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ORIGINAL ARTICLE

Male Infertility

Chlamydia trachomatis infection in the genital tract is associated with inflammation and hypospermia in the infertile male of China

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Chlamydia trachomatis **(CT) infection is the most prevalent sexually transmitted bacterial disease worldwide. However, unlike that in female infertility, the role of CT infection in male infertility remains controversial. The objective of this retrospective study was to explore the impacts of CT infection in the genital tract on sperm quality, sperm acrosin activity, antisperm antibody levels, and inflammation in a large cohort of infertile males in China. A total of 7154 semen samples were collected from infertile male subjects, 416 of whom were CT positive (CT+ group) and 6738 of whom were CT negative (CT− group), in our hospital between January 2016 and December 2018. Routine semen parameters (semen volume, pH, sperm concentration, viability, motility, morphology, *etc.*), granulocyte elastase levels, antisperm antibody levels, and sperm acrosin activity were compared between the CT+ and CT− groups. Our results showed that CT infection was significantly correlated with an abnormally low semen volume, as well as an increased white blood cell count and granulocyte elastase level (all $P < 0.05$) in the semen of infertile males; other routine semen parameters were not negatively impacted. The antisperm antibody level and sperm acrosin activity were not affected by CT infection. These findings suggested that CT infection might contribute to inflammation and hypospermia but does not impair sperm viability, motility morphology, and acrosin activity or generate antisperm antibodies in the infertile males of China.**

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INTRODUCTION

Worldwide, at least 30 million men suffer from infertility, and 15% of couples are affected.[1] The causes for male infertility vary, and genital tract infection is one of the major concerns.[2,3] *Chlamydia trachomatis* (CT) is the most common pathogen responsible for bacterial sexually transmitted infections.[4] However, unlike that in women, the role of CT infection in male infertility remains controversial. Some studies have claimed that CT infection contributes to male infertility by impairing sperm quality[5,6] or reducing acrosome reactivity;[7] however, some other studies have reported that CT infection does not directly affect sperm quality but induces an inflammatory response, leading to obstruction of the ejaculatory duct[8] or generation of antisperm antibodies.[9] Moreover, some other studies have found that it has no negative impact on semen quality and male fertility.[10,11]

In this study, we explored the impacts of CT infection in the genital tract on sperm quality, sperm acrosin activity, antisperm antibody levels, and inflammation in a large cohort of 7154 infertile males in China. Our analysis results support that CT infection is significantly correlated with an abnormally low semen volume, as well as increased white blood cell and seminal plasma granulocyte elastase levels, but it is not associated with the deterioration of sperm quality, reduction in sperm acrosin activity, or induction of antisperm antibodies. Therefore, our study provides new insights into the role of CT infection in male infertility.

PARTICIPANTS AND METHODS

Study population

This study was approved by the Ethics Review Board of The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China (No. 2018-142). From January 2016 to December 2018, semen samples were collected from male patients seeking infertility treatment in the Reproductive Center of The Third Affiliated Hospital of Guangzhou Medical University. Male infertility was diagnosed as the inability of a woman to conceive after regular sexual activity with no contraception for more than 1 year due to factors associated with her partner.[12] In 30%–45% of male infertility cases, the patients did not have abnormal semen parameters, which were defined as idiopathic male infertility.[13] Female partners were evaluated for infertility, and

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this study excluded couples who could not conceive due to female factors.

All the patients were strictly abstinent for 2 days to 7 days, and all the semen from one ejaculation was collected. The exclusion criteria were as follows: (1) foreigners and men not from mainland China; (2) those with missing information; (3) those whose semen samples were collected in an incorrect container, partially lost, or sent for examination after more than 30 min; or (4) those who were on antibacterial treatment. Eventually, a total of 7154 infertile male semen samples were selected for analysis.

