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Original article

Seroprevalence and Incidence of *Chlamydia Trachomatis* on Sperm Quality in Men of Procreating Age in Brazzaville

David Charles Roland M0UKALA[1,2]; Gabriel AH0MB0 [2]; Raoul AMPA [2]; Jacques François SILOU MASSAMBA [3]; Etienne NGUIMBI [2]; Yvon Sévérin K0MB0 [4]

[1]Medical Analysis Laboratory/Surgical Medical Clinic COGEMO
[2]Faculty of Science and Technology/University Marien NGOUABI
[3]Mother and Child Clinic
[4]WHO-Afro Medical Center

*Corresponding author: David Charles Roland M0UKALA; *davidmouk46@gmail.com*

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Summary

Chlamydia trachomatis is responsible for male infertility. The mechanism of action of C. *trachomatis* on male reproductive function is still controversial. The present study aims to evaluate seroprevalence and its influence on sperm quality in a male sample of **307** patients requesting serological examination for *C. trachomatis*, **73 of** whom were examined by sperm analysis - spermocytogram and sperm culture. The patients were aged between 17 and 71 years, consulting for infertility or urology. The analysis of *C. trachomatis* serologies, based on the enzyme immunoassay dosage of immunoglobulin G, yielded **111** cases of positive serology, i.e. a seroprevalence of **36.2%. The** age group over 60 years of age, the least represented group, **had** the highest frequency at **52.9%.** Analysis of the 73 spermograms and spermocytograms revealed a non-significant difference in mean sperm concentration, **49.67** million sperm per milliliter, standard deviation **±45.71, in** *Chlamydia* negative subjects versus **7.66** million sperm per milliliter, standard deviation **±10.98, in** *Chlamydia* positive subjects. A non-significant change in total motility of **39.83%,** standard deviation **±27.49,** in Chlamydia-negative subjects versus **36.03%,** standard deviation **±24.58, in** Chlamydia-positive subjects, a **3%** drop. Abnormal sperm forms, **51.86%, standard** deviation **±19.60,** in *Chlamydia* negative versus **57.17%,** standard deviation **±21.41,** in *Chlamydia* positive, an increase of **6%.** The global reading of the average spermogram of *Chlamydia* positive individuals revealed **oligospermia** and **asthenospermia** as abnormalities. This suggests that there is a possible link between *C. trachomatis* infection and decreased sperm quality in *Chlamydia* positive patients.

Keywords: ***Chlamydia trachomatis, seroprevalence, incidence, spermatozoa, spermogram.***

Introduction

Sexually transmitted infections (STIs) are frequently implicated in infertility and the reproductive health of populations, and are a major focus of public health policy around the world. *Chlamydia trachomatis* infection, a urogenital infection that is cited as the most common STI before gonorrhea and syphilis, has been the focus of increasing interest in reproductive health research [1] for several years.

In Africa, the prevalence of *C. trachomatis* infection by country shows some disparity, with the highest rates being 25% in KENYA (1985), 15.4% in The Gambia (1982), 12% in Senegal (1989), and Cameroon (1986) [2]. More recent studies indicate that In Senegal (2002), the prevalence was established at 1.2% in a study population of 578 people, with a male prevalence of 1.1% out of 265 samples [3]; in Côte d'Ivoire (1992), the highest seroprevalence among women in the 30-39 age group was 33.3% among pregnant women and 70.1% among prostitutes, whereas among men over 39 years of age, the peak was 64.3% in the control population and 83% among prisoners [4]; in Tunisia (1998) a prevalence of 34% in a male population of 97 people [5]; in Nigeria (2011) in a study of seroprevalence in a female population established a prevalence of 29.4%, with the most affected age groups being 20-24 years among female students and 25-29 years among non-students [6].

Infertility disorders due to *C. trachomatis* infection in women and men are no longer proven. Complications such as cervicitis, salpingitis, endometritis in women and urethritis,

epididymitis in men, lymphogranulomatous venereal disease, and genital ulceration in both sexes are common complications of C. *trachomatis* infection [7;8].

The study by J. SILOU MASSAMBA (1986) establishes the involvement of C. *trachomatis* in

Epididymitis and prostatitis in infertility consultants subjects in the Republic of Congo [9].

However, the influence of *C. trachomatis* on the physiological parameters of semen remains controversial [10;5].

