clinical specimen such as a urethral swab (Mardh *et al.*, 1980). However, with the introduction of non-invasive samples such as urine, cell culture was too insensitive (Taylor-Robinson & Thomas, 1991) and it was quickly discovered that components of semen were toxic to the growth and maintenance of the monolayer. One solution to this toxicity problem was the dilution of the semen to decrease the toxic effect, but this was achieved at the expense of sensitivity to detect *C. trachomatis* (Tjiam *et al.*, 1987).

Other non-molecular methods

Other non-molecular methods include the detection of *C. trachomatis* antigen by either enzyme immunoassay (EIA) test or direct immuno-fluorescence (DIF). The method of EIA was widely applied to the testing of semen and urine (Wolff *et al.*, 1994; Cengiz *et al.*, 1997), but was found to have poor overall sensitivity (Black, 1997) because of cross-reactivity with antigens from staphylococci, *Bacteroides* species and *Escherichia coli* (Eggert-Kruse *et al.*, 1995; Ivanov *et al.*, 2009). Using DIF, the visual identification

Table 1 A comparison of different methodologies for detection of *Chlamydia trachomatis* in clinical specimens

	Sensitivity	Specificity	Ease of test	Speed of test	Cost
Culture	+++	++++	+	+	++++
Enzyme immunoassay	++	++	++++	+++	++
Direct immunofluorescence	+++	+++	++	++++	++
Electron microscopy	+	+++	+	+	++++
Immunoperoxidase staining/in situ hybridization	++	+++	++	+	+++
Serological (serum/semen) usually IgG, IgA antibodies	++	++	++	++	+++
NAATs, e.g. PCR, LCR, SDA, TMA, real-time PCR and multiplex PCR	++++	++++	+++	++	+++

NAAT, nucleic acid amplification testing; PCR, polymerase chain reaction; SDA, strand displacement amplification; TMA, transcription-mediated amplification; Ig, immunoglobulin; + to ++++, low to high scores.

of fluorescent elementary bodies (which are only just visible under light microscopy) in clinical samples is subject to error and difficult to control for quality. Moreover, the performance of the test is dependent on the number of elementary bodies that are used to define a positive sample (Thomas *et al.*, 1993). Interestingly, the incidence of *C. trachomatis* in semen samples using DIF in two studies was 21.7 and 41.6%, respectively (Jungwirth *et al.*, 2003; Veznik *et al.*, 2004), highlighting the difficulty of applying this test to screen clinical samples, rather than using it as a confirmatory test (Ostergaard & Moller, 1995).

In a small number of studies, electron microscopy has been used and this has shown a close association between chlamydial elementary bodies and spermatozoa (Villegas *et al.*, 1991; Erbengi, 1993) and later studies appear to suggest that *C. trachomatis* can be internalized by sperm (Vigil *et al.*, 2002; Gallegos-Avila *et al.*, 2009). However, whilst such results are of academic interest, the expense and technical expertise required largely preclude its use in routine diagnostic procedures. Finally, immunoperoxidase (IP) staining allows for direct detection of chlamydial antigen, usually in tissue specimens, and was used in a single study to examine the semen of male sexual partners of women with laparoscopic diagnosis of pelvic inflammatory disease or bacterial vaginosis (Toth *et al.*, 2000). Despite all 28 semen cultures (see before) for *C. trachomatis* being negative, there was an overall IP chlamydial positivity rate of 36%.

Detection of *Chlamydia trachomatis* antibodies

The identification of chlamydial antibodies is clearly an indirect approach to detect *C. trachomatis* in any clinical specimen. However, sadly, there is no single sensitive and specific *C. trachomatis* antibody test that has been consistently used to investigate chlamydial serology (Johnson & Horner, 2008). Amongst commercially available assays, there are often variable sensitivity and specificity as there may be cross-reactivity with *Chlamydophila* (*Chlamydia*) *pneumoniae* (Gijsen *et al.*, 2001) – a common respiratory pathogen. It is obvious therefore that there are several limitations with the use of chlamydial serology (in whatever test specimen) in general to detect the presence of *C. trachomatis*.

Until recently, there was no consensus on the detection of chlamydial immunoglobulin (Ig)G and IgA in serum and the presence of *C. trachomatis* in the male genital tract. It was generally believed that detection of serum IgG and IgA was of no diagnostic value in male infertility (Wolff *et al.*, 1994; Dieterle *et al.*, 1995). Studies by Radouani *et al.* (1996), Weidner *et al.* (1996) and Levy *et al.* (1999) confirmed a lack of correlation between serological results and direct detection of *C. trachomatis* in semen. However, three recent studies (Idahl *et al.*, 2004, 2007; Joki-Korpela *et al.*, 2009) have shown that the presence of *C. trachomatis* IgG and IgA antibodies in serum from the male partner of an infertile couple was not only correlated with pregnancy (as confirmed by routine ultrasound at gestation weeks 15–17) but also associated with subtle negative changes in semen characteristics.

The role of chlamydial IgG and IgA antibodies in semen and their relationship with semen quality are perhaps more controversial. Many studies have shown no association (Eggert-Kruse *et al.*, 1997, 1998; Habermann & Krause, 1999; Penna Videau *et al.*, 2001; Liu & Zhu, 2003), whereas others studies have shown that there is one (Wolff *et al.*, 1991; Ochsendorf *et al.*, 1999). A more recent study also showed no relationship between past or present *C. trachomatis* infection, defined by positive direct and serological markers (in either serum and/or semen), and quality of semen defined according to WHO parameters (de Barbeyrac *et al.*, 2006). However, a new, large study which looked at *C. trachomatis* infection in young prostatitis patients found a strong correlation between mucosal anti-*C. trachomatis* IgA and sperm concentration, sperm motility and normal morphology (Mazzoli *et al.*, 2010). Perhaps the fundamental problem is the presence of chlamydial IgG or IgA antibodies in serum or semen does not allow a distinction between past or present genital infection (Dieterle *et al.*, 1995). Therefore, an appropriate current summary suggests that the determination of *C. trachomatis* antibodies in serum or seminal plasma seems to be of limited diagnostic value in male infertility work-up (de Barbeyrac *et al.*, 2006).

Molecular methods

Early methods to detect *C. trachomatis* DNA in clinical specimens used in situ hybridization (ISH). In a study of 94 semen samples, *C. trachomatis* was detected in eight (9%) cases (Yoshida *et al.*, 1994). However, the technique is not particularly sensitive and does not really lend itself to a routine diagnostic application. Therefore, once nucleic acid amplification testing (NAAT) became widely available (see next), ISH was superseded.

