ISSN-0976-0245 (Print) • ISSN-0976-5506 (Electronic)

Volume 10 | Number 02 | February 2019

Indian Journal of Public Health Research & Development

An International Journal

SCOPUS IJPHRD CITATION SCORE

Indian Journal of Public Health Research and Development Scopus coverage years: from 2010 to 2017 Publisher: R.K. Sharma, Institute of Medico-Legal Publications ISSN:0976-0245E-ISSN: 0976-5506 Subject area: Medicine: Public Health, Environmental and Occupational Health

CiteScore 2015- 0.02

SJR 2015- 0.105

SNIP 2015- 0.034

Covered by Scopus ★ EMBASE ★ Approved by MCI

Website:

www.ijphrd.com

Indian Journal of Public Health Research & Development

Print-ISSN: 0976-0245-Electronic-ISSN: 0976-5506, Frequency: Monthly

Indian Journal of Public Health Research & Development is a double blind peer reviewed international journal. It deals with all aspects of Public Health including Community Medicine, Public Health, Epidemiology, Occupational Health, Environmental Hazards, Clinical Research, and Public Health Laws and covers all medical specialties concerned with research and development for the masses. The journal strongly encourages reports of research carried out within Indian continent and South East Asia.

The journal has been assigned International Standards Serial Number (ISSN) and is indexed with Index Copernicus (Poland). It is also brought to notice that the journal is being covered by many international databases. The journal is covered by EBSCO (USA), Embase, EMCare & Scopus database. The journal is now part of DST, CSIR, and UGC consortia.

Website : www.ijphrd.com

Editor

Dr. R.K. Sharma
Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)

Printed, published and owned by

Dr. R.K. Sharma
Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)

Published at

Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall,
Sector- 32, Noida - 201 301 (Uttar Pradesh)

The Role of Chlamydial Infection in Male Infertility

Jabbar S. Hassan[1], Reyam F. Salah[2], Ayad M. Gaidan[2]

[1]Ph.D in Microbiology, College of Medicine/Al-Nahrain University/ Baghdad/Iraq,
[2]Ph.D in Microbiology, College of Science/[1] Tikrit University/ Tikrit/Iraq

ABSTRACT

Background: *Chlamydia trachomatis* is an obligate intracellular bacterium most commonly associated with sexually transmitted diseases. In females, infection with this bacterium is well-documented as a cause of infertility. However, in males this effect is controversial. The aim of current study was to investigate the association of *C. trachomatis* infection in infertile male genital tract with some sperm parameters. **Method**: This is a case-control study including 100 seminal fluid specimens from infertile men and other 100 specimens from fertile men as controls. Specimens from both groups were subjected for macroscopic and microscopic examinations. Bacterial DNA was extracted from each sample and 16S ribosomal gene of *C. trachomatis* was amplified with specific primers using conventional polymerase chain reaction (PCR). **Results:** Seventeen specimens from infertile men were positive for *C. trachomatis* DNA versus one specimen from fertile men. A comparison of sperm quality between *C. trachomatis*-positive and –negative infertile men revealed a significant association between the presence of *C. trachomatis* in male genital tract and low sperms concentration and motility. **Conclusion:** This study provided further evidence indicating that the motility and morphology of sperm are significantly influenced by the presence of *C. trachomatis* in male genital tract.

Keywords: *Chlamydia trachomatis, sexually transmitted diseases, male infertility, prostatitis, sperm motility, bacterial DNA.*

INTRODUCTION

Infertility is a common reproductive disorder affecting about 15% of couples worldwide [1]. Male infertility is believed to account for at least half of all cases of infertility. Although congenital, hormonal disorders, lifestyle, environmental hazards and psychological state are well-documented causes, the etiology of about 55% of male infertility is not obvious [2]. Infectious agent, especially bacteria, have a very important role in this regard. Of the most implicated bacteria are *Neisseria gonorrheae, Treponema pallidum* and *Chlamydia trachomatis* [3].

C. trachomatis is one of the most common causes of STDs in males and females, causing different genito-urinary tract pathologies such as urethritis, prostatitis, cervicitis, epididymitis, pelvic inflammatory disease (PID), tubal factor infertility and ectopic pregnancy [4]. The primary site for infection with *C. trachomatis* in males is the penile urethra from which the bacteria can ascend to the epididymis and testis [5]. In one study, such infection was found to be the most common cause of epididymitis compared to other STDs [6]. If the epididymis is involved, it will be a matter of time that orchitis and prostatitis are followed with subsequent canilicular system damage, testicular atrophy and obstructive azoospermia [7].