Sample collection and analysis

After abstinence for 2 days to 7 days, a semen sample was collected by masturbation in a sterile wide-mouth semen collection cup, sent to the outpatient semen examination room within 30 min, and immediately placed in a 37°C incubator until it was completely liquefied. According to the World Health Organization (WHO) Laboratory Manual for the Examination and Processing of Human Semen (5th edition),[14] manual and computer-aided sperm analysis (CASA; HTCasa II 1.10.3, Hamilton Thorne, Beverly, MA, USA) techniques were used to analyze semen volume, sperm concentration, sperm motility, and other parameters. Eosin staining and Papanicolaou staining were performed to assess sperm viability and sperm morphology, respectively.

Seminal plasma granulocyte elastase level

Polymorphonuclear granulocyte (PMN) elastase levels in the seminal plasma were detected with a Seminal Plasma PMN-Elastase Quantitative Assay Kit (ELISA; Huakang Biomedical Engineering Co., Ltd., Shenzhen, China), according to the manufacturer's instructions. In brief, after semen liquefaction, 10 μl of each sample was diluted to 1 ml. Serial dilutions of standards were prepared (3000 ng ml^{-1}, 1500 ng ml^{-1}, 750 ng ml^{-1}, 375 ng ml^{-1}, and 0 ng ml^{-1}). Standards and diluted samples were added to the ELISA plate at 100 μl per well. Then, the plate was incubated on a shaker (20–25*g*) for 60 min at room temperature. After incubation, the wells were washed for 5 times, and 100 μl of horseradish peroxidase (HRP)-conjugated substrates was added to each well. Then, the plate was incubated on a shaker (20–25*g*) for 45 min at room temperature. After incubation, the wells were washed for 5 times, and 100 μl of color development solution was added to each well. Then, the plate was incubated in the dark for 20 min at room temperature. After incubation, 100 μl of termination solution was added to each well and mixed thoroughly. Finally, the light absorbance of each well was measured at wavelengths of 450/630 nm. The standard curve was fitted by the 4-parameter logistic method, and the concentrations of PMN elastase in each sample were calculated. The WHO reference values[14] were as follows, normal: <290 ng ml^{-1}; latent infection: 290–1000 ng ml^{-1}; and confirmed infection: >1000 ng ml^{-1}.

Sperm acrosin activity

Sperm acrosin activity was determined using a Human Spermatozoa Acrosin Activity Quantitative Assay Kit (modified Kennedy method; Huakang Biomedical Engineering Co., Ltd.). In brief, after fresh semen was liquefied, the sperm concentration was counted. A total of 7.5×10^6 sperm were added to each tube and centrifuged at 2000*g* for 20 min (Centrifuge 5418, Eppendorf, Hamburg, Germany). The seminal plasma was removed. One hundred μl of inhibitor was added to each tube and mixed well. Then, 1000 μl of reaction solution was added to each tube and incubated at 24°C for 1 h. After incubation, 100 μl of termination solution was added to each tube and centrifuged at 2000*g* for 15 min. Finally, the light absorbance of all tubes was measured at a wavelength of 410 nm. At 24°C, hydrolysis of 1.0 μmol substrate per min was defined as 1 IU of acrosin activity. The reference range was 48.2–18.7 μIU per 10^6 spermatozoa.[15]

Mixed antiglobulin reaction (MAR) test

The antisperm antibody level was examined by the MAR test using a Spermatozoa IgG Antibody-Coating Assay Kit (Huakang Biomedical Engineering Co., Ltd.), according to the manufacturer's instructions. In brief, 5 μl of liquefied semen and 5 μl of antihuman IgG beads were added to the slide and mixed well. Then, 5 μl of antiserum was added and mixed well. The slides were covered with glass coverslips and incubated at room temperature for 3 min. After incubation, the slides were observed under a phase-contrast optical microscope (Axio Lab.A1, Carl Zeiss, Oberkochen, Germany), and at least 200 active spermatozoa per slide were counted. Finally, the percentage of motile sperm attached to beads was calculated (ignoring tail-tip binding). The reference values were as follows: negative (<10%), weakly positive (10%–50%), and positive (≥50%).

