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

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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 [10-13].

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 hemiometer 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 10 µL 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 then dried, 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.

| Setting                        | Low reference value |
|-------------------------------|---------------------|
| Volume (ml)                   | 1.5 (1.4-1.7)       |
| Number of spermatoza (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-methylene-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 hominis 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.
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    | 55 (78.6%)  | 15 (21.4%) |
| 30 - 39 years   | 68 (66.7%)  | 34 (33.3%) |
| 40 - 49 years   | 46 (55.4%)  | 37 (44.6%) |
| 50 - 59 years   | 19 (54.3%)  | 16 (45.7%) |
| More than 60    | 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 1/16th and 1/32nd are higher in the 30-39 and 40-49 age groups, these results are shown in Figure 2.

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         |         |
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 [25] 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 [26].

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 affected 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% [27] and in the Congolese population (1986) the highest frequency was noted in the 30-35 years old at 50% [28]. 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 [29,30]. 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 [21,22,23], 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 [21,22]. 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 [25]. 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%. FAOUOSAZUWA 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, trachomatis 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 [19;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. trachomatis 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.

Bibliography

[1] B. DE BARBEYRAC, O. PEUCHANT, C. Le ROY, M. CLERC, L. IMOUNGA, C. BEREAR, 2012, Infection à Chlamydia trachomatis: quoi de neuf, Feuillrets de Biologie vol.III, n°306 : 33-38.

[2] NDOYE, S. MBOUP, M.L. SAKHO, E. COUILLON, C.S. BOYE, H. GUEYE, 1990, Traitement de l'œsthré masculine à Chlamydia trachomatis par une Doxycycline Longamycine, Médecine d’Afrique noire 1990, 37 (12) : 807-808

[3] CISSE Cheikh, 2002, Détermination de la prévalence de l'infection à Chlamydia trachomatis et Neisseria gonorrhoeae en zone rurale (NIKAHR) par la technique d'AMPLIFICOR, Thesis - Université CHEIKH ANTA DIOP, Dakar

[4] STORCHANT born BENABBBOU Leila, 1992, Chlamydia trachomatis: Serodiagnosis and prevalence study in Ivory Coast, Doctoral thesis in Pharmacy - University of Limoge

[5] LAMMAR-KESKES, R.GDOUFA, R.BOUZID, F.BEN SALAH, D.SELLAMI, H.HAKIM, A.HAMMAM, T.REBA, S.REKIK, J.ORF LA. 1998, Repercussion of genital chlamydia trachomatis infection on semen in men consulting for infertility in couples, Andrology 8, No. 1, 25-35.

[6] AC Ikeme, HU Ezegwui, LC Ikeako, I Agbata, E Agbata, 2011, Seroprevalence of Chlamydia trachomatis in Enugu, Nigeria, Nigerian Journal of Clinical Practice - Apr-Jun - Vol 14 -2, pp 176 -180

[7] BAL François (2004), CHLAMYDIA TRACHOMATIS IN SEXUALLY TRANSMISSIBLE INFECTIONS, PhD thesis in Pharmacy, Univ. Henri Poincare Nancy I

[8] K. Jaton G. Greub (2005), Chlamydia: Signs of calling, diagnosis and treatment, Rev Med Switzerland; Volume 1. 30280

[9] J. SILOU MASSAMBA, 1986, Involvement of Chlamydia trachomatis in two African and European populations, consultant for infertility, Diploma of Studies and Research in Human Biology, University C. B. Lyon I

[10] Okoror LE, Agbonlahor DE, 2012, High prevalence of Chlamydia trachomatis in the sperm of males with low sperm count, Medical Microbiology and diagnostic, 1:3, pp 1-5

[11] Abdellatif EL FAROUKI, 2015, Place du spermogram en l'exploration de l'infertilité à l'Avicenne military hospital of Marrakech, Thesis - University CADI AYYAD- Marrakech

[12] Johanne Dussault, 2009, Le spermogramme, Sommaire Scientifique (OPTMQ), vol.16, n°2, pp 1-7

[13] Geneviève GRIZARD, Clément JIMENEZ, 1997, Les examens du sperme dans l'exploration de la fertilité masculine, Progress in Urology, 7, 496-504

[14] Leila STÖCHAN BENABBOU, 1992, Chlamydia trachomatis: serodiagnosis and seroprevalence study in Ivory Coast, thesis - University of Limoge

[15] F. CATALAN, 1996, LES INFECTIONS A CHLAMIDIA TRACHOMATIS, Bioforma-Cahier de formation n° 04, pp 141.

