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# Detection of *Chlamydia trachomatis* Immunogloblins in Seminal Plasma by Microimmunofluorescence Test and Their Effect on Semen Parameters of Infertile Males in Iraq

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**Abstract:**
**Background:** There is controversy over the role of asymptomatic genital tract infection of Chlamydia trachomatis (*C. trachomatis*) in the etiology of male infertility.
**Aim of the Study:** Detection of chlamydia antibodies in seminal plasma of infertile men and its effect on semen parameters.
**patients and Methods :** Semen samples were collected from 144 infertile male, 35 normal fertile male ,seminal plasma was used for detection of chlamydia antibodies by Microimmunofluorescence test (MlF) using species-specific antigens (genital serotypes D-K)
**Results:** This study confirmed that antichlamydia lgA and/ or lgG that were detected in seminal plasma of infertile males appeared to be *C. trachomatis* specific and associated with an inflammatory response. Semen parameters were severely affected in infertile patients with positive titer of antichlamydia lgA and or lgG that were detected in seminal plasma.
**Conclusions:** chlamydia trachomatis play an important role in male infertility
**Keywords:** *C. trachomatis*, infertility, immunoglobulines, semen parameters, microimmunofluorescence test

## Introduction

There are numerous studies showing an important role of Chlamydia trachomatis male infertility[1]. ln men, the pathogen can induce prostatitis ,epididymitis [2] and persistent low level inflammation of the male been genital tract [1].Chlamydia trachomatis can impair fertility by deterioration of spermatogenesis , impairment of sperm function and obstruction of seminal tract [3]. Reliable detection of *C.trachomatis* in genital tract is essential. Unfortunately the classical cell culture method cannot be used in semen because seminal plasma components are cytotoxic for McCoy cells [4].The detection of immunoglobulins against chlamydia seminal plasma has put forward as an alternative[1].

The objective of this study was to define the significant relation of antichlamydial lgA and or lgG in seminal plasma with semen parameters of infertile males

## Materials and Methods:

### Patients

One hundred forty four infertile males were examined in Kamal Al-Samarae hospital for infertility from November 2000 to November 2001 (96 patients with primary infertility and 48 patients with secondary infertility) and 35 normal fertile volunteers were eligible in this study. Ethics committee of Baghdad University, College of Medicine, approved the present research. At first the aim of study was explained for all participants and after obtaining their oral and signed consent they have been investigated. The age of the patients ranged (22-55) years and the duration of infertility problem ranged (1-18) years.

### Materials

a) Semen samples were collected from patients and control group according to recommendations of the World Health Organization (WHO) manual [5].
b) Species specific antigen of C.trachomatis (genital serotype D-K) (Rhone-Merieux- France)
c) Phosphate buffer saline. d) Glycerin buffer[6].

### Methods

Semen analysis (sperm concentration, motility, morphology) were performed according to recommendation of WHO manual[5].Seminal plasma was separated from semen sample by centrifugation at 300-500g which is enough to remove the seminal plasma from cellular components of seminal fluid. Seminal plasma was dispensed in aliquots and was kept at -20$^{O}$C until used [5].MIF technique was used to detect anti *C.trachomatis* immunoglobulins in seminal plasma of patients and control group[7, 8] . Ten µl of *C.trachomatis* antigen (genital serotypes D-K) was applied to each circle of specific slide. The slide was air dried for at least 30 min. at room temperature. The fixed slide was incubated for 30 min. at 37C with appropriate seminal plasma dilution (two-fold dilution from 1:2 to 1:256) diluted in phosphate buffer saline PBS, pH 7.2. Circle with antigen control was included. Before being washed by dipping in two PBS jar for 5 min. and in distilled water for 5 minutes. Ten µl fluorescein isothiocyanate conjugate at 1 :10 dilution and stained with 1% Evans blue (50µl conjugat+50

 

µl Evan blue +400 µl PBS).The slide was incubated for 30 min at 37C in moist chamber and dark place, then washed with PBS and distilled water. Glycerin buffer[6] was used in mounting step by adding small drop in each slide circle and covered with cover slip for examination by fluorescent microscope with 40X lens. The highest dilution giving specific fluorescence associated with elementary bodies was regarded as end point.[9]