This study aims to evaluate the responsibility of *C. trachomatis* on sperm quality and its impact on male infertility in Congo – Brazzaville.

Materials and Methods

1. Study framework

This work was carried out in the laboratory department of the COGEMO Medical-Surgical Clinic in Brazzaville.

2. Sampling

The sample consisted of patients who came for medical tests and follow-ups at various public and private hospitals and medical centers in the city. The patients were men consulting for couple infertility and/or urology.

3. Type and period of study

Prospective study started in 2017 and ended in 2019.

4. Conditions for patient recruitment

Inclusion Criteria:

- Male subjects living in Brazzaville, of procreating age, consulting for infertility and/or urology.
- The presentations of a prescription form including: spermogram - spermocytogram, sperm culture and serology for C. *trachomatis.*
- Subjects with a prescription report card that includes only *C. trachomatis* serology.

Exclusion Criteria:

- Subjects with positive sperm culture

5. Methods

5.1- Survey

The database of our study was constituted thanks to the establishment of an individual bench sheet where all the patient's information was collected as well as the stages of realization of the various analyses, with the following epidemiological data:

- Patient identity
- Patient file number
- Age
- Marital status
- Number of children
- Clinical information (reason for consultation and/or clinical signs-symptoms)
- Previous Medication
- Spermogram - spermocytogram results, sperm culture and chlamydial serology results

5.2 - Semen collection conditions

The semen used for the various tests (spermogram-spermocytogram, sperm culture) was collected in sterile spermogram jars after masturbation with simple water without the use of soap or condoms.

5.3 - Semen analysis

5.3.1 - The spermogram [11-16,20].

The spermogram examinations were performed on freshly collected semen according to the following procedure:

a) Pre-analytical stage:

After a period of abstinence of 3 to 7 days, the semen was collected in sterile pots, after masturbation, in the laboratory or at home. In the latter case, the specimen should be sent to the laboratory within 30 minutes of collection.

b) - Analytical stage:

It was carried out in two phases: macroscopic and microscopic evaluation, which are carried out within 30 to 60 minutes following sperm collection.

Macroscopic evaluation:

The aspect: it was a question of making a visual appreciation of the color and the limpidity of the seminal plasma.

The volume: it was measured using a graduated plastic pasteur pipette.

The pH: it was evaluated by depositing a drop of semen on a strip of Fisher Scientific pH paper, with a wide range of reference (1.0 to 14.0).

Viscosity: this was assessed by observing the flow of semen at the tip of a pipette. It is normal when the semen flows in separate drops.

Microscopic evaluation

Cytological examination: a drop of semen was deposited on a slide and covered by a lamina, to be observed on 5 to 10 fields at x400 magnification to evaluate the importance of the cellular elements: spermatozoa, round cells (germ cells), leukocytes, spontaneous agglutinates (specific attachment of motile spermatozoa) and aggregates (non-specific attachment of immobile spermatozoa).

Motility study: It consisted in estimating the percentage of sperm motility according to the 3 categories defined by the WHO: progressive motility or motility, non-progressive motility, immobile spermatozoa on two 10µL drops of semen observed between slide and lamina at x400 magnification on 5 to 10 fields.

For sperm concentrations below 15 million/mL and/or mobilities of less than 10%, a count was performed. The final result was the average of the estimates or counts performed.

Sperm count: it was carried out using a Malassez hematimeter on a drop of semen previously diluted in a physiological solution to which formalin (40%) was added at 1/5. The count was carried out on a minimum of 10 squares of the Malassez cell and the result was obtained from the average number of spermatozoa counted multiplied by 100 and by reverse dilution factor.

5.3.2 - The spermocytogram [11,13,14,16].

It is the study of sperm morphology after staining on slide. The pre-analytical stage is the same as for the spermogram and the analytical stage is carried out as follows:

Spreading on slide: two smears were made from 10µL of sperm respectively and then fixed with ethanol.

Staining: the staining technique we have applied is the Diff-Quick technique according to which, after fixing with alcohol, the blade is first dipped in 2% eosin and after draining it, it is dipped in methylene blue and then washed with tap water and dried.

Reading: the slide mounted under the microscope was examined at the highest magnification x1000 and in immersion. The count was performed on 200 spermatozoa.

5.3.3 - Reference values of the spermogram and spermocytogram

The reference values used to interpret our results are those of the World Health Organization (WHO, 2010) [29,30], whose low values are summarized in the table below.

