Original Research Article

Antibiotic treatment of asymptomatic Ureaplasma infection improves semen parameters in infertile men

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A R T I C L E   I N F O

Article history:
Received 26 September 2016
Accepted 15 November 2016
Available online 23 December 2016

Keywords:
Ureaplasma species
Asymptomatic infections
Male infertility
Semen parameters
Antibiotic treatment

A B S T R A C T

The role of asymptomatic infections caused by Ureaplasma species in male infertility and the efficacy of antibiotics in treatment of this failure is not yet definitely determined. A total of 165 infertile males having abnormal semen parameters (study group) as well as 165 healthy fertile men (control group) were included in this study. Semen samples were taken from all participants and after analyzing, undergone real-time PCR, microbial culture, and reactive oxygen species (ROS) as well as total antioxidant capacity (TAC) assays. Infected individuals of study group were treated with antibiotic. One month after the treatment completion, second semen samples were taken and undergone all the tests mentioned. The frequency of Ureaplasma spp. was significantly higher in the infertile men compared with the fertile ones (36.4% versus 11.5%; p < 0.001). Most of semen parameters were improved (p < 0.05) and reached their normal range, the level of TAC elevated (p < 0.001), and ROS level (p = 0.003) as well as ROS/TAC ratio (p = 0.003) reduced after antibiotic treatment. Moreover, wives of 37 infertile men (61.7%) became pregnant six months after the treatment completion. These findings indicate that Ureaplasma species are correlated with male infertility and that antibiotic therapy can improve the semen parameters and treat the male infertility.

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Introduction

Ureaplasma species are considered among the most prevalent sexually transmitted pathogens that have a global distribution. They are usually found as part of the normal microbiome of the human urogenital tract (Bharat et al., 2015; Razin 2006). These bacteria are associated with some symptomatic and asymptomatic genitourinary tract infections in both males and females, such as non-gonococcal urethritis (NGU), endometritis, bacterial vaginosis, preterm delivery, postpartum or postabortal fever, pelvic inflammatory disease (PID), ectopic pregnancy, as well as perinatal disorders such as low birth weight and neonatal bacteremia/meningitis (Ahmadi et al., 2016; Taylor-Robinson and Lamont, 2011; Viscardi 2010; Waites et al., 2005).

Some investigators believe that Ureaplasmas could negatively change various semen parameters, such as sperm motility, count, and morphology, and/or cause oxidative damage to spermatozoa via producing of ROS and causing the imbalance between ROS and TAC in seminal fluid, thereby leading to male infertility (Han et al., 2003; Huang et al., 2014; Lee et al., 2013; Nunez-Calongo et al., 1998; Potts et al., 2000; Reichart et al., 2000; Rybar et al., 2012; Sharma et al., 1999; Smith et al., 1996; Xu et al., 1997; Zhang et al., 2014), while other researchers have reported no influence of Ureaplasmal infections on semen quality (Günayli et al., 2011; La Vignera et al., 2011). However, the effect of urogenital Ureaplasma infections particularly asymptomatic ones on spermatozoa and seminological variables as well as their role in male or female infertility is still controversial and remains unclear (Knox et al., 2003; Ochsendorf, 2008; Potts et al., 2000; Sanocka-Maciejewska et al., 2005; Wang et al., 2006a).

http://dx.doi.org/10.1016/j.jab.2016.11.004
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There are many clinically asymptomatic men colonized by these bacteria where these microorganisms are potentially pathogenic and may play a role in urogenital tract infection or affect fertility potential as an opportunistic pathogen, under certain circumstances (Ahmadi et al., 2016; Al-Dagheri and Abdel-Dayem, 2010; Güneyli et al., 2011; Klein et al., 1969; Pannekoek et al., 2000; Salmeri et al., 2012). Nevertheless, the majority of asymptomatic infections may remain undetected and consequently untreated (Ahmadi et al., 2016).

The aims of the present study were to: 1) compare the frequency of Ureaplasma species between the study group (clinically asymptomatic infertile men with abnormal semen parameters) and the control group (healthy fertile men with normal semen parameters), 2) elucidate the association of asymptomatic Ureaplasma infections with male infertility, 3) assess the semen level of ROS, TAC, and the ROS/TAC ratio in both study and control groups, and 4) evaluate the effect of antibiotic treatment on improvement of spermatozoa parameters, semen levels of ROS and TAC, and ROS/TAC ratio in infected infertile men.