CT detection

A CT real-time quantitative polymerase chain reaction (qPCR) Nucleic Acid Detection Kit (Zhijiang Biotechnology Co., Ltd., Shanghai, China) was used to detect the presence of CT in the samples according to the manufacturer's instructions. In brief, nucleic acids were extracted from the samples. Fifty microliters of a positive control substance and 50 μl of a negative control substance (H_2O) were prepared. Fifty microliters of nucleic acid extract was mixed with boiling water for 10 min and then centrifuged at 18 000*g* for 5 min. Four microliters of each sample or the control was used as the qPCR template. qPCR was performed with an ABI Prism® 7000 (Thermo Fisher Scientific, Waltham, MA, USA) using the following program: 37°C for 2 min, 94°C for 2 min, 93°C for 15 s, and 60°C for 60 s for 40 cycles. Fluorescence intensity was detected in the FAM channel. A SLAN fluorescence quantitative detection system (Shanghai Hongshi Medical Technology Co., Ltd., Shanghai, China) was used to judge the results: (i) if the CT value of the sample was lower than the detection threshold, the sample was judged as CT−; (ii) if the CT value was ≤38 and the amplification curve was a typical "S" type curve, the sample was judged as CT positive (CT+); and (iii) if the CT value was between 38 and 40, repeated measurements were performed. After repeated measurements were taken, if the CT value was still between 38 and 40 and the amplification curve showed a typical "S" type curve, the sample was judged as CT+. However, if the amplification curve was not a typical "S" type curve, the sample was judged as CT negative (CT−), as shown in **Supplementary Figure 1**.

Statistical analyses

Statistical Product and Service Solutions (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA) was used to record all data. The results are presented as mean ± standard deviation (s.d.). Comparisons between variables (semen parameters) were performed using the Mann–Whitney U test because the data distribution according to the Kolmogorov–Smirnov test was not normal. Categorical variables were evaluated using the Chi-square and Fisher's exact test. Statistical significance was set as $P < 0.05$.

RESULTS

Associations between CT infection and routine semen parameters

Semen analysis was performed in the abovementioned population from 2016 to 2018 according to the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition).[14] Based on the CT detection results, 416 subjects, with a mean age of 33.5 (s.d.: 5.8) years, were included in the CT+ group, and 6738 subjects, with a mean age of 34.5 (s.d.: 6.0) years, were included in the CT− group.

Supplementary Table 1 shows the WHO lower reference limits for routine semen parameters in fertile males; these limits were applied to compare the proportions of males with various routine semen parameters in each subgroup between the CT+ and CT− groups. Our results showed that the proportion of males with a normal semen volume (≥1.5 ml) in the CT+ group (87.5%) was significantly lower (P = 0.005) than that in the CT− group (91.5%). The proportion of males with normal sperm viability (≥58%) in the CT+ group (94.0%) was significantly higher (P = 0.002) than that in the CT− group (90.3%). The proportion of males with normal progressive sperm motility (PR; ≥32%) in the CT+ group (83.7%) was significantly higher (P = 0.011) than that in the CT− group (78.4%). The proportion of males with normal total sperm motility (PR + nonprogressive motility [NP] ≥40%) in the CT+ group (79.1%) was significantly higher (P = 0.037) than that in the CT− group (74.5%). The proportion of males with a normal white blood cell count (<1 × 10^6 ml^{-1}) in the CT+ group (88.2%) was significantly lower (P < 0.001) than that in the CT− group (97.5%). Other parameters, including sperm concentration and normal forms, were not significantly different (P > 0.05) between the CT+ and CT− groups (**Table 1**). Then, our results showed comparisons of sperm motility parameters between the CT+ and CT− groups. There were no significant differences (P > 0.05) in sperm motility parameters between the two groups (**Table 2**).