**Results**

ln this study, out of 144 infertile male ,108 (75%) have positive titer of lgA and \ or lgG in their seminal plasma. Out of 108 infertile males with positive titer in seminal plasma, four were azoospermic infertiles and were excluded from the comparison. Sperm concentration less than 20x106/ml of semen was detected in (49.03%) among infertile males with positive titer. Sperm motility with less than 50%linear progressive type was 100% among infertile males with positive titer. Sperm motility with more than 50%linear progressive was detected more frequently among normal fertile. The sperm abnormal forms with more than 25% abnormality were detected in (58.66%) among infertile males with positive titer while they were detected only in (8.58%) of normal fertile males Out of 36 infertile males with negative titer in seminal plasma 9 azoospermic infertiles and were excluded from the study as shown in Table (1).

Table (2) shows that Sperm concentration less than 20x106 /ml of semen was detected in (18.52%) infertile males with negative titer. Sperm motility with less than 50% linear progressive type was detected in (81 .48%) infertile male with negative titer. Sperm abnormal form more than 25% abnormality were detected in (55.55%) infertile male with negative titer as shown in (Table 2) .Out of 63 primary infertile males with positive titer in seminal plasma, three were azoospermic and only one was azoospermic secondary infertile out of (45) that were excluded from the comparison as shown in table (3). Sperm concentration less than $20x10^6$/ml was detected in (56.670/o) among primary infertile males and (38.64%) among secondary infertile males. Sperm motility with less than 50% linear progressive type was detected in (95%) of primary infertile males and in (97.73%o) of secondary infertile males The sperm abnormal forms with more than 25%abnormality were detected more frequently among primary infertiles as shown in table (3).

**Table (1):Relationship between antichlamydial immunoglobulins in seminal plasma and semen parameters of infertile and fertile males.**

| Semen characteristics | Infertile male with positive titer * No. (%) | Fertile male with negative titer No. (%) |
|---|---|---|
| **Sperm Count** | | |
| **< 20 million/ml** | **51(49.03%)** | **2(5.72%)** |
| **≥ 20 million/ml** | **53(50.7%)** | **33(94.28%)** |
| **Total** | **104(100%)** | **35(100%)** |
| **Sperm Motility** | | |
| **<50%** | **100(96.15%)** | **0(0%)** |
| **≥50%** | **4(3.85%)** | **35(100%)** |
| **Total** | **104(100%)** | **35(100%)** |
| **Sperm Abnormal form** | | |
| **<25%** | **43(41.34%)** | **32(91.42%)** |
| **≥25%** | **61(58.66%)** | **3(8.58%)** |
| **Total** | **104(100%)** | **35(100%)** |

*** Four a zoo spermic patients with positive titer are not included**

 

**Table (2): Relationship between antichlamydial immunoglobulins in seminal plasma and semen parameters of infertile males with positive and negative titer.**

| Semen characteristics | Infertile male with positive titer * No. (%) | Infertile male with negative titer** No. (%) |
|---|---|---|
| **Sperm Count** | | |
| **< 20 million/ml** | **51(49.03)** | **5(18.52)** |
| **≥ 20 million/ml** | **53(50.7)** | **22(81.48)** |
| **Total** | **104(100)** | **27(100)** |
| **Sperm Motility** | | |
| **<50%** | **100(96.15)** | **22(81.48)** |
| **≥50%** | **4(3.85)** | **5(18.52)** |
| **Total** | **104(100)** | **27(100)** |
| Sperm Abnormal form | | |
| **<25%** | **43(41.34)** | **12(44.45)** |
| **≥25%** | **61(58.66)** | **15(55.55)** |
| **Total** | **104(100)** | **27(100)** |

***Nine azoospermic patients with negative titer are not included**
**** Four azoospermic patients with positive titer**

**Table (3): Relationship between antichlamydial immunoglobulins in seminal plasma of infertile males with primary and secondary infertility**

| Semen characteristics | Primary Infertile male with positive titer * No. (%) | Secondary infertile male with positive titer** No. (%) |
|---|---|---|
| **Sperm Count** | | |
| **< 20 million/ml** | **34(56.67)** | **17(38.64)** |
| **≥ 20 million/ml** | **26(43.3)** | **27(61.36)** |
| **Total** | **60(100)** | **44(100)** |
| **Sperm Motility** | | |
| **<50%** | **57(95)** | **43(97.73)** |
| **≥50%** | **3(5)** | **1(2.27)** |
| **Total** | **60(100)** | **44(100)** |
| **Sperm Abnormal form** | | |
| **<25%** | **24(40)** | **19(43.18)** |
| **≥25%** | **36(60)** | **25(56.82)** |
| **Total** | **60(100)** | **44(100)** |