[16] J. Tuch, 2012, Antisperm antibodies: indications, etiologies and applications in 2011, from the exploration of infertility to the concept of male immunocontraception, Andrology 22:20-28.

[17] OPTMQ, 2016, GUIDE SUR L’EXAMEN ET LA PREPARATION DE SPERME, Ordre Professionnel des Technologistes médicaux du Québec (Ed. October) pp 27-57.

[18] Tatiana LEGKOBYT, Sophie DESPEYROUX, Yasmine LOMBRY, Shérزادe MEBARKI, Stéphanie BANKOISSOU, 2010, DIAGNOSTIC BIOLOGIQUE DE L’INFECTION À CHLAMYDIA TRACHOMATIS : Avis sur les actes, Haute Autorité de Santé, NABM Classifications : sous-chapitres'6-03, 7-04,16 – 01, pp 15-16, www.has-sante.fr

[19] KOUAKOU Nouaman, 2006, SPERMATIC INFECTIONS: Clinical and Bacteriological Aspects, Thesis - University of BAMAKO.

[20] F. BOITREILLE, P. CLEMENT, 2014, Le spermogramme : un outil de choix dans l’exploration de l’infertilité masculine et du couple, Feuillrets de Biologie VOL LV N° 320 :41-46.

[21] BAL François, 2004, CHLAMYDIA TRACHOMATIS IN SEXUALLY TRANSMISSIBLE INFECTIONS, PhD thesis in Pharmacy, Univ. Henri Poincare Nancy I.

[22] BEBEAR. C. 2002, Mycoplasmas and Chlamydiae, Elsevier-Masson, pp 49-88.
[23] Gold M, Schillinger JA, Markowitz L, Saint Louis MOI. 2000. Duration of untreated genital infections with Chlamydia trachomatis: a review of the literature. *Sex Transm Dis*; 12(4): 329-337.

[24] Fairley CK, Gurrin L, Walker J, Hocking JS. 2007, "Doctor, how long has my chlamydia been there?" Answer: "... years". *Sex Transm Dis*; 34: 727-728.

[25] Ruijs GJ, Kauer FM, Jager S, Schroder FP, Schirm J, Kremer J. 1990, Epidemiological aspects of chlamydial infections and tubal abnormalities in infertile couples. *Eur J Obstet Gynecol Reprod Biol*; 36: 107-116.

[26] KANETI J, SAROV B, SAROV I., 1988, IgG and IgA antibodies specific for Chlamydia trachomatis in acute epididymitis, *Europ. Urol.* 14: 323-327.

[27] FAVOUR OSAZUWA, OSAMUDIAMEN I, AIGUOBARUEGHIAN, LOUIS ALEKWE, PAUL E. IMADE, KENNEDY O. IBADIN and LEWIS O. ABERARE, 2013, The prevalence of Chlamydia trachomatis infection among infertile males and its association with abnormal semen characteristics in Delta State, Nigeria, *Tanzania Journal of Health Research, Volume 15, Number 2:* 1-5.

[28] Guadalupe Gallegos, M.D., Benito Ramos, M.D., Rebeca Santiso, Ph.D., Vicente Goyanes, M.D., Ph.D., Jaime Gosalvez, Ph.D., and Jose Luis Fernandez, M.D., Ph.D., 2008, Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma, Fertility and Sterility, *Vol. 90, No. 2:* 328-334.

[29] WHO, 2010, WHO laboratory manual for the Examination and processing of human semen, WHO 5TH EDITION, pp 7-113.

[30] Trevor G. Cooper, Elizabeth Noonan, Sigrid von Eckardstein, Jacques Auger, H.W. Gordon Baker, Hermann M. Behre, Trine B. Haugen, Thinus Kruger, Christina Wang, Michael T. Mbizvo, and Kirsten M. Vogelsong, 2010, World Health Organization reference values for human semen characteristics, *Human Reproduction Update, Vol.16, No.3 pp. 231-245.*

[31] Auger J, Eustache F. 2000, Standardization of the morphological classification of human spermatozoa according to the modified David's method. *Andrology; 10:* 358-373.

[32] Fellous M., Siffroi J.P. 2003, Genetics of male infertility, new approaches. *13:1480157.*