Materials and methods

Patient enrolment

Ethics approval for this study was obtained from the Ethics Committee of Royan (No. IR.ACECR.ROYAN.REC.1395.52). The patients in this study were selected from men consecutively admitted to the Royan Institute for Reproductive Biomedicine, Tehran, Iran. All participants as well as their sexual partners provided written informed consent. All the patients were clinically examined and asked for past medical, sexual, and social histories.

Inclusion and exclusion criteria

Included patients (the study group) were those that had abnormal semen analysis results in which at least one semen parameter (sperm count, motility, or normal morphology) being below the latest reference value recommended by (WHO, 1999). Patients displaying any symptoms of urogenital tract infections or having endocrine disorders, chromosomal anomalies, reproductive system abnormalities (varicocele, hydrocele, or undescended testis), testicular tumors, systemic diseases, sperm autoantibodies, or those who had history of antibiotic use within the previous week were not included in our study. Males with azoospermia, heavy use of alcohol, heavy smoking, continuous exposure to chemical or physical agents with known adverse reproductive effects (e.g., benzene and radiation), as well as patients whose semen samples tested positive for Ureaplasma spp. by real-time polymerase chain reaction (real-time PCR), but negative by microbial culture, were also excluded from the study.

In all, 165 clinically asymptomatic men with abnormal semen parameters having infertility of at least one-year duration fulfilled the eligibility criteria for inclusion in this study. In addition, 165 healthy fertile men with normal semen parameters attending for routine check-up were enrolled in a consecutive manner over the study period and designated as the control group. The female partners of all participants had normal results on fertility examination.

Semen collection and analysis

Given written and verbal instructions to the participants to follow the procedure, 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/ml and total count), motility (classes A, B, A+B, C, and total), and normal morphology, as indicated by WHO (1999) manual for semen analysis. Semen analysis was confirmed using a light microscope equipped with a Computer-Aided Semen Analysis (CASA; Test Sperm 2.1, Videotest, St. Petersburg, Russia) system.

Afterward, seminal fluids collected from study and control groups were divided to four parts for performing: 1) real-time PCR (200 μl), 2) microbial culture (500 μl, inoculated into Transport-PPLO broth medium), 3) ROS level assay (100–500 μl), and 4) TAC test (100–200 μl, stored at −70 °C till time of the test).

DNA extraction

Ureaplasma DNA was extracted from semen samples using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer’s instructions. Negative Control of Extraction was used in the extraction procedure and internal control, which serves as an amplification control, was directly added to the sample/lysis mixture during the DNA isolation for each individually processed specimen to identify possible reaction inhibition.

Real-time PCR

Ureaplasma species Real-TM commercial kit (Sacace Biotechnologies Srl, Como, Italy) was used for qualitative detection of Ureaplasma species. Reagent preparation was performed according to the manufacturer’s instructions and real-time PCR was performed on a Rotor-Gene™ 6000 (Corbett Life Science, Sydney, Australia). The temperature profile of amplification was as follows: step 1) 95 °C for 15 min, followed by step 2) five cycles of 95 °C for 5 s, 60 °C for 20 s and 72 °C for 15 s, and step 3) the last step of 40 cycles of 95 °C for 5 s, 60 °C for 20 s and 72 °C for 15 s. Fluorescent signal detection was accrued at the second stage of the step 3 (60 °C). Amplification data were analyzed by the instrument software (Rotor-Gene software series, version 1.7), and cycle threshold (Ct) values ≤ 33 were considered positive.

Microbial culture for Ureaplasma spp. detection

Semen samples found positive in real-time PCR, underwent microbial culture in order to ensure that the detected bacteria were viable and could grow and form the colonies.

Media for Ureaplasma culture and isolation including Transport-PPLO broth, Urea-PPLO broth, and Urea-PPLO agar, were prepared using a commercial PPLO Broth/Agar Base Without Crystal Violet (Conda, Madrid, Spain). Supplements and additives including 10% Yeast extract (Conda, Madrid, Spain), 20% horse serum (Baharafshan, Tehran, Iran), 0.1% urea (Carlo Erba, Italy), 1000 IU/ml of penicillin G, 0.002% phenol red (Sigma, St. Louis, MO, USA) as a pH indicator, and 0.01% MnSO4 solution (for agar medium), were added to the suspension, and final pH was adjusted to 6.0 for Urea-PPLO broth/agar, and to 7.0 for Transport-PPLO broth (Tully and Razin, 1983).