***Three azoospermic patients with positive titer are not included**
**** Only one azoospermic patient with positive titer is not included**

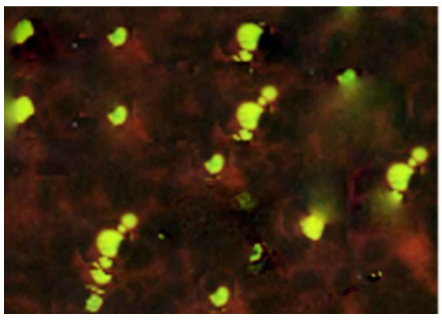

**Figure (1): Specific fluorescence of *C.trachomatis* (100X) by microimmunofluorescence test in seminal plasma of infertile males**

**Discussion**

 

The high incidence (75%) of *C. trachomatis* specific lgA and /or lgG in seminal plasma of infertile men using MlF, support the fact that they had previously encountered with sexually transmitted *C. trachomatis* in the form of chronic asymptomatic infection within genital tract. With respect to semen parameters of infertile males, there was an obvious difference between infertile males with positive titer of antichlamydial immunoglobulins in seminal plasma and normal fertile males. Sperm count with less than $20x10^6$ sperm/ml was recorded in (49.03%) among infertile males with positive titer (Table 1). This count was recorded in (56.67%) among primary infertile and in (38.64%) among secondary infertile males with positive titer in seminal plasma. Among infertile males with negative titer, the sperm count less than $20x10^6$ sperm/ml was recorded in (18.52%) while among normal fertile males this count was recorded in (5.72%) only.(Table 1 and 2) .

Sperm count > $20x10^6$ sperm/ml was recorded in (50.7%) among infertile with positive titer. This count was recorded in (43,3%) among primary infertile and in (61.36%) among secondary infertile males with positive titer in seminal plasma.(Table 1 and 2). Among infertile males with negative titer, the sperm count $\geq 20x10^6$ was recorded in (81.48%) while among normal fertile males this count was recorded in (94.28%). These findings were in agreement with [1, 10, 11] that approved that infertile males with positive antichlamydial immunoglobulins (lgA and/or lgG) in seminal plasma had sperm count significantly lower than that of infertile with negative titer or normal fertile control .

Detection of specific immunoglobulins in seminal plasma do not rule out the presence of *C. trachomatis* in male genital tract even in culture negative semen which may be harbored in accessory glands and leads to direct effect on spermatogenesis and number of mature spermatozoa beside hormonal factors which effect on the number of spermatozoa [1, 11, 12].

This study revealed that linear progressive motility 250% were detected in (3.85%) among infertile males with positive titer. This type of motility was recorded in (5%) among primary infertile and in (2.27%) among secondary infertile males with positive titer in seminal plasma.(Table 1 and 2). Among infertile males with negative titer, linear progressive motility >50% was detected in (18.52%) while among normal fertile this type of motility was detected in 100%.

The important outcomes with respect to linear progressive motility less than 50% was detected in (96.15%) of infertile males with positive titer. This type of motility was recorded in (95%) among primary infertile males and in (97.73%) among secondary infertile males. Among infertile males with negative titer in seminal plasma linear progressive motility, less than 50% was recorded in (81.48%) while among normal fertile this type of motility was not recorded. (Table 1 and 2) . Similar results were reported by others [11, 13, 14] , they found that there was a significant differences among infertile with positive titer in seminal plasma and those with negative titer as well as among normal fertile in sperm motility with special regard to linear progressive motility.

