

# The Effect of Post Chlamydia Trachomatis Infection Treatment on Reactive Oxygen Species and Sperm Parameters of Infertile Men

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Chlamydia Trachomatis (CT) infection is often mentioned as a silent disease. Reactive Oxygen Species (ROS) can also cause Sperm apoptosis and have negative impact on Sperm parameters. The objectives of this study were to elucidate the association between Sperm parameters and ROS caused by CT infections resulting in male Infertility as well as evaluating the role of antibiotic therapy. A total of 848 infertile males having normal and abnormal Sperm parameters were included. After Semen sampling, the CT IgA were measured by Elisa and confirmed by Nested PCR. ROS was determined by Chemiluminescence. After treatment under the direct supervision of the private urologists. Then, the second Semen samples were taken and subjected to tests on Sperm parameters and ROS levels as assessed again. The levels of ROS and morphology were improved following the treatments ( $P < 0.05$ ). Antibiotic therapy due to CT infection, could reduce ROS, improve normal morphology and recover some of Semen parameters. Our findings indicate that CT infection and Sperm parameters were associated with the rate of ROS in infertile men. However, after treatment, ROS value dropped allowing the recovery of certain Sperm parameters.

Antibiotic therapy can improve some Semen quality parameters and treat the male Infertility.

**Keywords:** Antibiotic treatment; Chlamydia trachomatis; Male under the tm 0 3(a); Rm(t; C)-5 c

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Frequency of C. trachomatis between the study groups

Of 848 patients (with normal and abnormal Sperm) included in the study group (infertile men), seven (0.83%) were tested positive for C. trachomatis. All infected patients in the study group were resolved from the infection after the treatment completion.

Semen parameters

Table 1 and 2 compares the Semen parameters before and after the antibiotic therapy, respectively. Figure 1 shows the Semen parameters in infected infertile men before and after the antibiotic therapy. There was not statistically significant difference in the Semen parameters.

The count of leukocytes, Sperm count, total motility, Class A (rapid progressive), class D (non-motile), volume of Semen and pH Semen after treatment, the parameters of recovery showed improvement with no significant differences (P>0.05.). Motility of the Class B (progressive) and class C (non-progressive) had no significant differences. The mean count of white blood cells in the pre-treatment sample was 0.41 ± 0.518 million per ml, and the mean count of white blood cells in the samples after treatment was 0.09 ± 0.146 million per ml, the count of WBC was decreased after treatment compared with before treatment, but this reduction was not significant (P-value=0.144).

The Sperm count was increased numerically after treatment, but this increase was not significantly different (P-value=0.128). The Sperm motility was increased numerically after treatment compared with before treatment, but this increase was not significantly different (P-value=0.398). Sperm motility including class A, class B, class C and class D before and after treatment was not significant (P-value=0.138).

The volume of Sperm samples was not statistically significant before and after treatment (P-value=0.249). The pH Semen samples were not statistically significant before and after treatment (P-value=0.157). The normal morphology after treatment compared with the baseline was significantly increased (P-value=0.024).

After ROS total/Before ROS total	2.023a	0.043*
After ROS WBC/Before ROS WBC	2.023a	0.043*
After ROS base/Before ROS base	2.023a	0.043*
After pH/Before pH	1.414a	0.157 ns
After normal morphology	2.251b	0.024*
After volume/Before volume	1.153b	0.249ns2.251b1.414a

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Mill/ml	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
WBC before treatment							

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**Inclusion-exclusion criteria, semen collection and analysis**

All the patients were clinically examined and asked for past medical, sexual, and social histories. The study population consisted of men who referred to Royan Institute with Chlamydia trachomatis infection. Eight hundred and forty-eight patients with normal and abnormal Sperm parameters (low Sperm count, pyoSpermia, low Sperm progressive motility, low normal morphology) were included.

Patients with symptoms such as genitourinary tract infections, reproductive system abnormalities, varicocele, testicular tumors, systemic diseases, non-compliance with Spermogram test conditions, and those with a history of antibiotic use in the previous week were not included in our study.

This study was a cross-sectional study, Semen samples were collected into sterile sample cups through self-administered masturbation, after 3-7 days of sexual abstinence. Samples were put in the incubator directly for liquefaction and then manually analyzed by the same person for volume, viscosity, pH, presence of White Blood Cells (WBCs), Sperm concentration (count per ml and total count), motility (classes A, B, A+B, C, and total), and normal morphology, as indicated by the latest WHO manual for Semen analysis (Organization, 2010). Semen analysis was confirmed using a light microscope equipped with a Computer-Aided Semen Analysis (CASA; Test Sperm2.1, Video test, St. Petersburg, Russia) system. The presence of leukocytes in seminal fluid was detected by peroxidase test. Sperm morphology was detected by staining papanicolaou procedure.

In the first appointment, Semen samples from infertile men were collected in sterile containers and each sample was divided into two parts; the first part for Semen analysis and the next part for Sperm parameters. To evaluate the first part, Sperms were kept into sterile vials in order to perform the Elisa test and PCR at -70°C until testing was contained. After the centrifugation, plasma samples were analyzed by Elisa test samples and sediment samples were used for DNA extraction. After the infection confirmation by PCR, patients were asked to visit the second visit interval of 3 days from the last ejaculation for Sperm analysis and ROS tests. Antibiotics (every 12 h for two weeks) were also prescribed for them. After the completion of antibiotic usage, if the patients were not resolved from the infection, the treatment continued taking the same antibiotic with the same dose for another week. In order to assess the effect of the empirical antibiotic treatment on Semen parameters ROS levels, as well as clearance from infection, a subsequent Semen sample was taken 30 days after completion of the antibiotic therapy, by considering the 3-7 days of sexual abstinence.

**Elisa test**

To detect specific IgA, antibodies to C. 

this bacterium and its treatment. Our findings will also be helpful for infected patients who have abnormal Semen parameters to maintain fertility and reproductive health.

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### Disclosure statement

The other authors have no competing interests to disclose.

### Ethics Approval

The ethical approval for patient enrolment in this study was obtained from the Ethics Committee of Royan institute (IR.ACECR. ROYAN.REC.1394.84).

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### Author contribution

Analyzed the data, evaluated the results, and wrote the paper: RA

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