A systematic review in 2002 established that both polymerase chain reaction (PCR) and ligase chain reaction (LCR) showed better sensitivities than non-molecular methods (Watson *et al.*, 2002). The authors also concluded that these NAATs used on non-invasive samples such as urine were more effective in detecting asymptomatic chlamydial infection and that these tests performed well in low prevalence populations. Skidmore *et al.* (2006) document the two NAATs currently in widespread use in the United Kingdom: the Becton Dickinson ProbeTec (Oxford, UK), which uses strand displacement amplification (SDA) technology (Spears *et al.*, 1997), and the

Roche Cobas Amplicor PCR (West Sussex, UK), which is now being superseded by a real-time PCR method, the Roche Cobas Taqman CT. A third NAAT currently available in the United Kingdom is transcription-mediated amplification (TMA) by GenProbe (Manchester, UK) (Pasternack *et al.*, 1997). It is now generally accepted that in *C. trachomatis* detection, NAATs have become the method of choice (Hamdad & Orfila, 2005; Gaydos *et al.*, 2008), so much so that they must be used in the UK national chlamydia screening programme (Department of Health, 2003).

The first NAAT to be used successfully for the detection of *C. trachomatis* in semen was PCR (Van den Brule *et al.*, 1993) using in-house methodology. This has been used subsequently in a number of studies (Ochsendorf *et al.*, 1999; Hosseinzadeh *et al.*, 2004; Gdoura *et al.*, 2008). Since then, PCR has been developed commercially and hence the method is standardized, as for example, in the Roche Cobas Amplicor PCR, which also has an in-built control for specimen inhibitors (as LCR has; Hamdad-Daoudi *et al.*, 2004; de Barbeyrac *et al.*, 2006). A more recent development has been the introduction of real-time or quantitative PCR, which allows detection of *C. trachomatis* DNA copy numbers (Al-Mously *et al.*, 2009). In the 1990s, the LCR, which was a commercial system (Abbott), also proved to be successful in detecting *C. trachomatis* in semen (Eggert-Kruse *et al.*, 1997, 2002a,b; Fujisawa *et al.*, 1999). However, LCR was recently discontinued and is no longer available.

Choice of test specimen

First void or first catch urine (usually first 15–50 mL) has been shown to be an acceptable specimen, for the detection of *C. trachomatis* genital infection in men, since the early 1990s (Chernesky *et al.*, 1990). Apart from the above changes in methodology for improved *C. trachomatis* detection in urine, other factors have emphasized the advantages of using urine. It has been known for a while that NAAT inhibitors can be present in many clinical specimens, especially urethral swabs, although they are quite rare in urine (Toye *et al.*, 1998). Moreover, a recent study has shown that there is no significant difference in organism load between FVU and urethral swabs in men when assessed by quantitative PCR (Michel *et al.*, 2007). Therefore, detection of *C. trachomatis* in urine is advantageous as the sample is self-taken, non-invasive, produces a high organism load and has a low incidence of NAAT inhibitors. However, in an infertility setting, semen is a readily available fluid and therefore we approached this review by raising the question whether semen could be a valid test specimen. We particularly wanted to study the detection of *C. trachomatis* in patients who had provided samples of urine and semen, so that a direct comparison could be made between urine, which is the recommended specimen, and semen. The decision to focus on this topic was influenced by our own research findings (e.g. Eley *et al.*, 2005b) as well as the apparent lack of helpful advice in the literature on whether and how to test semen for *C. trachomatis*.

Materials and methods

A literature search was performed of the US National Library of Medicine PubMed database using the keywords: *Chlamydia trachomatis* AND male infertility (8 October 2009). This returned 322 publications that were selected for further review if they met the following criteria: (i) the study population comprised men undergoing investigations for infertility; (ii) a urine specimen was tested in parallel with a semen sample for *C. trachomatis*. Studies were excluded if: (i) only serological tests (either serum and/or semen) were performed; (ii) only semen or urine samples were tested.

Briefly, the first reviewer (A. Eley) scanned the abstracts and/or full papers using the criteria described before and categorized them into two libraries: 'papers for further analysis' and 'papers not relevant'. A total of 14 papers were selected for a detailed review and the second reviewer (A. A. Pacey) assessed these papers for inclusion in the study and confirmed those papers that were not relevant.

Results

When details of the 14 studies (Table 2) were analysed, a number of key features were observed. First, a majority of papers were more than 5 years old and this has an obvious bearing on the results of new diagnostic developments in the detection of *C. trachomatis*. Secondly, common features of men patients were that their mean or median ages were from 30 to 37 years. The median duration of infertility for the couples, where reported, was about 4 years and sexual abstinence prior to testing (when mentioned) was between 3 and 5 days (data not shown).

One important difference between the studies was that in only four were there details of whether patients were being investigated for primary or secondary infertility. In those where details were included, the proportion of patients with secondary infertility ranged from 21% to 49%. This may be of some relevance as it has been reported that there is an increased risk of *C. trachomatis* infection in patients with secondary infertility (Malik *et al.*, 2009).

A further difference in the studies was the lack of consistency with regard to including men with azoospermia. Some studies included these men (Bornman *et al.*, 1998;

Table 2 Summary of studies on detection of *Chlamydia trachomatis* in urine and semen of infertile and subfertile men

		Patients				Methodology		Semen			Urine				Semen + urine
		Age (years)													
Authors/date	Country of study	Mean	Median	Range	No.	Extraction method	Test	% positive	C	I	FVU	% positive	C	I	% positive
Bollmann *et al.*, 2001	Germany	NK	30	19–58	77	[a]	LCR	3.89	x	0	✔	4.54	x	0	NK
Bornman *et al.*, 1998	South Africa	33	NK	25–51	131	–	EIA	26.71	D[e]	–	✔[f]	25.19	D	–	34.35
de Barbeyrac *et al.*, 2006	France	35	NK	NK	260	[b]	Amplicor	0.38	x	7	✔	0.76	x	1	1.15
Eggert-Kruse *et al.*, 1997	Germany	NK	33	21–53	150	[a]	LCR	0.66	x	NK	✔	0	x	NK	0.66
Eggert-Kruse *et al.*, 2002a	Germany	NK	34	19–59	256	[a]	LCR	NK	x	NK	✔	NK	x	NK	1.86
Eggert-Kruse *et al.*, 2002b	Germany	NK	34	22–53	202	[a]	LCR	NK	x	NK	✔	NK	x	NK	1.00
Eggert-Kruse *et al.*, 2003	Germany	NK	33	NK	707	[a]	LCR	0.70	X	NK	✔	1.55	X	NK	1.83
Fujisawa *et al.*, 1999	Japan	NK	NK	NK	98	[a]	LCR	1.02	x	NK	✔	0	x	NK	1.02
Gdoura *et al.*, 2008	Tunisia	37	NK	26–58	104	[c]	In-house PCR	42.30	x	0	✔	39.42	x	0	43.26
Hamdad-Daoudi *et al.*, 2004	France	NK	33	24–44	111	[b]	Amplicor	2.70	S	8	✔	5.40	S	4	6.30
Kokab *et al.*, 2010	Iran	35	NK	NK	255	NK	SDA	7.05	S+D	0	✔	3.52	S+D	0	7.05
Mania-Pramanik *et al.*, 2001	India	31	NK	25–38	15	–	EIA	13.33	Dup	–	✔	33.33	Dup	–	33.33
Ochsendorf *et al.*, 1999	Germany	NK	33	25–55	125	[d]	In-house PCR	1.60	x	NK	✔	1.60	x	NK	2.40
Rosemond *et al.*, 2006	France	NK	NK	NK	100	[b]	Amplicor	1.0	X	9	✔	0	X	NK	1.0