Table I: Low reference values of the spermogram - spermocytogram [30].

Settings	Low reference value
Volume (ml)	1.5 (1.4-1.7)
Number of spermatozoa (x106 per ejaculate)	39 (33—46)
Sperm concentration (x106 per ml)	15 (12-16)
Total mobility (PR + NP, %)	40 (38—42)
Progressive mobility (PR, %)	32 (31-34)
Vitality (%)	58 (55-63)
Normal spermatozoon shape (%)	4 (3.0-4.0)

However, the reference value for the percentage of spermatozoa of normal shape considered in this study is that of the classification of David et al (1974), which is greater than 30% [31,23],

5.3.4- Sperm culture [17]

The pre-analytical stage is the same as for the spermogram, with greater rigor on the hygiene to be applied before sampling, in particular the cleaning of the glans with soap and water.

However, the analytical step was carried out according to the following steps:

Macroscopic analysis

In addition to the macroscopic parameters found in the spermogram analysis, emphasis was placed on the smell of the semen.

Microscopic analysis

It consisted of a cytological examination of the constituents of the seminal plasma as in the analysis of the spermogram completed by a parasitological examination oriented towards the search for *Trichomonas vaginalis* trophozoites and in rare cases Bilharzie eggs.

Three slides were thus prepared, from a 10 µL drop of semen each, the first one covered with a slide for a fresh reading at x400 magnification, the last two were used to make a smear which was then stained, one with Gram and the other with methylene blue or May Grünwald - Giemsa, for a reading at x1000 magnification when immersed.

Culture; Two techniques were the subject of bacteriological examination of seminal plasmas

- Seeding on agar culture media; the media on which the semen was seeded are : manitol salt agar (MSA), eosin-methylen-levin (EMB), uriselect 4, blood agar, chocolate gelose for the search for common (enterobacterium, staphylococcus) and demanding (enterococcus, gonococcus) bacterial germs, chloramphenicol sabouraud for the isolation of fungi *(Candida albicans).*
- Liofilchem's AF Genital-System gallery seeding for the isolation, identification of common and demanding germs and isolation of *Mycoplasma homonis* and *Ureaplasma urealyticum.*

The inoculated media were placed in the oven for incubation for 24 hours for common and demanding germs and 48 hours for Mycoplasma. Samples with positive cultures were systematically excluded from the study.

5.4- Chlamydiae serology

Chlamydia serology was performed by semi-quantitative enzyme immunoassay techniques using ORGENICS' ImmunoComb *Chlamydia trachomatis* IgG antibody assay.

Obtaining serums and/or blood plasmas used for *C. trachomatis* serology.

The serums and/or blood plasmas were obtained after blood was drawn by venipuncture from the subjects concerned. The collected blood was collected in dry or anticoagulant tubes where serum/plasma separation with the red cell pellet was obtained by centrifugation at **3000 rpm** for 10 minutes, 30 to 60 minutes after collection. The serum/plasma was then aliquoted into cryotubes for final analysis.

Enzyme ImmunoComb [18,19,8,15].

The test begins with the distribution of serum or plasma samples into the wells of compartment A of the development tray. Then the following steps are observed:

1. The comb is inserted into the wells of compartment A. Anti-C. *Trachomatis* antibodies that may be present in the tested samples specifically bind to the *C. trachomatis* antigens immobilized on the comb surface. Simultaneously, immunoglobulin's present in the samples are captured by the anti-human immunoglobulins on the upper spot (internal control).
2. Any antibodies not specifically bound in this first step are removed in a washing step in compartment B.
3. In compartment C, fixed anti-C. *Trachomatis* IgG class antibodies are recognized by anti-human IgG antibodies conjugated to alkaline phosphatase.
4. After further washing steps in compartments D and E.
5. In compartment F, alkaline phosphatase reacts with a chromogenic compound. This reaction leads to the visualization of the results in the form of grey-blue spots on the surface of the comb teeth.

Results

Number of patients received for Chlamydia serology is **307 of** which **73** with Spermogram- spermocytogram and sperm culture

1. Age distribution of the study population

This age distribution shows that the most represented age group is 30 to 39 years old, followed by 40 to 49 years old, and the least represented is over 60 years old. This distribution is shown in Figure 1.