Seminal fluid (500 μl) inoculated into Transport-PPLO broth medium, was immediately transported to microbiology laboratory, where the medium was passed through a 0.45 μm pore size disposable syringe filter (Minisart, Sartorius, Goettingen, Germany) and inoculated into Urea-PPLO broth medium. The latter medium was incubated aerobically at 35–37 °C. The tubes were held for seven days and inspected once daily for color changes in the broth. As soon as a noticeable alkaline shift in pH (yellow to orange-red) was observed, the broth was immediately
subcultured to the agar medium. Then, agar plate was put in a Candle Jar (to provide an atmosphere of 5–7% CO₂) and incubated at 35–37°C for seven days and daily checked under a light microscope for *Ureaplasma* colonies.

**ROS measurement**

Measurement of reactive oxygen species in semen specimens was performed according to the WHO laboratory manual for the examination and processing of human semen (World Health Organization, 2010). Spermatozoa were washed in Krebs–Ringer medium (KRM) and adjust to 10 × 10⁶ spermatozoa per ml. Chemiluminescent probes, including luminal, formyl-methionyl-leucyl-phenylalanine (FMLP), and Phorbol 12 myristate 13-acetate (PMA) were utilized to detect extracellular, and WBCs and spermatozoa generated ROS, respectively.

Chemiluminescent signals were monitored using a luminometer (Synergy™ H4 Hybrid Multi-Mode Microplate Reader, BioTek®, USA), and final ROS level was calculated as relative light units (RLU)/s × 10⁶ sperm.

**Assessment of TAC**

Total antioxidant capacity of semen samples was assessed using a Total Antioxidant Capacity Assay kit (abcam®, Cambridge, UK) by colorimetric method. TAC was analyzed by means of a Microplate Reader (Synergy™ H4 Hybrid Multi-Mode Microplate Reader, BioTek®, USA) and calculated as nmol/μl of semen. Subsequently, the ROS/TAC ratio of semen samples was determined for each participant.

**Antibiotic treatment and patients’ follow-up**

Patients of study group tested positive in both real-time PCR and microbial culture for *Ureaplasma* spp., as well as their sexual partner, were treated with doxycycline (Razak Laboratories, Tehran, Iran), 100 mg orally twice daily for seven days (Taylor-Robinson and Bebear, 1997). If the patients were not resolved from the infection, the treatment continued taking the same antibiotic with the same dose for one more week. In case of antibiotic resistance, azithromycin was administered for five days (500 mg on first day followed by once daily for next four days) (Ballow and Amsden, 1992). In order to assess the effect of the empirical antibiotic treatment on semen parameters, ROS and TAC levels, as well as resolving from infection, a subsequent semen sample was taken 30 days after completion of the antibiotic therapy (Pajovic et al., 2013), also respecting the 3–7 days of sexual abstinence. Semen analysis, real-time PCR, microbial culture, ROS and TAC assessment, as well as calculation of ROS/TAC ratio were performed on these specimens, as already described for the first samples.

**Statistical analysis**

Statistical analysis was performed using IBM SPSS statistical software, version 22.0 (SPSS Inc., Chicago, IL). Data of semen parameters, ROS, and TAC measurements between the study and control groups were compared using Independent-Samples T test. Comparison of the mentioned variables before and after the antibiotic therapy was performed using Paired-Samples T test. Chi-Square test was applied to assess statistical significant differences in qualitative variables, including age-group and viscosity, between the groups. In all of the analyses, p < 0.05 was considered statistically significant. The data were expressed as the mean value plus-minus the standard error of the mean (mean ± SEM).

**Results**

**Study population**

The average age of the included participants in the study and control groups was 34.3 ± 0.4 years (ranging from 24 to 59), and 33.6 ± 0.4 years (ranging from 24 to 49), respectively. Among the stratified age-groups including 24–29, 30–35, 36–41, 42–47, 48–53, and >53 years, most of the participants in both study (46.7%) and control (50.9%) groups were in 30–35 years age-group. The mean duration of infertility for study group was 54.8 ± 3.8 (ranging from 12 to 288) months. All spermatozoa of the included patients in the study group had abnormal motility. In addition to the abnormal motility, 52 patients (31.5%) had also abnormal sperm count, and 93 (56.4%) of them had abnormal sperm morphology.