LCR, ligase chain reaction; SDA, strand displacement amplification; Amplicor, Roche Cobas polymerase chain reaction (PCR); EIA, enzyme immunoassay; FVU, first void urine; C, confirmation (S, same test; D, different test; Dup, duplicate testing); I, inhibitors (number positive); NK, not known; ✔, correct; X, absent.

[a]Heating at 95–100 °C for 20 min; [b]lysis buffer and heating at 90–95 °C for 10 min; [c]cetyltrimethylammonium bromide (CTAB)/phenol-choloroform/isoamyl alchohol; [d]Celite and GIT (guanidinethiocyanate/Tris-HCl/EDTA/Triton-X) lysis buffer; [e]7/35 confirmed; [f]urine collected at least 2 h after previous micturition.

Fujisawa *et al.*, 1999; Gdoura *et al.*, 2008), whereas in others, they were excluded (Eggert-Kruse *et al.*, 2002a,b; de Barbeyrac *et al.*, 2006) or not mentioned (Eggert-Kruse *et al.*, 1997; Ochsendorf *et al.*, 1999; Bollmann *et al.*, 2001; Mania-Pramanik *et al.*, 2001; Hamdad-Daoudi *et al.*, 2004; Kokab *et al.*, 2010). In cases of obstructive azoospermia, sexually transmitted disease-associated infections have been considered a possible risk factor (Eggert-Kruse *et al.*, 2003) and hence large numbers of azoospermic patients in a study could have a bias on the findings.

Finally, the majority of studies focused on only detecting *C. trachomatis*, whereas in five studies (Eggert-Kruse *et al.*, 1997, 2002a,b; Rosemond *et al.*, 2006; Gdoura *et al.*, 2008), other organisms leading up to a full microbiological screen were investigated. Apart from the disadvantages of workload and cost, full microbiological screens are potentially useful as they shed light on possible interactions between organisms which may be of interest in trying to determine pathogenesis.

Of the 14 studies identified as being suitable for inclusion in the review (Table 2), three were omitted for detailed analysis (i.e. Bollmann *et al.*, 2001; Eggert-Kruse *et al.*, 2002a,b). In the case of Bollmann *et al.* (2001), no details were provided as to the identity of patients positive for *C. trachomatis* in semen (3/77) and urine (3/66); any comparisons therefore could not be made. However, despite no statement made in the manuscript on whether the urine was FVU, this was confirmed to have been first catch urine (U. Gobel, personal communication). Whereas, in the studies of Eggert-Kruse *et al.* (2002a,b), despite the overall *C. trachomatis* prevalence given (1.0 and 1.9%, respectively), there was no breakdown of how many individual semen and/or FVU samples were positive. When the remaining 11 studies were examined in

more detail, it was clear that in all of them except that of Ochsendorf *et al.* (1999), the numbers of samples positive for *C. trachomatis* in semen were different from those positive in FVU. On further examination of the work of Ochsendorf *et al.* (1999), it was realized that the data reported for the number of positive specimens in FVU and semen were not from the same cohort, thereby not allowing us to make the same comparison.

Table 2 shows that, of the 11 remaining studies, there are some important variations between them with regard to: (i) numbers of patients in the study; (ii) the categorization of patients; (iii) the geographical location of studies; and (iv) the diagnostic test used. Briefly, with the exceptions of the very small study of Mania-Pramanik *et al.* (2001) which examined only 15 men and the large study of Eggert-Kruse *et al.* (2003) which examined 707 men, all other studies looked at relatively modest size patient groups (i.e. between 98 and 260 men). Perhaps a more obvious difference between the studies was the categorization of patients, who are documented as either (i) infertile men; (ii) infertile couples; or (iii) subfertile couples (data not shown). However, it is not clear how these categories were defined and whether they represent similar or dissimilar subjects. With regard to the geographical location of the 11 studies, it is interesting that there is little difference in the proportion of *C. trachomatis*-infected individuals throughout northern Europe, Iran and Japan (combined semen/FVU mean of 2.7%), but much higher incidence of *C. trachomatis* infection is seen in Tunisia (combined semen/FVU of 43%), South Africa (combined semen/FVU of 34%) and India (combined semen/FVU of 33%). This may be in part related to the method of detection used. The oldest method used in these 11 studies was EIA (Bornman *et al.*, 1998; Mania-Pramanik *et al.*, 2001), which is known to have lower specificity and sensitivity than NAATs (Black, 1997). However, even the majority of the NAATs used here were by today's standards quite outdated. In the NAAT studies, commercial test systems were used in all but two (i.e. Ochsendorf *et al.*, 1999 and Gdoura *et al.*, 2008 who used an in-house PCR system) and interestingly, in some of these studies, no *C. trachomatis* was detected and the overall prevalence was consistently low (Eggert-Kruse *et al.*, 1997; Fujisawa *et al.*, 1999; Rosemond *et al.*, 2006). It should also be noted that in-house PCR is often not a standardized method as in commercial systems, where there are internal controls for extraction and amplification and this has a direct bearing on comparability of test results.

When the incidence of *C. trachomatis* infection in the semen and urine samples of the 11 papers was examined in more detail (Table 3), we observed that in 56% of patients, *C. trachomatis* could be detected in both semen and FVU, whereas 20% of patients were positive only in FVU and 23% of patients positive only in semen. This clearly emphasizes that a large number of *C. trachomatis* infections in these patients would remain undetected if semen samples were not tested.