In the comparisons of sperm morphological parameters between the CT+ and CT− groups, our results exhibited that the sperm midpiece deformity rate (M%; 15.2% ± 5.2%) in the CT+ group was significantly lower (P = 0.026) than that in the CT− group (15.8% ± 5.3%). There were no significant differences (P > 0.05) in the other morphological parameters between the two groups (**Table 3**).

In summary, our findings suggest that CT infection is significantly associated with an abnormally low semen volume and an increased white blood cell count in infertile males, but it does not negatively impact sperm viability, motility, or morphology. The lack of differences in viability, motility, and morphology is not attributable to similar prevalence rates of other causes of infertility (**Supplementary Table 2**).

CT infection induced increased PMN elastase levels in the seminal plasma

The seminal plasma PMN elastase level indicates the presence of bacterial infection. The assay results were classified into 3 subgroups: normal, latent infection, and confirmed infection groups. About the Chi-square test results of the proportion of males in each subgroup with abnormal PMN elastase levels between the CT+ and CT− groups, our results exhibited that the latent infection rate (32.0%) and the confirmed infection rate (38.0%) in the CT+ group were significantly higher (P < 0.001) than those in the CT− group (24.8% and 22.1%, respectively) as shown in **Table 4**. The results indicated that CT infection induced an increase in the level of PMN elastase in the seminal plasma, which is consistent with the abovementioned observation of increased white blood cell counts and indicates the initiation of inflammatory responses.

CT infection did not affect antisperm antibody levels

Antisperm antibody levels were determined by the mixed antiglobulin reaction (MAR) test, and the test results were classified as negative, weakly positive, or positive. Our results showed that there were no significant differences in antisperm antibody levels among each MAR result subgroup (P = 0.071) between the CT+ and CT− groups, indicating that CT infection had no effect on the antisperm antibody level (**Table 5**).

CT infection did not affect acrosin activity

The Chi-square test results of the proportions of normal and abnormal acrosin activity between the CT+ and CT− groups showed that there

Table 1: Chi-square test of semen routine parameters between the CT+ and CT− groups

Parameter	*CT+ (total=416), n (%)*	*CT− (total=6738), n (%)*	χ^2	*P*
Semen volume (ml)			7.708	0.005
<1.5	52 (12.5)	575 (8.5)		
≥1.5	364 (87.5)	6163 (91.5)		
pH			3.653	0.056
<7.2	23 (5.5)	549 (8.2)		
≥7.2	393 (94.5)	6189 (91.9)		
Sperm concentration (×10^6 ml^{-1})			0.154	0.695
<15	38 (9.1)	655 (9.7)		
≥15	378 (90.9)	6083 (90.3)		
Sperm vitality (%)			6.358	0.012
<58	25 (6.0)	657 (9.8)		
≥58	391 (94.0)	6081 (90.3)		
Normal forms (%)			2.404	0.121
>4	195 (46.9)	2897 (43.0)		
≤4	221 (53.1)	3841 (57.0)		
PR (%)			6.506	0.011
<32	68 (16.4)	1457 (21.6)		
≥32	348 (83.7)	5281 (78.4)		
PR + NP (%)			4.364	0.037
<40	87 (20.9)	1718 (25.5)		
≥40	329 (79.1)	5020 (74.5)		
White blood cells (×10^6 ml^{-1})			114.856	<0.001
≤1	367 (88.2)	6570 (97.5)		
>1	49 (11.8)	168 (2.5)		

PR: progressive motility; NP: nonprogressive motility; PR+NP: total motility; CT+: *Chlamydia trachomatis* positive; CT−: *Chlamydia trachomatis* negative

was no significant difference in the detection of acrosin activity between the two groups (P = 0.441) in the CT+ and CT– groups, indicating that CT infection exerted no effect on sperm acrosin activity (**Table 6**).