In a mouse model, [8] found that urethral inoculation of fertile male mice with 10^6 *C. trachomatis* inclusion-forming units caused remarkable alterations in semen parameters, mostly included higher DNA fragmentation, increase mean percentages of necrotic spermatozoa and a reduction in the reproductive performance of these mice compared with controls. In human, many previous studies had addressed this issue; however, the results were inconsistence. So, this study was aimed to investigate the association of *C. trachomatis* with some sperm parameters and thus with male infertility.

MATERIAL AND METHOD

The Study Population

A case-control study was conducted during the period from July 2016 to February 2017 including 100 males with primary and secondary infertility who were attending Kamal AL-Samaraay Hospital/Baghdad, Iraq. Inclusion criteria were married adult males having unprotected intercourses without conception for at least 1.5 years. Exclusion criteria were known disturbance in hormonal levels, anatomical problems, such as varicocele and cryptorchidism, karyotyping abnormalities, a previous or ongoing treatment for fertility disorders and the presence of sperm defects of supposed genetic origin. Other 100 fertile males, without genito-urinary tract anatomical deformities or infection were recruited as controls. Clinical manifestations were determined by consultation of a sterility and urinary tract specialist and from verification of the information in the medical record. A written consent letter or verbal agreement was taken from all participants. The study was approved by the Institutional Review Board (IRB) at College of Medicine/Al-Nahrain University, Baghdad, Iraq..

Samples collection and Processing:

Seminal fluid specimens were collected from each subject in the laboratory by masturbation after 3-5 days of sexual abstinence. The ejaculate was deposited in a sterile wide-mouth screw-capped plastic container. The seminal fluid was examined according to World Health Organization (WHO) criteria [9]. Liquefaction time, volume, color, viscosity and pH were assessed. All samples underwent microscopic examination under a phase contrast microscope to assess the following parameters:

Spermatozoa motility which was calculated as a percentage through observing the speed at which 200 sperms move with a flagellar movement. According to the type of movement, four grades were assigned [10]:

A: rapid progressive movement,

B; Slow progressive movement,

C: Non-progressive movement and

D: no movement.

Sperm concentration was measured by haemocytometer.

The percentage and type of morphologically abnormal spermatozoa.

Identification of other cell types within the ejaculate.

Bacterial DNA Extraction and Gene Amplification

One hundred µl of seminal fluid specimen were used for bacterial DNA extraction using a ready commercial kit (DNA-sorb-B (Sacace/Italy) Kit) according to the manufacturer's protocol. A specific pair of primers was used in conventional PCR to detect the presence of 16S ribosomal gene of *C. trachomatis* [11]. The forward and reverse primers were 5'-TGG CGG CGT GGA TGA GGC AT-3' and 5'-CTC AGT CCC AGT GTT GGC GG-3', respectively, with a fragment length of 300 bp. The reaction tube (GoTaq® Green Master Mix/ Promega/USA) was set to contain 0.4µmol-l from each primer, DNA template (2ng), 4mmol MgCl2, Taq DNA polymerase (0.05µl), and dNTPs 0.4mmol each. Non-template negative control was used to validate the reaction. The tubes were placed in the thermo-cycler (Cleaver Scientific Thermal Cycler TC32/80) which was previously programmed with the following PCR conditions: 94°C for 5min followed by 40 cycles of 94°C for 20sec, 65°C for 20sec, and 72°C for 20sec, terminating in 72°C for 5min. Ten µL of PCR product were subjected to 1% agarose gel electrophoresis with ethidium bromide (0.5µg /ml; Sigma). Amplicon visualization was performed using an UV light transilluminator and then photographed using digital camera.

Statistical Analysis

Quantitative variables were expressed as mean±standard deviation (SD) and analyzed with student *t*-test while binomial variables were expressed as frequency and percentage and analyzed with Chi-squared test whenever possible. The statistical significant was set at P value ≤0.05.

RESULTS

Seminal fluid characteristics

Seminal fluid characteristics of the studied population were shown in Table (1). In all studied parameters with no exception, there were significant differences between fertile and infertile men. Interestingly, there were remarkable variations in most of these parameters in infertile men reflected by a relatively high standard deviation.