Table 3 Summary of results of semen and urine testing for *Chlamydia trachomatis* in the 11 selected papers

	Semen/FVU samples			
Authors/date	+/+	−/−	+/−	−/+
Bornman *et al.*, 1998	23	86	12	10
de Barbeyrac *et al.*, 2006	0	257	1	2
Eggert-Kruse *et al.*, 1997	0	149	1	0
Eggert-Kruse *et al.*, 2003	3	694	2	8
Fujisawa *et al.*, 1999	0	97	1	0
Gdoura *et al.*, 2008	40	59	4	1
Hamdad-Daoudi *et al.*, 2004	2	104	1	4
Kokab *et al.*, 2010	9	237	9	0
Mania-Pramanik *et al.*, 2001	2	10	0	3
Ochsendorf *et al.*, 1999	1	122	1	1
Rosemond *et al.*, 2006	0	99	1	0
Total (% positive)	80 (56)	1914 (0)	33 (23)	29 (20)

FVU, first void urine.

Discussion

To date, it has generally been considered that a sample of urine from a man is a suitable specimen for determining whether or not he currently has *C. trachomatis* infection of the urethra (Wisniewski *et al.*, 2008). However, an obvious alternative test specimen to consider in an infertility setting is semen, as this is already available when the patient has a semen analysis. Moreover, the analysis of semen may provide additional information about whether there is a chlamydial infection of the testicles and epididymis. It has been recorded how transmission of *C. trachomatis* by donor insemination is possible (Nagel *et al.*, 1986; Van den Brule *et al.*, 1993) and we are aware of the fact that this organism has the ability to adhere to sperm (Wolner-Hanssen & Mardh, 1984) and is not always removed from sperm by density centrifugation prior to intrauterine insemination (IUI) or IVF/intracytoplasmic sperm injection (ICSI) (Al-Mously *et al.*, 2009). Sperm–chlamydia interaction may be an unrecognized cause of fertilization failure during IVF (Pacey & Eley, 2004), and therefore supports the argument that testing semen for *C. trachomatis* may be of value in some settings.

If we accept the argument that testing of semen for *C. trachomatis* may provide additional information on the chlamydial status of the man, we need to know how best to perform such a test. However, it is clear from the literature that there is no approved methodology for testing of semen for *C. trachomatis* (Chernesky, 2005; Peeling & Embree, 2005). All that can be recommended is

that validated methods are used. However, this is not very helpful to the practitioner as there are many factors that need to be considered in the validation of a diagnostic method. Ideally, it would be best to use a commercial system so that the same methodology is universally adopted. During the development of NAATs, the first test used to detect *C. trachomatis* in clinical samples was in-house PCR, and after a period of time, so many variations existed that it became difficult to make comparisons between them. A particular problem with in-house PCR is that there is a wide variation in results as typified by the two studies used in this review (Ochsendorf *et al.*, 1999; Gdoura *et al.*, 2008). The current commercial methods in use in the United Kingdom for routine detection of *C. trachomatis* include real-time PCR, SDA and TMA. However, these methods have rarely been applied to the detection of *C. trachomatis* in semen and therefore initially, there need to be studies that make comparisons between tests and test specimens to determine which performs better. This should include an assessment of best methods for DNA and/or RNA extraction, as, in commercial systems, the technical details of how this is done are often undisclosed.

An important consideration in selecting semen as a test specimen, if NAAT testing is used, is that it is already known that there are more NAAT inhibitors in semen than in urine (Hamdad-Daoudi *et al.*, 2004; de Barbeyrac *et al.*, 2006). In one detailed study (which was not included in this search; Pannekoek *et al.*, 2003), experiments showed low sensitivity of LCR in the detection of *C. trachomatis* from semen when compared with urine. This could have been because of the presence of inhibitory components in semen that might have interfered with the LCR and was supported by the observation that none of the 19 *C. trachomatis*-spiked samples from donors tested positive by LCR. In comparison, when in-house PCR was used on the same spiked samples, *C. trachomatis* was detected in all of them. Further experiments showed that DNA extracted from specimens prior to testing by LCR greatly improved the detection rate with 11 of the 19 *C. trachomatis*-spiked samples becoming positive. This confirms the importance of DNA extraction, as mentioned before, in improving the performance of *C. trachomatis* detection in a commercial testing system.

Therefore, it is essential that in every NAAT of semen, there is an in-built inhibitor control. Methods such as SDA incorporate such an internal standard (Skidmore *et al.*, 2006), but very few of the studies in Table 2 commented on the presence of NAAT inhibitors. In those that did, more inhibitors in semen than FVU were recorded. In addition to the issue of NAAT inhibitors, it is important to note that a significant proportion of men with an infection of the upper genital tract will be asymptomatic and hence there may be fewer bacteria to detect (Witkin, 2002). Therefore, test sensitivity will be of additional importance if semen is used as the test specimen. Unfortunately, for whatever reason, we know that there have been very large differences in the chlamydial positivity rate detected in semen from infertile men: typically ranging from between 1% and 5% (Hamdad-Daoudi *et al.*, 2004; Hosseinzadeh *et al.*, 2004) to as high as 39–42% (Witkin *et al.*, 1993; Gdoura *et al.*, 2008). These differences are difficult to understand, although variations in test methodology may be the most important factors to consider. In a study where specimens of semen from the same patients were assessed with two different NAAT methods (Pannekoek *et al.*, 2000), there were very marked differences in the results.

Since the introduction of NAATs in the detection of *C. trachomatis*, much discussion has taken place on how to evaluate a new highly sensitive test when compared with a less sensitive, older test (McAdam, 2000). One of the ways to address this has been the introduction of a minimum testing algorithm (Skidmore *et al.*, 2006). Such an algorithm recommends that every positive test be confirmed either by repeat on an original specimen where available, or by repeating an original extract from the first test. In a majority of the studies in this review (Table 2), no confirmatory tests were performed except in the case of Bornman *et al.* (1998), Hamdad-Daoudi *et al.* (2004) and Kokab *et al.* (2010) who confirmed their results with either the same or a different test, respectively and Mania-Pramanik *et al.* (2001) who confirmed their testing on duplicate samples. Future studies on NAATs for semen should incorporate the guidelines of the minimum testing algorithm. Moreover, with the recent introduction of a new variant *C. trachomatis* strain (Alexander & Ison, 2008), it is important that any testing platform is able to detect this new strain.

From a practical standpoint, if it is accepted that commercial NAAT methods are the most appropriate ones to use to detect *C. trachomatis* in semen, it is necessary to consider the process of sample collection, transport and storage of the specimen. WHO (1999) recommends an abstinence period of 2–7 days for semen analysis, but it is not clear if this would be appropriate for the diagnosis of *C. trachomatis* infection. A fundamental difficulty with testing of semen for *C. trachomatis* is the possibility that during ejaculation, semen may become contaminated with elementary bodies in the urethra (Ochsendorf *et al.*, 1999). This further complicates any comparison between testing of semen and urine. However, once the optimal methodology for specimen collection and transport has been determined, it would be advantageous to measure the chlamydial load in semen and this could be related to

other such studies of urine (Michel *et al.*, 2007). This would provide useful information in longer-term investigations to measure the duration of both symptomatic and asymptomatic chlamydial infections of the upper genital tract, as there are few data on the duration of chlamydial infection in men (Golden *et al.*, 2000). Using a recommended and universally accepted NAAT method for detecting *C. trachomatis* in semen would also allow for studies to be conducted on the relevance of chlamydial seminal IgA, given the current difficulties in interpreting the studies.