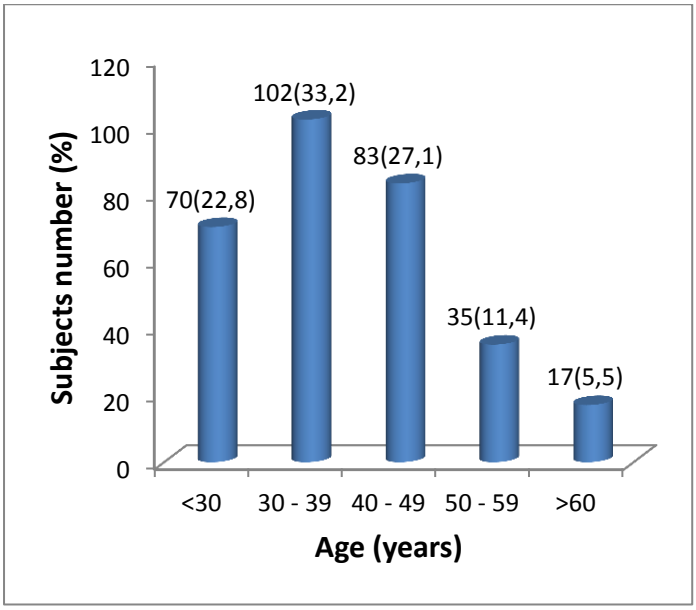

Figure 1: Distribution of Study Population by Age Group

2. Prevalence of Chlamydia trachomatis

Chlamydia serology results determined a 36.2% frequency of Chlamydia positive individuals in the study population. These results are reported in Table II

Table II: Prevalence of *Chlamydia trachomatis*

Serology	Staff	%
Négative	196	63,8
Positive	111	**36,2**

3. Distribution of Chlamydia serology results by age

Chlamydia serology results show the highest frequency of positivity in the over-60 age group significantly (threshold of significance: $p < 0.05$), these results are presented in Table III.

Table III: Distribution of Chlamydia serology results by age

Age Group	Results (%)		*p*-value
	Negative	Positive	
Less than 30 years old	55 (78,6%)	15 (21,4%)	
30 - 39 years old	68 (66,7%)	34 (33,3%)	
40 - 49 years old	46 (55,4%)	**37 (44,6%)**	0,012
50 - 59 years old	19 (54,3%)	**16 (45,7%)**	
More than 60 years old	8 (47,1%)	9 (52,9%)	

4. Distribution of positive serologies according to Immunoglobulin G (IgG) titration

Based on the anti-Chlamydia immunoglobulin G titration, the highest frequency of 49.55% is for 1/16 and 1/32 IgG levels, these results are shown in Table IV.

Table IV: Distribution of positive serologies according to IgG titration: 1/16; 1/32; 1/64

IgG titers	Staff	%
1/16	55	**49,55**
1/32	55	**49,55**
1/64	1	0,9

5. Distribution of Chlamydia-positive individuals by IgG titers and by age

IgG titrations at l/16th and l/32nd are higher in the 30-39 and 40-49 age groups, these results are shown in Figure 2.