**Frequency of Ureaplasma spp. between the groups**

All real-time PCR positive specimens underwent the microbial culture for detection of viable *Ureaplasma* species. Under a light microscope, *Ureaplasma* colonies were generally between 10 and 50 μm in diameter, round, coarsely granular with a rough edge (Sea urchin-shaped) and golden to brown in color (Fig. 1). Four patients in the study group (2.4%) and six participants in the control group (3.6%) tested positive for *Ureaplasma* spp. by real-time PCR, but negative by microbial culture, thus were excluded from the study.

Of 165 patients included in the study group, 60 (36.4%) were tested positive both in real-time PCR and microbial culture for *Ureaplasma* spp., whereas in the control group 19 (11.5%) individuals were *Ureaplasma* positive. There was a significant

![Fig. 1. Colonies of *Ureaplasma* spp. growing on Urea-PPLO agar medium after 3 days of incubation. The colonies are between 10 and 50 μm in diameter, round, coarsely granular with a rough edge (typical “Sea urchin” morphology), and a brownish appearance. Arrows show the colonies. (Magnification: a: 100×; b: 200×; c: 400×).](image-url)
difference between these two groups in the frequency of these bacteria \( (p < 0.001) \), and the odds ratio (OR) was 4.4 (95% confidence interval (CI) = 2.5–7.8). Most of the infected patients in both study (50.0%) and control (52.6%) groups were in 30–35 years age-group.

Semen samples of three patients (5% of positive patients) in the study group were still positive by real-time PCR and microbial culture, one month after treatment completion though, with increased Ct values in real-time PCR. Their semen parameters were also still in abnormal range. The treatment of these patients continued taking the same antibiotic with the same dose for one more week. After one month of the second treatment completion, two patients were resolved from the infection but one of them was still positive for *Ureaplasma* spp. with both real-time PCR and culture methods. This patient was treated with azithromycin for five days (500 mg on first day followed by once daily for next four days); after that, the patient was resolved from infection. The semen parameters of all these three patients, after eradication of the infection, were improved and reached the WHO normal ranges.

We used qualitative real-time PCR; however, comparison of Ct value between the groups showed that the mean Ct value for 60 infected patients in the study group was 27.5 ± 0.3, and for 19 infected participants in the control group was 32.7 ± 0.3; which indicates the higher value in the control group \( (p < 0.001) \).

**Semmen parameters**

Table 1 compares the semen parameters between study and control groups. There was statistically significant difference in all of the semen parameters, except the volume, pH, and viscosity between the groups.

Comparison of semen parameters in infected patients of the study group, before and after the antibiotic therapy, is presented in Table 2, which indicates the statistically significant difference in all of the semen parameters, except in the volume and pH, before and after the antibiotic therapy. These data indicate that the semen parameters have been improved after the treatment. Antibiotic treatment also eliminated the leukocytes (WBCs) in seminal fluid of the patients in the study group. Moreover, most of the parameters reached their normal range after antibiotic therapy, as well (Table 3).

The wives of 37 infected patients (61.7%) in the study group became pregnant after an average of six months of antibiotic treatment completion. Their semen parameters as well as ROS and TAC levels had been reached WHO normal ranges.

### Table 1

Comparison of semen parameters between the study and control groups.

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>Study group* (Infertile men) ( N = 165 )</th>
<th>Control group (Fertile men) ( N = 165 )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>3.1 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Semen pH</td>
<td>7.7 ± 0.0</td>
<td>7.8 ± 0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>33.9 ± 2.0</td>
<td>84.8 ± 2.7</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Total sperm count (million)</td>
<td>108.8 ± 8.2</td>
<td>264.6 ± 11.6</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Total sperm motility class A + B + C (%)</td>
<td>22.7 ± 0.6</td>
<td>66.5 ± 0.9</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Progressive motility class A (%)</td>
<td>1.1 ± 0.1</td>
<td>9.3 ± 0.6</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Progressive motility class B (%)</td>
<td>13.0 ± 0.6</td>
<td>39.0 ± 0.6</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Total progressive motility class A + B (%)</td>
<td>14.3 ± 0.6</td>
<td>49.0 ± 0.5</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Non-progressive motility class C (%)</td>
<td>8.6 ± 0.4</td>
<td>17.4 ± 0.7</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Normal sperm morphology (%)</td>
<td>3.4 ± 0.2</td>
<td>5.5 ± 0.1</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>WBC (x/million/ml)</td>
<td>0.3 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>ROS (RLU/s × 10⁶ sperm)</td>
<td>11.3 ± 1.6</td>
<td>5.2 ± 0.1</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>TAC (mmol/µl)</td>
<td>69.4 ± 1.3</td>
<td>73.2 ± 1.1</td>
<td>0.03</td>
</tr>
<tr>
<td>ROS/TAC ratio</td>
<td>0.2 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>(&lt; 0.001)</td>
</tr>
</tbody>
</table>