Data from this review suggest that testing semen for *C. trachomatis,* which should result in more antibiotic treatment, could be related to a better clinical outcome in terms of semen quality and pregnancy rates. To our knowledge, there have been few studies performed to provide us with the necessary evidence. However, recent publications do support this concept. Epidemiological data showed that men with a history of penile discharge, painful micturition and genital ulcers who did not seek adequate treatment for these symptoms were more likely to be infertile than men without the symptoms or men who were adequately treated (Okonofua *et al.*, 2005). In a study by Idahl *et al.* (2007), *C. trachomatis* in the man reduced the chance of the couple achieving pregnancy. In the absence of randomized controlled trials, it could not be concluded whether or not treatment with antibiotics would increase the likelihood of conceiving spontaneously. However, the authors hypothesized that such treatment may inherently improve the fecundity of the man. Finally, in a study by Gallegos *et al.* (2008), they showed that antibiotic therapy was successful in decreasing the percentage of spermatozoa with *C. trachomatis*-induced fragmented DNA and in a small group of patients, it was found to improve pregnancy rates.

Conclusion

It is clear from this review that there are only dated and relatively small, comparative studies on the detection of *C. trachomatis* in semen and urine. Therefore, it has been impossible to answer the question whether or not urine or semen is the best specimen for testing of men of infertile couples for *C. trachomatis*. However, from the limited data presented in Tables 2 and 3, it is clear that a significant number of *C. trachomatis* infections will be missed if urine is the only test specimen and semen is not tested as well. When the data for Tables 2 and 3 were collected, statistical advice was sought to see if a meta-analysis could be performed. However, it was concluded that this could not be performed. Nevertheless, it is clear that: (i) study-specific methodology affects the detection of *C. trachomatis* and (ii) the results of the two test specimens are not 100% concordant. The deviation from concordance might suggest that the tests on semen detect infections that tests on urine cannot detect. However, this pattern of data is also consistent with a low but non-zero error rate in both tests (semen and urine). A combination of the two factors is also plausible. Therefore, to conclude that testing semen gives added value could only be formally assessed when the sensitivity and specificity of each test are precisely evaluated and to date this has not been done. Further studies are needed to compare the use of urine and semen to diagnose *C. trachomatis* infection. To assist the design of these studies, we make a number of recommendations (Table 4).

Table 4 Recommendations for future testing of semen for *Chlamydia trachomatis*

1. Make a comparison of existing commercially available NAATs to establish the best, taking note of different methodologies for extracting nucleic acids
2. Try to establish a validated protocol with a specific NAAT
3. Depending on the type of NAAT, use an internal control for inhibitors
4. Confirm any positive results for *C. trachomatis* using the minimum testing algorithm
5. Combine semen testing with testing of FVU using an appropriate NAAT
6. Use a large enough patient group to give sufficient power to demonstrate statistical significance
7. Establish the chlamydial load in semen over a period of time to provide information on the natural history of infection in males
8. Establish the relevance of chlamydial seminal immunoglobulin A

NAAT, nucleic acid amplification testing; FVU, first void urine.

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References

Alexander S & Ison C. (2008) Is new variant *Chlamydia trachomatis* present in England and Wales? *Sex Transm Infect* 84, 29–31.

Al-Mously N, Cross NA, Eley A & Pacey AA. (2009) Real-time polymerase chain reaction shows that density centrifugation does not always remove *Chlamydia trachomatis* from human sperm. *Fertil Steril* 92, 1606–1615.

Association of Biomedical Andrologists, Association of Clinical Embryologists, British Andrology Society, British Fertility Society, Royal College of Obstetricians and Gynaecologists. (2008) UK guidelines for the medical and laboratory screening of sperm, egg and embryo donors. *Hum Fertil* 11, 201–210.

de Barbeyrac B, Papaxanthos-Roche A, Mathieu C, Germain C, Brun JL, Gachet M, Mayer G, Bebear C, Chene G & Hocke C. (2006) *Chlamydia trachomatis* in subfertile couples undergoing an in vitro fertilization program: a prospective study. *Eur J Obstet Gynaecol Reprod Biol* 129, 46–53.

Black CM. (1997) Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev* 10, 160–184.

Bollmann R, Engel S, Petzoldt R & Gobel UB. (2001) *Chlamydia trachomatis* in andrologic patients – direct and indirect detection. *Infection* 29, 113–118.

Bornman MS, Ramathuga TN, Mahomed MF, Greef AS, Crewe-Brown HH & Reif S. (1998) Chlamydial infection in asymptomatic infertile men attending an andrology clinic. *Arch Androl* 41, 203–208.

British Association for Sexual Health and HIV. (2006) *Sexually Transmitted Infections: UK National Screening and Testing Guidelines*. Available at: http://www.bashh.org/documents/59/59.pdf; accessed 3 March 2010.

Cengiz T, Aydoganli L, Baykam M, Mungan NA, Tuncbilek E, Dincer M, Yakupoghu K & Akalin Z. (1997) Chlamydial infections and male infertility. *Int Urol Nephrol* 29, 687–693.

Chernesky MA. (2005) The laboratory diagnosis of *Chlamydia trachomatis* infections. *Can J Infect Dis Med Microbiol* 16, 39–44.

Chernesky M, Castriciano S, Sellors J, Stewart I, Cunningham I, Landis SJ, Seidelman W, Grant L, Devlin C & Mahony J. (1990) Detection of *Chlamydia trachomatis* antigens in urine as an alternative to swabs and cultures. *J Infect Dis* 161, 124–126.

Custo GM, Lauro V, Saitto C & Frongillo RF. (1989) Chlamydial infection and male infertility: an epidemiological study. *Arch Androl* 23, 243–248.

Department of Health. (2003) *Chlamydia Screening Programme Roll Out: Core Requirements*. Available at: http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4006809; accessed 3 March 2010.

Dieterle S, Mahony JB, Luinstra KE & Stibbe W. (1995) Chlamydial immunoglobulin IgG and IgA antibodies in serum and semen are not associated with the presence of *Chlamydia trachomatis* DNA or rRNA in semen from male partners of infertile couples. *Hum Reprod* 10, 315–319.