Linear progressive motility is severely affected not only as a result of *C.trachomatis* infection in which the organism may adhere to spermalozoa and then enter to spermatozoan head or tail which lead to impairment of normal movement ,but also as a result of elevated concentration of leukocytes in semen samples of infertile males with positive titer in seminal plasma which had a deleterious effect on spermatozoa because of their ability to stimulate the release of reactive oxygen species (ROS), there by affecting sperm motility [15, 16]

Regarding the sperm abnormal forms, this study revealed that abnormal sperm more than 25% was detected in (58.66%) of infertile males with positive titer in seminal plasma. Among primary infertile males sperm abnormal form more than 25% was recorded in (60%) while among secondary infertile it was detected in (56.82%).Abnormal sperm > 25% was detected in (55.55%) among infertile males with negative titer in seminal plasma and only (8.58%) was detected in normal fertile males. Abnormal sperm less than 25%was detected in (91 .42%) normal fertile and in (44.45%) infertile males with negative titer ,while among infertile males with positive titer, sperm abnormal form less than 25% was (41 .43%). (Table 1 and 2) .These findings coincide with that of [11, 17, 18] who reported that sperm abnormal forms among primary infertile males with positive titer in seminal plasma were significantly higher than that of infertile males with negative titer. This result come in contrary with that reported by[19] they mentioned that there was no difference in sperm abnormal forms among infertile males with positive and those with negative chlamydial genital infection. This could be due to the fact that they used a small sample composed from 40 infertile males, that made the difference between positive and negative infertile males statistically not significant .

The higher incidence of abnormal forms among infertile males with positive titer may be attributed to the presence of chlamydial genital infection in which elementary bodies adhere and enter into spermatozoan head or tail leading to abnormal morphology of spermatozoa beside the fact that the majority of infertile males with positive titer in seminal plasma have significant elevation in the number of white blood cells (WBCs) in semen more than $1x10^6$ cell/ml and this will stimulate the production of hydrogen peroxide, oxygen radical and reactive nitrogen intermediate by activated macrophage and granulocytes. This products have a highly deleterious effect on spermatozoa, and the degree of sperm damage by WBCs products depends on the location of inflammatory reaction and, the duration of exposure of sperm to these products, The possibility of genetic defects in the formation of sperms during spermatogenesis also must be keep in mind. [12, 15]

 

With respect to semen volume, this study revealed that there was no obvious difference among infertile males with positive titer in seminal plasma and those with negative titer while the difference was clear between infertile males with positive titer and normal fertile males with negative titer in seminal plasma. (Table 3). This could be due to the fact that genital infection with *C. trachomatis* is asymptomatic or silent in large proportion of infertile males and the organism may be reside in epididymis, seminal vesicle or prostate gland which lead to direct effect on spermatogenesis also it may cause reduction of secretory activities of accessory glands which subsequently leads to reduction in semen volume beside other factors like, level of sexual hormones and, days of abstinence.[18, 20]

Regarding mean of non-motile spermatozoa this study revealed that percentage of non-motile spermatozoa among infertile males with positive titer was higher than that of both infertile males with negative titer in seminal plasma and normal fertile males. (Table 3) .This comes in agreement with that reported by [21] where they found that percentage of non-motile spermatozoa was higher among those with positive evidence of chlamydial infection either by direct or indirect detection method, when compared with infertile with negative evidence or normal fertile males. This could be due to direct effect of chlamydial elementary bodies ( EBs) that impaired motility of sperms by its adherence and entry to head or tail of spermatozoa or as a result of indirect effect of chlamydial infection that cause elevation of leukocytes in semen even in the absence of signs which reflect an inflammatory response , leukocytes play an important role in impairment of sperm motility by producing reactive oxygen species ( ROS) which have a deleterious effect on spermatozoa impairment of sperm motility may be due to presence of anti-sperm antibodies as a result of chlamydial infection or other genital infections [12] [15, 22]

This study revealed that among infertile males with positive titer in seminal plasma there were four (3.7%) azoospermic males with previous history of urogenital infection (UGl) in which 3 of them were primary infertiles and only one was secondary infertile. Among infertile males with negative titer in seminal plasma there was 9/36 (25%) azoospermic males in which only two azoospermic males with previous history of UGl. (Table 1 and 2). the presence of four azoospermic males with positive titer in seminal plasma and previous history of UGI may be attributed in part to chronic genital infection with *C. trachomatis* which may be reside in epididymis and subsequently lead to tubal occlusion of the duct system[1, 22].

In conclusion this study confirmed that antichlamydial lgA and /or lgG that detected by MIF in seminal plasma of infertile males were *C. trachomatis* specific and associated with an inflammatory response. Also semen parameters severely affected in infertile Patients with positive titer of antichlamydial IgA and or lgG in seminal plasma ; suggesting that *C. trachomatis* play an important role in male infertility.

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