ROS: reactive oxygen species; TAC: total antioxidant capacity; WBCs: white blood cells. Data are presented as mean ± standard error of the mean (SEM); \( p < 0.05 \) is considered statistically significant.

* Study group (infertile men) before antibiotic therapy.

Comparison of semen viscosity (normal, somewhat thick, or thick) showed no statistically significant difference neither between the study and control groups nor before and after the treatment. Most of the semen samples in the study as well as the control group had normal viscosity (88.5% and 92.7% for study and control groups, respectively).

**ROS and TAC analyses**

As shown in Tables 1 and 2, semen ROS level was higher in the study group rather than the control group \( (p < 0.001) \), and significantly reduced in the study group after the antibiotic therapy \( (p = 0.003) \). TAC value was lower in the study group compared with the control group \( (p = 0.003) \), which increased after antibiotic treatment in the study group \( (p < 0.001) \). The ROS/TAC ratio was higher in the study group in comparison with the control group \( (p < 0.001) \), which decreased after the treatment in the study group \( (p = 0.003) \).

### Discussion

*Ureaplasma* species, along with the other urogenital mycoplasmas are the smallest self-replicating organisms primarily associated with mucosal surfaces, inhabiting the urogenital tracts of their hosts in close relation with epithelial cells. Their adhesion to the host cells is a prerequisite for colonization and subsequent infection (Waites et al., 2005, 2009). They may invade and reside intra-cellularly in the host cells; such intracellular localization may give them the ability to cause chronic infections, and to evade the host immune response (Ammar et al., 2014; McAuliffe et al., 2006; Waites et al., 2005).

These bacteria are considered to be commensal microorganisms silently colonizing the genitourinary tract; hence, the majority of infected individuals will be clinically asymptomatic carriers, remaining undetected and consequently untreated (Pannekoek et al., 2000). However, under certain conditions, the bacteria could be opportunistic pathogens, that may cause dysfunction of accessory sex glands (Gimenes et al., 2014).

In the present study, we investigated the genus *Ureaplasma* without discriminating between *U. urealyticum* and *U. parvum*. Most studies have discussed the role of ureaplasmas in male infertility without distinguishing between these species, as well (Gimenes et al., 2014).

In our study, *Ureaplasma* species were detected of a higher frequency in the semen of infertile men (36.4%) in comparison to healthy control (fertile) men (11.5%), with the OR being 4.4 (95%
The detection rate for Ureaplasma species in infertile men was more than three-fold higher than that in the healthy fertile men which is in accordance with Lee et al. (2013) who reported that the detection rate for Ureaplasma spp. in infertile men was approximately two-fold higher than that in fertile ones. Moreover, in real-time PCR, we found that the mean Ct value for detection of these bacteria was significantly higher in infected healthy fertile men compared with infected infertile ones, indicating that the copy number of Ureaplasma DNA in semen samples of infected fertile men increased in the presence of these bacteria and male infertility.

The prevalence of Ureaplasma species in semen samples of infertile males varies from 5% to 42% in different studies (Abusarah et al., 2013; Wang et al., 2006b); our result of Ureaplasma prevalence rate in the study group (infertile male) is also in this range. The frequency of these bacteria in semen samples of healthy fertile men in the literature ranges from 0% to 26.1% (Al-Sweih et al., 2012; Gnarpe and Friberg, 2010; Al-Sweih et al., 2012; Gnarpe and Friberg, 2010). The frequency of these bacteria in semen samples of healthy fertile men ranges from 0% to 26.1% (Al-Sweih et al., 2012; Gnarpe and Friberg, 2010; Al-Sweih et al., 2012; Gnarpe and Friberg, 2010). These findings suggest a correlation between these bacteria and male infertility.