Dohle GR, Diemer T, Giwercman A, Jungwirth A, Kopa Z & Krausz C. (2010) *EAU Guidelines on Male Infertility. European Association of Urology*. Available at: http://www.uroweb.org/gls/pdf/Male%20Infertility%202010.pdf; accessed 28 May 2010.

Eggert-Kruse W, Gerhard I, Naher H, Tilgen W & Runnebuam B. (1990) Chlamydial infection – a female and/or male infertility factors? *Fertil Steril* 53, 1037–1043.

Eggert-Kruse W, Rohr G, Strock W, Pohl S, Schwalbach B & Runnebaum B. (1995) Anaerobes in ejaculates of subfertile men. *Hum Reprod Update* 1, 462–478.

Eggert-Kruse W, Buhlinger-Gopfarth N, Rohr G, Probst S, Aufenanger J, Naher H & Runnebaum B. (1996) Antibodies to *Chlamydia trachomatis* in semen and relationship with parameters of male infertility. *Hum Reprod* 11, 1408–1417.

Eggert-Kruse W, Rohr G, Demirakca T, Rusu R, Naher H, Petzoldt D & Runnebaum B. (1997) Chlamydial serology in 1303 asymptomatic subfertile couples. *Hum Reprod* 12, 1464–1475.

Eggert-Kruse W, Rohr G, Probst S, Rusu R, Hund M, Demirakca T, Aufenanger J, Runnebaum B & Petzoldt D. (1998) Antisperm antibodies and microorganisms in genital secretions – a clinically significant relationship? *Andrologia* 30(Suppl. 1), 61–71.

Eggert-Kruse W, Neuer A, Clussman C, Boit R, Geissler W, Rohr G & Strowitzki T. (2002a) Seminal antibodies to human 60 kd heat shock protein (HSP60) in male partners of subfertile couples. *Hum Reprod* 17, 726–735.

Eggert-Kruse W, Zwick E-M, Batschulat K, Rohr G, Armbruster FP, Petzoldt D & Strowitzki T. (2002b) Are zinc levels in seminal plasma associated with seminal leukocytes and other determinants of semen quality? *Fertil Steril* 77, 260–269.

Eggert-Kruse W, Rohr G, Kunt B, Meyer A, Wondra J, Strowitzki T & Petzoldt D. (2003) Prevalence of *Chlamydia trachomatis* in subfertile couples. *Fertil Steril* 80, 660–663.

Eley A, Hosseinzadeh S, Hakimi H, Geary I & Pacey AA. (2005a) Apoptosis of ejaculated human sperm is induced by co-incubation with *Chlamydia trachomatis* lipopolysaccharide. *Hum Reprod* 20, 2601–2607.

Eley A, Pacey AA, Galdiero M, Galdiero M & Galdiero F. (2005b) Can *Chlamydia trachomatis* directly damage your sperm? *Lancet Infect Dis* 5, 53–57.

Erbengi T. (1993) Ultrastructural observations on the entry of *Chlamydia trachomatis* into human spermatozoa. *Hum Reprod* 8, 416–421.

Fujisawa M, Nakano Y, Matrui T, Okada H, Arakawa S & Kamidono S. (1999) *Chlamydia trachomatis* detected by ligase chain reaction in the semen of asymptomatic patients without pyospermia or pyuria. *Arch Androl* 42, 41–44.

Gallegos G, Ramos B, Santiso R, Goyanes V, Gosalvez J & Fernandez JL. (2008) Sperm DNA fragmentation in infertile men with genitourinary infection by *Chlamydia trachomatis* and *Mycoplasma*. *Fertil Steril* 90, 328–334.

Gallegos-Avila G, Ortega-Martinez M, Ramos-Gonzalez B, Tijerina-Menchaca R, Ancer-Rodriguez J & Jaramilo-Rangel G. (2009) Ultrastructural findings in semen samples of infertile men infected with *Chlamydia trachomatis* and mycoplasmas. *Fertil Steril* 91, 915–919.

Gaydos CA, Ferrero DV & Papp J. (2008) Laboratory aspects of screening men for *Chlamydia trachomatis* in the new millennium. *Sex Transm Dis* 35, S45–S50.

Gdoura R, Kchaou W, Ammar-Kerkes L, Chakroun N, Sellemi A, Znazen A, Rebai T & Hammami A. (2008) Assessment of *Chlamydia trachomatis, Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* in semen and first void urine specimens of asymptomatic male partners of infertile couples. *J Androl* 29, 198–206.

Gijsen AP, Land JA, Goossens VJ, Leffers P, Bruggeman CA & Evers JL. (2001) *Chlamydia pneumoniae* and screening for tubal factor subfertility. *Hum Reprod* 16, 487–491.

Golden MR, Schillinger JA, Markowitz L & St Louis ME. (2000) Duration of untreated genital infections with *Chlamydia trachomatis*: a review of the literature. *Sex Transm Dis* 27, 329–337.

Gregoriou O, Vitoratos N, Papadias C, Gregoriou G & Zourias PA. (1989) The role of chlamydial serology in fertile and subfertile men. *Eur J Obstet Gynaecol* 30, 53–50.

Habermann B & Krause W. (1999) Altered sperm function or sperm antibodies are not associated with chlamydial antibodies in infertile men with leukocytospermia. *J Eur Acad Dermatol Venereol* 12, 25–29.

Hamdad F & Orfila J. (2005) Diagnosis of urogenital infection by *Chlamydia trachomatis.* Contribution of genetic amplification techniques. *Prog Urol* 15, 598–601.

Hamdad-Daoudi F, Petit J & Eb J. (2004) Assessment of *Chlamydia trachomatis* infection in asymptomatic male partners of infertile couples. *J Med Microbiol* 53, 985–990.

Hosseinzadeh S, Brewis IA, Pacey AA, Moore HDM & Eley A. (2000) Co-incubation of human spermatozoa with *Chlamydia trachomatis* in vitro causes increased tyrosine phosphorylation of sperm proteins. *Infect Immun* 68, 4872–4876.

Hosseinzadeh S, Brewis IA, Eley A & Pacey AA. (2001) Co-incubation of human spermatozoa with *Chlamydia trachomatis* serovar E causes premature sperm death. *Hum Reprod* 16, 293–299.

Hosseinzadeh S, Eley A & Pacey AA. (2004) Semen quality of men with asymptomatic chlamydial infection. *J Androl* 25, 104–110.

ICSI. (2004) *The Diagnosis and Management of Infertility. The Institute for Clinical System Improvement*. Available at: http://www.icsi.org/infertility/diagnosis_and_managementofinfertility_2301.html; accessed 3 March 2010.