Table 1 Seminal fluids analysis between infertile and fertile men according to different parameters

Parameters	Infertile	Fertile	P-value
Age/ years (mean±SD)	28.92±5.9	27.73±3.9	0.095
Volume/ mL (mean±SD)	2.06±0.7	2.51±0.8	0.024
Motility% (mean±SD) Grade A Grade B Grade C Grade D	 1.31±4.2 5.95±8.9 18.25±12.1 50.65±27.1	 45.15±7.1 20.97±3.2 15.44±3.5 11.18±2.9	 <0.001 <0.001 0.027 <0.001
Concentration× 10^6 (mean±SD)	21.54±15.2	63.08±9.8	<0.001
Abnormality% (mean±SD)	53.70±15.3	8.94±2.3	<0.001
Pus cells, No.a/HPF (mean±SD)	1.26±2.8	0.39±0.2	0.003

SD: standard deviation, HPF: high power field

Molecular Detection of *C. trachomatis*

Gel electrophoresis of PCR product was shown in Figure (1). Out of 100 seminal fluid specimens from infertile men, 17(17%) were positive for *C. trachomatis*. On the other hand, only one specimen (1%) from control group had positive result (P<0.001).

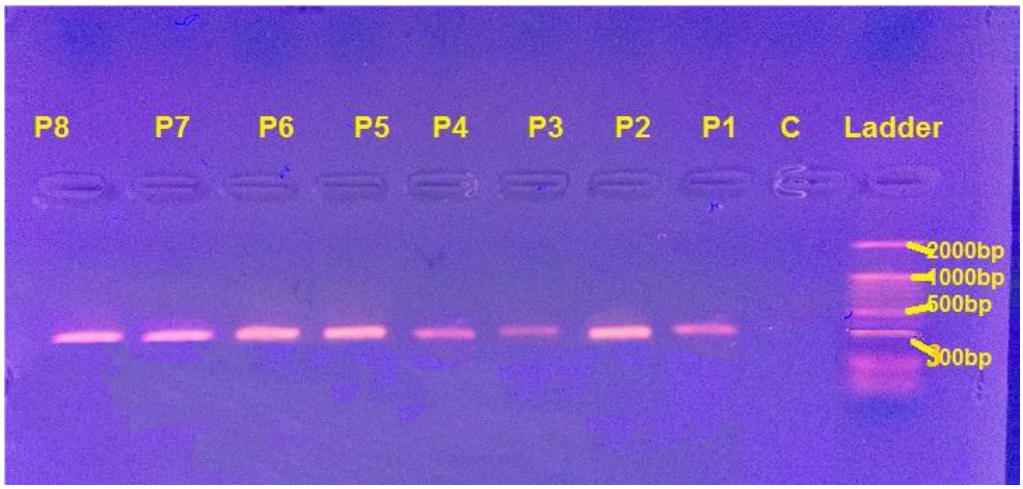

Figure 1 Gel electrophoresis of PCR products of *Chlamydia trachomatis* S ribosomal gene. P1,2,3,4,5,6,7,8: positive results with 300bp fragment length. Lane C: negative control.

Association of Chlamydial Infection with Seminal Fluid Parameters

The comparison between chlamydia-positive and –negative infertile men regarding seminal fluid characteristics was shown in Table (2). Both motility and abnormality were significantly affected by chlamydial infection. The percentage of grade C (no progressive movement of sperms) is higher in infected than non-infected men (19.79±13.1 versus 17.76±11.9, P=0.018; Table 2). In addition, the percentage of grade A (rapid progressive movement of sperms) was very low (0.79±0.62) compared with that in non-infected men (1.47±0.8) with a difference that was very close to significant (P= 0.059). The percentage of abnormal sperms was also found to be significantly different

between infected and non-infected (43.13±15.1 and 53.88±15.4, respectively, P=0.034; Table 2).