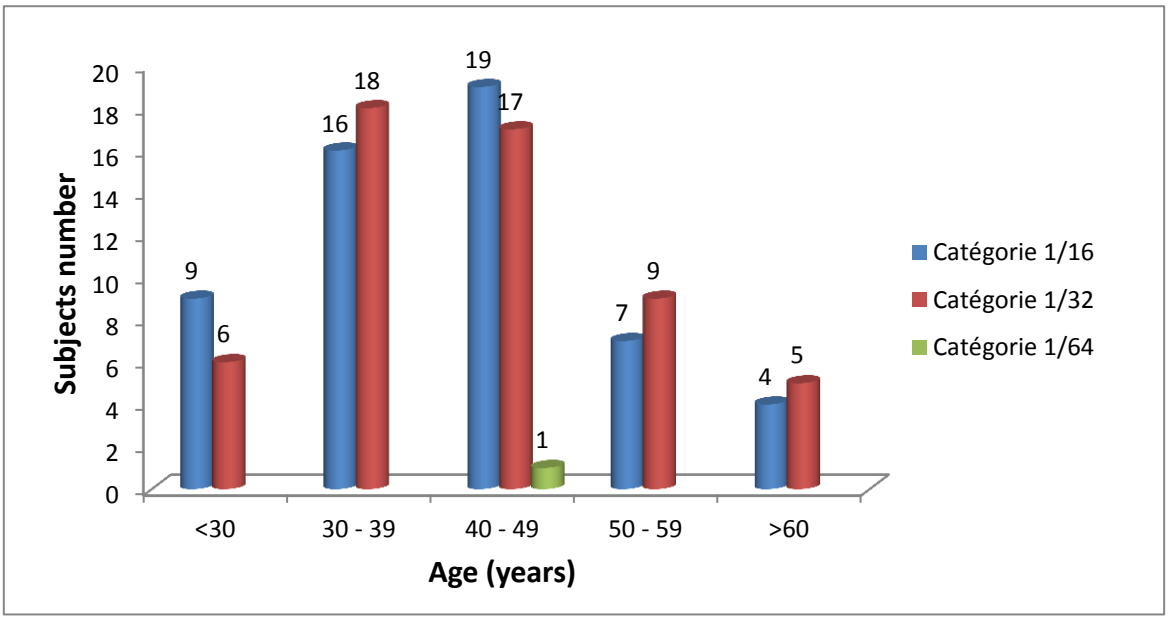

Figure 2: Distribution of Chlamydia positive individuals by IgG titration

6. Impact of Chlamydia positive serologies on spermogram parameters

The results of the mean spermogram show non-significant variations to the disadvantage of Chlamydia-positive subjects on some quantitative parameters such as sperm concentration, motility and abnormal sperm forms (Threshold of Significance: $p < 0.05$), these results are presented in Table V

Table V: Impact of Chlamydia positive serologies on spermogram parameters

Spermogram parameters	Chlamydia Result		p-value
	Négative	Positive	

Average semen volume	**2,61±1,32**	**2,63±1,22**	**0,942**
Viscosity n (%)			
Normal	**28(58,3)**	**20(41,7)**	
Heterogenous	**7(43,8)**	**9(56,2)**	**0,088**
Hyperviscosity	**8(88,9)**	**1(11,1)**	
Leukocyte n (%)			
absent	**10(52,6)**	**9(47,4)**	
rare	**11(57,9)**	**8(42,1)**	**0,810**
some	**14(66,7)**	**7(33,3)**	
many	**9(64,3)**	**5(35,7)**	
Average sperm count/mL	**49668571,41 ± 45707873,56**	**7655844,83 ± 10978467,14**	**0,271**
Abnormal spermatozoa (average %)	**51,86± 19,60**	**,17±21,41**	**0,221**
Mobility (average %)	**39,83±27,49**	**36,03±24,58**	**0,544**
Motility (average %)	**27,43±21,20**	**29,28±22,72**	**0,731**

Discussion

• Situation *C. trachomatis* in the target population

307 patients requesting *C. Trachomatis* serology were enrolled in this study. Seventy-three (73) of these patients also requested spermogram and sperm culture examinations. Patients ranged in age from 17 to 71 years and the most represented age groups were 30 to 39 and 40 to 49 years.

The analysis of C. *trachomatis* serologies, based on the search for IgG class immunoglobulins, performed on the 307 patients determined a prevalence of infection of **36.2%,** or 111 cases of *Chlamydia* positive serology in the target population; a prevalence close to that of L. Ammar-Keskes et al (1998) found **a prevalence of 34%** in a study on the impact of C. *trachomatis* on semen [5] and J. F. Silou Massamba (1986) found a prevalence of **37% in a** Congolese population in a study on the involvement of C. trachomatis in two populations of men, African and European[9].

In our study, the highest peak prevalence was found in the over-60s, the least representative group with **52.9% (9/17** patients), followed by the 50-59 year olds **47.9% (16/35)** and the 40-49 year olds **44.6% (37/83), The most** infected age groups, whereas in the target population of Okoror LE and Agbonlahor DE (2012) the most affected age group is the 30-40 years old **38.52%,** the over-60 years old being affected only **10.4%** [10] and in the Congolese population (1986) the highest frequency was noted in the 30-35 years old at 50% [9]. This suggests that in our target population, *C. trachomatis* infection affects older men. Two approaches may help us to avoid this. The first, more subjective, suggests that the over-60 age group is the most sexually active. More older men may be at risk of infection through risky behaviors and unprotected sex. This could be the case for the 50-59 and 40-49 age groups, whose seroprevalence is also high in this study.