Idahl A, Boman J, Kumlin U & Olofsson JI. (2004) Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. *Hum Reprod* 19, 1121–1126.

Idahl A, Abramsson L, Kumlin U, Liljeqvist JA & Olofsson JI. (2007) Male serum *Chlamydia trachomatis* IgA and IgG, but not heat shock protein 60 IgG, correlates with negatively affected semen characteristics and lower pregnancy rates in the infertile couple. *Int J Androl* 30, 99–107.

Ivanov IB, Kuzmin MD & Gritsenko VA. (2009) Microflora of the seminal fluid of healthy men and men suffering from chronic prostatitis syndrome. *Int J Androl* 32, 462–467.

Johnson AM & Horner P. (2008) A new role for *Chlamydia trachomatis* serology. *Sex Transm Infect* 84, 79–80.

Joki-Korpela P, Sahrakerpi N, Halttunen M, Surcel H-M, Paavonen J & Tiitinen A. (2009) The role of *Chlamydia trachomatis* infection in male infertility. *Fertil Steril* 91, 1448–1450.

Jungwirth A, Straberger A, Esterbauer B, Fink K & Schmeller N. (2003) Acrosome reaction in *Chlamydia*-positive and negative patients. *Andrologia* 35, 314–316.

Kokab A, Akhondi MM, Sadeghi MR, Modarresi MH, Aarabi M, Jennings R, Pacey AA & Eley A. (2010) Raised inflammatory markers in semen from men with asymptomatic chlamydial infection. *J Androl* 31, 114–120.

Levy R, Layani-Milon M-P, Giscard D'Estaing S, Najioullah F, Lornage J, Aymard M & Lina B. (1999) Screening for *Chlamydia trachomatis* and *Ureaplasma urealyticum* infection in semen from asymptomatic male partners of infertile couples prior to in vitro fertilization. *Int J Androl* 22, 113–118.

Liu DB & Zhu PY. (2003) Detection of IgG and IgM antibodies against *Chlamydia trachomatis* in semen of asymptomatic infertile patients (English abstract, journal article). *Zhonghua Nan Ke Xue* 9, 197–199.

Malik A, Jain S, Rizvi M, Shukla I & Hakim S. (2009) *Chlamydia trachomatis* infection in women with secondary infertility. *Fertil Steril* 91, 91–95.

Mania-Pramanik J, Gokral J & Meherji PK. (2001) *Chlamydia trachomatis* infection among asymptomatic males in an infertility clinic. *Indian J Dermatol Venereol Leprol* 67, 242–245.

Mardh P-A, Colleen S & Sylan J. (1980) Inhibitory effect on the formation of chlamydial inclusions in McCoy cells by seminal fluid and some of its components. *Investig Urol* 17, 510–513.

Mazzoli S, Cai T, Addonisio P, Bechi A, Mondaini N & Bartoletti R. (2010) *Chlamydia trachomatis* infection is related to poor semen quality in young prostatitis patients. *Eur Urol* 57, 708–714.

McAdam A. (2000) Discrepant analysis: how can we test a test? *J Clin Microbiol* 38, 2027–2029.

Michel C-EC, Sonnex C, Carne CA, White JA, Magbanua JPV, Nadala ECB & Lee HH. (2007) *Chlamydia trachomatis* load at matched anatomic sites: implications for screening strategies. *J Clin Microbiol* 45, 1395–1402.

Nagel TC, Tagatz GE & Campbell BF. (1986) Transmission of *Chlamydia trachomatis* by artificial insemination. *Fertil Steril* 46, 959–960.

Nagy B, Corradi G, Vajda Z, Gimers R & Romor S. (1989) The occurrence of *Chlamydia trachomatis* in the semen of men participating in an IVF programme. *Hum Reprod* 4, 54–56.

NICE. (2004) *Fertility: Assessment and Treatment for People with Fertility Problems*. National Institute for Health and Clinical Excellence, UK. Available at: http://www.nice.org.uk/nicemedia/pdf/cg011fullguideline.pdf; accessed 3 March 2010.

Ochsendorf FR. (2008) Sexually transmitted infections: impact on male fertility. *Andrologia* 40, 72–75.

Ochsendorf FR, Ozdemir K, Rabenau H, Fenner T, Oremek R, Milbradt R & Doerr HW. (1999) *Chlamydia trachomatis* and male infertility: *Chlamydia*-IgA antibodies in seminal plasma are *C. trachomatis* specific and associated with an inflammatory response. *J Eur Acad Dermatol Venereol* 12, 143–152.

Okonofua F, Menakaya U, Onemu SO, Omo-Aghoja LO & Bergstrom S. (2005) A case-control study of risk factors for male infertility in Nigeria. *Asian J Androl* 7, 351–361.

Ostergaard L & Moller JK. (1995) Use of PCR and direct immunofluorescence microscopy for confirmation of results obtained by Syva MicroTrak *Chlamydia* enzyme immunoassay. *J Clin Microbiol* 33, 2620–2623.

Paavonen J & Eggert-Kruse W. (1999) *Chlamydia trachomatis*: impact on human reproduction. *Hum Reprod Update* 5, 433–447.

Pacey AA & Eley A. (2004) *Chlamydia* and male fertility. *Hum Fertil* 7, 271–276.

Pannekoek Y, Westernberg SM, de Vries J, Repping S, Spanjaard L, Eijk PP, van der Ende A & Dankert J. (2000) PCR assessment of *Chlamydia trachomatis* infection of semen specimens processed for artificial insemination. *J Clin Microbiol* 38, 3763–3767.

Pannekoek Y, Westenberg SM, Eijk PP, Repping S, van der Veen F, van der Ende A & Dankert J. (2003) Assessment of *Chlamydia trachomatis* infection of semen specimens by ligase chain reaction. *J Med Microbiol* 52, 777–779.

Pasternack R, Vuorinen P & Miettinen A. (1997) Evaluation of the Gen-Probe *Chlamydia trachomatis* transcription-mediated amplification assay with urine specimens from women. *J Clin Microbiol* 35, 676–678.

Peeling R & Embree J. (2005) Screening for sexually transmitted infection pathogens in semen samples. *Can J Infect Dis Med Microbiol* 16, 73–76.

Penna Videau S, Cermeno Vivas J & Salazar N. (2001) IgA antibodies to *Chlamydia trachomatis* and seminal parameters in asymptomatic infertile males. *Arch Androl* 46, 189–195.

Radouani F, Bennani A, Takourt B, Had N, Ibrahimi S, Boutaleb Y, Guinet R & Benslimane A. (1996) Chlamydial infections and male infertility in Morocco. *Contracept Fertil Sex* 24, 779–783.