Table 2 Association of chlamydial infection with seminal fluid parameters

Variables	Infected (n=17)	Non-infected (n=83)	P-value
Volume/ mL (mean±SD)	2.17±0.9	2.02±0.6	0.375
Motility %, (mean±SD) Grade A Grade B Grade C Grade D	 0.79±0.62 5.42±3.9 19.79±13.1 51.46±26.4	 1.47±0.8 6.12±4.2 17.76±11.9 50.39±27.5	 0.059 0.038 0.018 0.868
Concentration× 10^6(mean±SD)	20.96±14	21.72±15.6	0.831
Abnormality%(mean±SD)	43.13±15.1	53.88±15.4	0.034
Pus cells, No./HPF(mean±SD)	1.34±1.2	1.24±0.9	0.880

SD: standard deviation, HPF: high power field

DISCUSSION

Current study indicated the important role of *C. trachomatis* in affecting sperms morphology and progressive motility and thus male infertility. This indicates the involvement of prostate in chlamydia infection, because recent evidence suggested that prostatitis induced by *C. trachomatis* was associated with low sperm concentration and motility [12]. These results are comparable to a recent study conducted by [13] on Italian patients with chronic prostatitis. The authors found that beside human papillomavirus (HPV), *C. trachomatis* had a key role in male infertility especially that is associated with sperms motility and morphology. Many other epidemiological studies reported that the presence of *C. trachomatis* in male genital tract was significantly associated with low sperms concentration, viability and motility, as well as with an alteration in sperms morphology [12,14]. Moreover, [15] have linked the detection of *C. trachomatis* DNA in the semen with poor sperm motility. Furthermore, in a recent Iranian study including 1080 subfertile patients, [16] showed that sperms motility, concentration, and morphology were negatively associated with *C. trachomatis* infection. However, many other studies reported non-significant association between *C. trachomatis* infection of the male genital tract and altered sperms quality [17,18].

Several mechanisms have been proposed for this association between chlamydial infection and male infertility. *C. trachomatis* could affect the sperms either directly or indirectly. Direct impacts involve a contact of the pathogen or its soluble products with the sperms while indirect effects are believed to be through induction of inflammatory reaction accompanied with release of toxic mediators such as reactive oxygen species (ROS) and cytokines [19]. Interestingly, both proposed mechanisms were subjected for intensive investigation although with conflicting results.

In an in vivo study, [20] found that there was a small but significant reduction in the ability of sperms from chlamydia-positive men to undergo the acrosome reaction (an essential part of the process of fertilization) compared with sperm from men without an infection. Another study showed that lipopolysaccharide (LPS) of the bacteria can bind to CD14 on the sperm surface and induce ROS production causing marked reduction in sperms motility and even a caspase-mediated apoptosis [21]. Supporting these findings is a recent study by [22] who demonstrated through flow cytometry analysis that there was a significant increase in the activity of caspase-3 in semen of infertile men positive for *C. trachomatis*.

On the other hand, [23] studied the incidence of *C.*

trachomatis infection among male partners of infertile couples and found that there were no differences in sperms concentration, motility and morphology between infected and non-infected men. More recently, [19] had *in vitro* incubated human sperms with *C. trachomatis* (serovar E or LGV) for 6 to 24h at 37°C, after which they analyzed several sperm quality parameters. Interestingly, they did not report any significant changes in sperms motility, viability, DNA fragmentation, ROS production, peroxidation level and mitochondrial potential compared to controls. These discrepancies in results can be attributed to several factors such as differences in experimental conditions, type and number of samples used and detection assay.

Noteworthy, detection of *C. trachomatis* in married males who failed to have conception does not only indicate the possible cause of this infertility, but also could serve as a marker for involvement of female partner in this infection. In this regard, sperms can transport *C. trachomatis* to the female genital tract [24] with induction of immune response against sperms in women [15]. Thus, even if there is no significant association of *C. trachomatis* with male infertility, there is no doubt that this pathogen can transmit to the female partner where there is a general agreement it has such notorious impact.

Despite the wide discrepancies about the possible effect of *C. trachomatis* on sperm, herein, we provide further evidence indicating that the motility and morphology of sperms are significantly affected by the presence of *C. trachomatis* in the male genital tract.

Acknowledgement: The authors highly appreciate the efforts of all staff in Al-Imamain Al-Kadhumain Medical City during samples collection.