DISCUSSION

Male genital tract infections occur in approximately 15% of males with infertility. Because infections affect different parts of the male genital tract, from the testis to the urethra, sperm may be affected to different degrees during development, maturation, and transport.[16] CT infection in the genital tract is the most common bacterial sexually transmitted infection.[17] A large number of previous studies have shown that CT infection impacts female fertility. For example, studies conducted by Rantsi *et al.*[18,19] showed that CT+ women had a longer natural pregnancy time than CT– women, and persistent CT infection led to tubal infertility in women. Tang *et al.*[20] performed a meta-analysis of the association between pregnancy and CT infection with data derived from 3 public databases and found that CT infection was associated with an increased risk of adverse outcomes associated with multiple pregnancies and fertility. Therefore, it is certain that CT infection affects female fertility.[11] However, whether CT infection affects male fertility remains controversial. These controversial results are reflected in epidemiological studies and *in vitro* and *in vivo* experiments.

Some epidemiological studies have shown that CT infection is significantly correlated with azoospermia and oligozoospermia,[21,22] while other reports have shown that CT infection is not associated with decreased semen quality or male infertility.[23,24]

In vitro experiments in different studies evaluating the effects of CT infection on sperm functions have produced conflicting results. Eley *et al.*[25] incubated highly active semen with normal spermatozoa with CT or CT lipopolysaccharide and found that the presence of CT induced premature spermatozoa death. Satta *et al.*[26] showed that CT infection caused phosphatidylserine externalization and DNA fragmentation in spermatozoa. Wolner-Hanssen and Mardh showed that CT adhered to spermatozoa as they developed.[27] In contrast, Puerta Suarez *et al.*[11] showed that CT did not adhere to spermatozoa *in vitro*. The contrasting results of these *in vitro* experiments might be due to technology accuracy.[28] Another limitation of these *in vitro* experiments is that the impacts of CT infection on sperm at earlier developmental stages remain uninvestigated. Induced pluripotent stem cells (iPSCs) have the potential to differentiate into various cell types in 3 germ layers;[29,30] therefore, it would be of interest to employ iPSCs to model the spermatogenesis process *in vitro* and examine the impacts of CT infection.

In vivo studies on CT infection in men also produced contradictory results. Some studies showed that CT infection led to high sperm DNA fragmentation[31,32] and an impaired acrosome reaction,[7] which might negatively impact male fertility. In contrast, some other studies found that sperm DNA fragmentation and an impaired acrosome reaction were not related to CT infection.[6,9,33] In our analysis, there was no significant difference in acrosin activity between the CT+ group and the CT– group (**Table 6**). Therefore, our results support that CT infection does not affect sperm acrosin activity. Some researchers have hypothesized that although CT infection in the male genital tract does not greatly impair the quality of semen, it might serve as a reservoir for infection and increase the risk of infection in women, which in turn leads to infertility.[24,34,35]

Some studies have indicated that genital tract inflammation could contribute to male infertility.[36] The seminal plasma PMN elastase level is a very sensitive indicator of inflammation.[37] Our results showed that the proportions of males with an abnormally high white blood

Table 2: Comparison of sperm motility parameters between the CT+ and CT– groups

Parameter	*CT+ (n=416)*	*CT– (n=6738)*	*P*
VAP (μm s^{-1}), mean±s.d.	54.74±11.47	55.16±11.70	0.321
VSL (μm s^{-1}), mean±s.d.	43.88±11.35	44.42±11.22	0.188
VCL (μm s^{-1}), mean±s.d.	88.60±18.55	88.87±19.77	0.984
ALH (μm s^{-1}), mean±s.d.	4.31±1.02	4.29±1.10	0.750
BCF (Hz), mean±s.d.	28.33±4.61	28.55±4.98	0.240
STR (VSL/VAP), mean±s.d.	77.29±6.92	77.48±6.97	0.588
LIN (VSL/VCL), mean±s.d.	49.71±8.13	50.10±8.00	0.242

VCL: curvilinear velocity; VSL: straight line velocity; VAP: average pathway velocity; ALH: amplitude of lateral head displacement; BCF: beat cross frequency; STR: straightness; LIN, linearity; s.d.: standard deviation; CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative

Table 3: Comparison of sperm morphology parameters between the CT+ and CT– groups

Parameter	*CT+ (n=416)*	*CT– (n=6738)*	*P*
SDI, mean±s.d.	1.23±0.11	1.24±0.12	0.073
TZI, mean±s.d.	1.29±0.10	1.30±0.11	0.111
Normal forms (%), mean±s.d.	4.25±3.77	4.18±4.06	0.347
H (%), mean±s.d.	95.46±4.17	95.60±4.27	0.369
M (%), mean±s.d.	15.19±5.18	15.81±5.33	0.026
P (%), mean±s.d.	6.30±3.56	6.58±3.67	0.192
C (%), mean±s.d.	6.30±4.46	6.39±4.64	0.962
Sperm head area (μm^2), mean±s.d.	5.43±2.08	5.28±2.10	0.087
Sperm head elongation (%), mean±s.d.	66.67±6.50	67.33±6.65	0.059

SDI: sperm deformity index; TZI: teratozoospermia index; H: sperm head piece deformity; M: sperm middle piece deformity; P: sperm principal piece deformity; C: sperm cytoplasm deformity; s.d.: standard deviation; CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative

Table 4: Chi-square test of polymorphonuclear granulocyte elastase level between the CT+ and CT– groups

PMN elastase level	*CT+ (total=337), n (%)*	*CT– (total=4452), n (%)*	χ^2	*P*
Normal	101 (30.0)	2364 (53.1)	73.253	<0.001
Latent infection	108 (32.0)	1106 (24.8)		
Confirmed infection	128 (38.0)	982 (22.1)		

CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative; PMN: polymorphonuclear granulocyte

Table 5: Chi-square test of antisperm antibody level between the CT+ and CT– groups

Antisperm antibody level	*CT+ (total=386), n (%)*	*CT– (total=6385), n (%)*	χ^2	*P*
Negative	323 (83.7)	5585 (87.5)	5.228	0.071
Weak positive	62 (16.1)	776 (12.2)		
Positive	1 (0.3)	24 (0.4)		

CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative

Table 6: Chi-square test of acrosin activity between the CT+ and CT– groups

Acrosin activity	*CT+ (total=386), n (%)*	*CT– (total=6506), n (%)*	χ^2	*P*
Normal	265 (68.1)	4308 (66.2)	0.598	0.441
Abnormal	124 (31.9)	2198 (33.8)		

CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative

cell count and PMN elastase level in the CT+ group were significantly higher than those in the CT– group (**Table 1** and **4**), which confirmed that CT infection induced an inflammatory response. Another study also confirmed that CT induced inflammation, as measured by PMN elastase levels, and increased inflammation was associated with disturbed sperm DNA integrity, which might hinder successful fertilization and induction of pregnancy.[38] Hence, there are potential impacts of inflammation on male fertility and the fertilizing capacity of spermatozoa. Recently, single-cell transcriptome analysis has been developed to investigate immune responses to microbial infection;[39,40] therefore, it would be of interest to employ single-cell sequencing technologies to explore the potential mechanism of genital tract inflammation induced by CT infection.

In this study, we analyzed a large cohort of 7154 infertile male subjects in China. A major finding was that the semen volume in the CT+ group was significantly lower than that in the CT– group (**Table 1**), indicating a higher rate of hypospermia in infertile males with CT infection in China. The association between CT infection and hypospermia has also been reported in the Mexican population, but the reason is still unknown.[41] In the current study, we observed a stronger inflammatory response in the CT+ group; thus, hypospermia might be caused by inflammation-associated obstruction of the ejaculatory duct.[8] Some studies have shown that in CT+ patients, significantly decreased fructose, citrate, and α-glucosidase levels indicate dysfunction of seminal vesicles, the prostate, and the epididymis to some extent[42] and might be alternative causes of hypospermia. It would be of interest to employ animal models and genetic modification tools such as the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system[43,44] to further investigate whether there is a causal relationship between CT-induced inflammation and hypospermia, as well as the underlying molecular mechanism at the gene level.