The second approach is based on the nature of the IgG class immunoglobulins sought in the analysis of sera for C. *trachomatis* serology. IgG, as a marker of the body's immune response to *C.* trachomatis infection, once produced, can persist for several months or even years [21;22]. However, *C. trachomatis* infection is known to be both symptomatic and asymptomatic, and sometimes even chronic, due to the persistence of the infection in the upper reproductive tract of women for more than 60 days to several years, resulting in its persistence for up to 4 years in a couple [23;24;25]; this could explain the positive immunoglobulin G status of the oldest individuals in our study population. The latter could have been in contact with the bacteria for a few years earlier. It should also be noted that age is a risk factor in the context of prostatitis of infectious or non-infectious origin. On a population of European men, in the same study cited above, Silou Massamba established the link between *C. trachomatis* infection and unilateral epididymal involvement in 62.4% of cases on the one hand and prostatic involvement in 66.7% of cases on the other.

The distribution of IgG-positive *Chlamydia* individuals by IgG titration showed as many 1/16 as 1/32 positive cases (49.55% respectively), with IgG levels associated with urethritis in humans [21;22] and 1/64 to 0.9% cases being associated with active infection if the presence of IgG is coupled with that of IgA and/or simply epididymitis [21;26]. The **30-39** year age group is more affected for the 1/16 rate and the **40-49** year age group for the 1/32 rate.

• Spermogram profile based on the serodiagnosis of *C. trachomatis*

We note in this study that the difference is not significant between the mean values of the numerical parameters of the spermogram of Chlamydia negative and Chlamydia positive subjects globally; L.Ammar-Keske et al (1998) makes the same observation when studying the repercussions of C. trachomatis infection on the semen of infertile men [5]. However, there is a considerable difference in the concentration of spermatozoa taken in isolation: **(49.67 ± 45.71) x 106** spermatozoa/mL in Chlamydia-negative subjects versus **(7.66 ± 10.98) x 106** spermatozoa/mL in Chlamydia-positive subjects, a decrease in the concentration of spermatozoa also noted by Okoror et al (2012) due to *C. trachomatis infection* [10], Other parameters such as total motility and abnormal sperm forms showed non-significant variation, respectively **(39.83+27.49) %** in Chlamydia negative subjects versus **(36.03±24.58)%** in Chlamydia positive subjects, and **(51.86±19.60)% in Chlamydia** negative men versus **(57.17±21.41)% in Chlamydia** positive men. We note here a drop in motility of about 3% in Chlamydia positive individuals and an increase in abnormal sperm shape of about 6%. FAVOUR OSAZUWA et al (2013) found a significant association between the prevalence of *C. trachomatis* and sperm abnormalities[27].

The predominance of abnormal sperm and decreased motility in Chlamydia-positive individuals may be related to the mechanism of action of C. *trachomatis* on sperm; J.L. Fernandez et

al (2008) establish a causal link between urogenital *C. trachomatis* infection, *trchomatis and Mycoplasma urogenital infection and* the deterioration of genomic integrity through sperm DNA fragmentation, decreased sperm motility and decreased sperm concentration [28].

• Chlamydia-positive subjects have two abnormalities on their spermogram

A global reading of the average spermogram of Chlamydia-positive subjects reveals two essential abnormalities: **oligozoospermia,** a decrease in sperm concentration, and **asthenozoospermia, an** impairment of sperm motility, with reference to WHO 2010 standards [29,30]. This table, which is consistent with the work undertaken by our predecessors [10;27] cited above, each in its own particularity, allows us to establish a probable link between the seroprevalence of Chlamydia infection and the decrease in sperm concentration in our study population. This with possible collateral damage affecting sperm shape and motility.

Since the present study does not allow us to define the mechanisms, a more in-depth study integrating the search for *C. trachomatis* DNA in semen and the search for fragmented sperm DNA could help us to better understand the phenomenon. The responsibility of *C. trachomatis* for infertility is no longer in doubt.

Conclusion

The present study suggests that the frequency of C. *trahomatis* infection in the target population is quite high (%) compared to the different studies on male prevalence of Chlamydia, already carried out by different authors, with individuals over 40 years of age being the most affected.

Analysis of the respective spermograms of Chlamydia negative and Chlamydia positive subjects revealed a possible link between Chlamydia infection and the decrease in the physiological quality of the semen of our study population.

The reduction in sperm concentration and motility could be consequences that affect the fertilizing capacity of the sperm of Chlamydia positive subjects.

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