Rosemond A, Lanotte P, Watt S, Sauget AS, Guerif F, Royere D, Goudeau A & Mereghetti L. (2006) Systematic screening tests for *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* in urogenital specimens of infertile couples. *Pathol Biol* 54, 125–129.

Royal College of Obstetricians and Gynaecologists Evidence-based Clinical Guidelines. (1999) Guideline Summary No. 2: the initial investigation and management of the infertile couple. *Br J Urol Int* 83, 636–645.

Satta A, Stivala A, Garozzo A, Morello A, Perdichizzi A, Vicari E, Salmeri M & Calogero AE. (2006) Experimental *Chlamydia*

trachomatis infection causes apoptosis in human sperm. *Hum Reprod* 21, 134–137.

Skidmore S, Horner P & Mallinson H. (2006) Testing specimens for *Chlamydia trachomatis*. *Sex Transm Infect* 82, 272–275.

Soffer Y, Ron-El R, Golan A, Herman A, Caspi E & Samra Z. (1990) Male genital mycoplasmas and *Chlamydia trachomatis* culture: its relationship with accessory gland function, sperm quality and autoimmunity. *Fertil Steril* 53, 331–336.

Sowerby E & Parsons J. (2004) Prevention of iatrogenic pelvic infections during in vitro fertilization – current practice in the UK. *Hum Fertil* 7, 135–140.

Spears PA, Linn CP, Woodard DL & Walker GT. (1997) Simultaneous strand displacement amplification and fluorescence polarization detection of *Chlamydia trachomatis* DNA. *Ann Biochem* 247, 130–137.

Taylor-Robinson D. (1997) Evaluation and comparison of tests to diagnose *Chlamydia trachomatis* genital infections. *Hum Reprod* 12(Suppl. 11), 113–120.

Taylor-Robinson D & Thomas BJ. (1991) Laboratory techniques for the diagnosis of chlamydial infections. *Genitourin Med* 67, 256–266.

The Male Infertility Best Practice Policy Committee of the American Urological Association and the Practice Committee of the American Society for Reproductive Medicine. (2006) Report on optimal evaluation of the infertile male. *Fertil Steril* 86, S202–S209.

Thomas BJ, MacLeod EJ & Taylor-Robinson D. (1993) Evaluation of sensitivity of 10 diagnostic assays for *Chlamydia trachomatis* by use of a simple laboratory procedure. *J Clin Pathol* 46, 912–914.

Tjiam KH, van Heijst BYM, Polak-Vogelzang AA, Rothbarth PH, van Joost T, Stolz E & Michel MF. (1987) Sexually communicable micro-organisms in human semen samples to be used for artificial insemination by donor. *Genitourin Med* 63, 116–118.

Toth M, Patton DL, Campbell LA, Carretta EI, Mouradian J, Toth A, Shevchuk M, Baergen R & Ledger W. (2000) Detection of chlamydial antigenic material in ovarian, prostatic, ectopic pregnancy and semen samples of culture-negative subjects. *Am J Reprod Immunol* 43, 218–222.

Toye B, Woods W, Bobrowska M & Ramotar K. (1998) Inhibition of PCR in genital and urine specimens submitted for *Chlamydia trachomatis* testing. *J Clin Microbiol* 36, 2356–2358.

Van den Brule AJC, Hemrika DJ, Walboomers JMM, Raaphorst P, Van Amstel N, Bleker OP & Meijer CJLM. (1993) Detection of *Chlamydia trachomatis* in semen of artificial insemination donors by the polymerase chain reaction. *Fertil Steril* 59, 1098–1104.

Veznik Z, Pospisil L, Svecova D, Zajicova A & Unzeitig V. (2004) Chlamydiae in the ejaculate: their influence on the quality and morphology of sperm. *Acta Obstet Gynaecol Scand* 83, 656–660.

Vigil P, Morales P, Tapia A, Riquelme R & Salgado AM. (2002) *Chlamydia trachomatis* infection in male partners of infertile couples: incidence and sperm function. *Andrologia* 34, 155–161.

Villegas H, Pinon M, Shor V & Karchmer S. (1991) Electron microscopy of *Chlamydia trachomatis* infection of the male genital tract. *Arch Androl* 27, 117–126.

Watson EJ, Templeton A, Russell I, Paavonen J, Mardh P-A, Stary A & Pedersen BS. (2002) The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol* 51, 1021–1031.

Weidner W, Floren E, Zimmermann O, Thiele D & Ludwig M. (1996) Chlamydial antibodies in semen: search for ''silent'' chlamydial infections in asymptomatic andrological patients. *Infection* 24, 309–313.

Wisniewski CA, White JA, Michel C-EC, Mahilum-Tapay L, Magbanua JPV, Nadala ECB, Barber PJ, Goh BT & Lee HH. (2008) Optimal method of collection of first-void urine for diagnosis of *Chlamydia trachomatis* infections in men. *J Clin Microbiol* 46, 1466–1469.

Witkin SS. (2002) Immunological aspects of genital *Chlamydia* infections. *Best Pract Res: Clin Obstet Gynaecol* 16, 865–874.

Witkin SS, Jeremias J, Grifo JA & Ledger WJ. (1993) Detection of *Chlamydia trachomatis* in semen by the polymerase chain reaction in male members of infertile couples. *Am J Obstet Gynecol* 168, 1457–1462.

Witkin SS, Kligman I & Bongiovanni AM. (1995) Relationship between an asymptomatic male genital tract exposure to *Chlamydia trachomatis* and an autoimmune response to spermatozoa. *Hum Reprod* 10, 2952–2955.

Wolff H, Neubert U, Zebhauser M, Bezold G, Korting HC & Meurer M. (1991) *Chlamydia trachomatis* induces an inflammatory response in the male genital tract and is associated with altered semen quality. *Fertil Steril* 55, 1017–1019.

Wolff H, Neubert U, Volkenandt M, Zochling N, Schlupin E-M, Bezold G & Meurer M. (1994) Detection of *Chlamydia trachomatis* in semen by antibody-enzyme immunoassay compared with polymerase reaction, antigen-enzyme immunoassay, and urethral cell culture. *Fertil Steril* 62, 1250–1254.

Wolner-Hanssen P & Mardh PA. (1984) *In vitro* tests of the adherence of *Chlamydia trachomatis* to human spermatozoa. *Fertil Steril* 42, 102–107.

World Health Organisation. (1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge University Press, Cambridge, UK.

World Health Organisation. (2001) Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections Overviews and Estimates. World Health Organisation, Geneva.

Yoshida K, Kobayashi N & Negishi T. (1994) *Chlamydia trachomatis* infection in the semen of asymptomatic infertile men: detection of the antigen by in situ hybridization. *Urol Int* 53, 217–221.