In conclusion, our study provides new evidence of the significant association of CT infection with hypospermia and inflammation in infertile males in China. CT infection did not exert deleterious effects on sperm viability, motility, or morphology and did not affect antisperm antibody levels or sperm acrosin activity.

AUTHOR CONTRIBUTIONS

YL and DZ conceived and designed the study. HZ, XT, GZ, JY, QL, YC, XS, and YL collected the samples, performed the assays, and acquired the data. SW, XX, YL, and DZ analyzed the data. SW, GZ, YL, and DZ wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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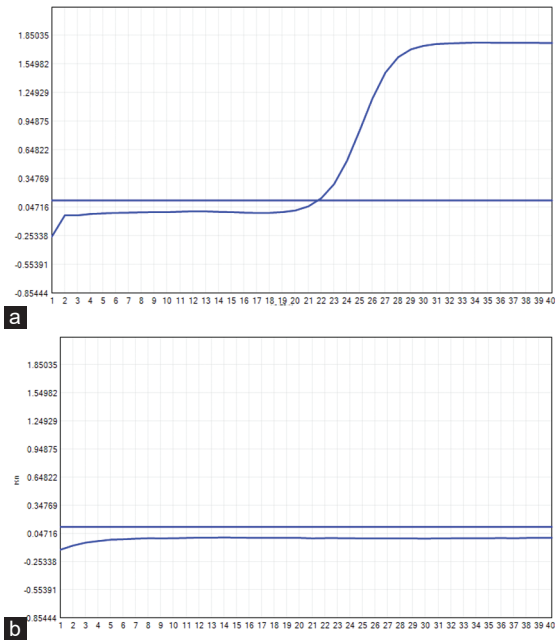

Supplementary Figure 1: Representative amplification curves of CT infection detection. (**a**) A typical S-shaped amplification curve indicating CT positive. (**b**) A flat amplification curve indicating CT negative. CT: *Chlamydia trachomatis.*

Supplementary Table 1: World Health Organization lower reference limits of semen parameters from fertile male

Parameter	*Lower reference limits (95% CI)*
Semen volume (ml)	1.5 (1.4–1.7)
pH	≥7.2
Sperm concentration ($\times 10^6$ ml^{-1})	15 (12–16)
Sperm vitality (%)	58 (55–63)
Normal forms (%)	4 (3–4)
PR (%)	32 (31–34)
PR + NP (%)	40 (38–42)

WHO: World Health Organization; CI: confidence interval; PR: progressive motility (WHO 1999, Grades a + b); NP: nonprogressive motility (WHO 1999, Grade c); PR + NP: total motility

Supplementary Table 2: Causes of infertility of the studied subjects

	CT+ (n=386), n (%)	*CT– (n=6,506), n (%)*
Mycoplasma infection	11 (2.57)	90 (1.34)
Other genital tract infections	8 (2.01)	110 (1.63)
Chronic prostatitis	10 (2.45)	171 (2.54)
Obesity	8 (2.01)	151 (2.24)
Varicocele	26 (6.32)	232 (3.44)
Hereditary factors	2 (0.57)	79 (1.17)
Oligozoospermia	109 (26.21)	1169 (17.35)
Asthenozoospermia	25 (6.03)	1161 (17.23)
Teratozoospermia	36 (8.62)	1005 (14.91)
Asthenoteratozoospermia	20 (6.9)	256 (3.80)
Oligoteratozoospermia	22 (5.17)	228 (3.39)
Oligoasthenoteratozoospermia	25 (6.03)	360 (5.34)
Others	104 (25.11)	1726 (25.62)

CT+: *Chlamydia trachomatis* positive; CT–: *Chlamydia trachomatis* negative