Ethical Clearance: The study was approved by the Institutional Review Board (IRB) at College of Medicine/Al-Nahrain University, Baghdad, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare

REFERENCES

1. Mascarenhas MN, Flaxman SR, Vanderpoel S, et al. National, regional and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PloS Med 2012; (12): e1001356.
2. Samplaski MK, Domes T, Jarvi KA. Chlamydial infection and its role in male infertility. Adv Androl 2014. ID 307950.
3. Roggeri M, Cannas S, Cubeddu M, et al. Bacterial agents as a cause of infertility in humans. New Microbiologica 2016; 39(3): 2006-209.
4. Brunham RC, Rekart ML. Considerations on Chlamydia trachomatis disease expression. FEMS Immunol Med Microbiol 2009; 55(2): 162-166.
5. Trojian TH, Lishnak TS, Heiman D. Epididymitis and orchitis: an overview. Am Family Physcian 2009; 79(7): 583-587.
6. Hedger MP. Immunophysiology and pathology of inflammation in the testis and epididymis. J Androl 2011; 32: 625-640.
7. Stephens AJ, Aubuchon M, Schust DJ. Antichlamydial antibodies, human fertility, and pregnancy wastage. Infec Dis Obstet Gynecol 2011. ID 525182.
8. Sellami H, Gdoura R, Mabrouk I, et al. A proposal mouse model to study male infertility provoked by genital serovar E, Chlamydia trachomatis. J Androl 2011; 32(1): 86-94.
9. Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010; 16(3); 231-245.
10. Vasan SS. Semen analysis and sperm function tests: how much to test? Indian J Urol 2011; 27(1): 41-48.
11. Ahmadi A, Khodabandehloo M, Ramaanzadeh R, et al. The relationship between Chlamydia trachomatis genital infection and spontaneous abortion. J Reprod Infertil 2016; 17(2): 110-116.
12. Mazzoli S, Cai T, Addonisio P, Bechi A, Mondaini N, Bartoletti R. Chlamydia trachomatis infection is related to poor semen quality in young prostatitis patients. Eur. Urol. 2010; 57: 708–714
13. Cai T, Wagenlehner FM, Mondaini N, et al. Effect of human papillomavirus and Chlamydia trachomatis co-infection on sperm quality in young heterosexual men with chronic prostatitis-related symptoms. BJU Int 2014; 113(2): 281-287.
14. Pajovic B, Radojevic N, Vukovic M, Stjepcevic A. Semen analysis before and after antibiotic treatment

of asymptomatic Chlamydia- and Ureaplasma-related pyrospermia. Andrologia 2013; 45: 266-271.

15. Witkin SS, Jeremias J, Grifo JA, Ledge WJ. Detection of Chlamydia trachomatis in semen by the polymerase chain reaction in male members of infertile couples. Am J Obstet Gynecol 1993; 168(5): 1457–1462.
16. Moazenchi M, Totonchi M, Salman R, et al. The impact of Chlamydia trachomatis infection on sperm parameters and male fertility: a comprehensive study. Int J STD AIDS 2017; 0(0): 1-8.
17. Weidner W, Floren E, Zimmermann O, Thiele D, Ludwig M. Chlamydial antibodies in semen: search for "silent" chlamydial infections in asymptomatic andrological patients. Infec 1996; 24: 309-313.
18. Dehghan ML, Aflatoonian A, Talei R, Ghasemadeh J, Pacey AA. Semen inflammatory markers and Chlamydia trachomais infection in male partners of infertile couples. Andrologia 2016; 48(7): 729-736.
19. Suarez J, Sanchez LR, Salazar FC, et al. Chlamydia trachomatis neither exerts deleterious effects on spermatozoa nor impairs male fertility. Sci Rep 2017; 7: 1126-1133.
20. Jungwirth A, Straberger B, Esterbauer K, Fink K, Schmeller N. Acrosome reaction in chlamydia-positive and negative patients. Andrologia 2003; 35: 314–16.
21. Hosseinzadeh S, Pacey AA, Eley A. Chlamydia trachomatis-induced death of human spermatozoa is caused primarily by lipopolysaccharide. J Med Microbiol 2003; 52: 193–200.
22. Sellami H, Znazen A, Sellami A, et al. Molecular Detection of Chlamydia trachomatis and Other Sexually Transmitted Bacteria in Semen of Male Partners of Infertile Couples in Tunisia: The Effect on Semen Parameters and Spermatozoa Apoptosis Markers. PLoS ONE 2014; 9(7): e98903.
23. Vigil P, Morales P, Tapia A, Riquelme R, Salgado AM. Chlamydia trachomatis infection in male partners of infertile couples: incidence and sperm function. Andrologia 2002; 34: 155–61.
24. Erbengi T. Ultrastructural observations on the entry of Chlamydia trachomatis into human spermatozoa. Hum Reprod 1993; 8(3): 416–421.