The prevalence of Ureaplasma species in semen samples of infertile men was more than three-fold higher than that in the healthy fertile men which is in accordance with Lee et al. (2013) who reported that the detection rate for Ureaplasma spp. in infertile men was approximately two-fold higher than that in fertile ones. Moreover, in real-time PCR, we found that the mean Ct value for detection of these bacteria was significantly higher in infected healthy fertile men compared with infected infertile ones, indicating that the copy number of Ureaplasma DNA in semen samples of infected infertile men was higher in comparison to that of infected healthy fertile men; these findings suggest a correlation between these bacteria and male infertility.

The prevalence of Ureaplasma species in semen samples of infertile males varies from 5% to 42% in different studies (Abusarah et al., 2013; Wang et al., 2006b); our result of Ureaplasma prevalence rate in the study group (infertile male) is also in this range. The frequency of these bacteria in semen samples of healthy fertile men in the literature ranges from 0% to 26.1% (Al-Daghistani and Abdel Dayem, 2010; Al-Sweih et al., 2012; Gnarpe and Friberg, 1972; Huang et al., 2015; Lee et al., 2013; Liu et al., 2014; Peerayeh et al., 2008; Xu et al., 1997; Zeighami et al., 2009). However, all of these studies except one (Al-Sweih et al., 2012), confirmed our findings of the higher frequency of these bacteria in the infertile men in comparison to the healthy fertile ones.

The variability in prevalence rates of Ureaplasma species in both fertile and infertile men, reported in different countries, is perhaps due to a variation in ethnic and social populations, differences in detection methods, types of samples studied (semen, urethral swab, first void urine), sample sizes, hygiene issues, socio-economic status, age of participants, and absence of regular screening, treatment, and control programs, particularly in some of the developing countries (Ahmadi et al., 2016).

The highest prevalence of urogenital Ureaplasmas in our study was observed in 30–35 years age-group in both infertile and healthy fertile men. However, most of the participants in both groups were also aged between 30 and 35 years. Other studies have reported a higher positive rate in young-aged patients as well (Gupta et al., 2009; Pónyai et al., 2013).

In the present study, four participants in the study group and six in control group found positive for Ureaplasma spp. by real-time PCR, but negative by microbial culture. As we wanted to include just individuals infected by viable bacteria and because DNA of dead bacteria may still be detected by real-time PCR, these participants were excluded from the study.

Tetracyclines (particularly doxycycline) are the first-line treatment and primary choice in empirical therapy of urogenital infections due to urogenital mycoplasmas, including Ureaplasma spp. (Cazanave et al., 2012; Song et al., 2014). Doxycycline along with azithromycin remains the reference treatment for acute NGU in males and for cervicitis in females, according to the 2009 European and 2008 French guidelines (Cazanave et al., 2012). However, some of the ureaplasmal strains isolated from patients may be resistant to tetracyclines, perhaps due to the acquisition of a streptococcal tetM gene (Taylor-Robinson and Bebear, 1997).

In the present study, three patients (5% of positive patients) weren’t resolved from the infection after the first treatment with doxycycline and their semen parameters were not improved as well. However, after the second treatment by the same dose of doxycycline, the infection was eradicated from two patients, but one patient still was positive for Ureaplasma spp. perhaps due to the presence of the antibiotic resistance mentioned. The treatment of these patients was continued taking azithromycin for five days (500 mg on first day followed by once daily for next four days). After this treatment, the patient resolved from infection and their semen parameters were improved and reached WHO normal ranges. Because the bacteria may be transmitted between the couples (Ahmadi et al., 2016), in all treatment procedures, the female partner of the patient was also treated by the same antibiotic with the same dose. However, because of ethical issues, neither could we perform this study as randomized controlled trial nor use placebo for treatment of the infected patients.

Some investigators reported that infection with Ureaplasma species may decrease sperm concentration (Wang et al., 2006b; Zeighami et al., 2009), motility and/or morphology (Al-Daghistani et al., 2010; Gdoura et al., 2008; Xu et al., 1997), thereby causing male infertility. These findings are in accordance with our results.

Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of semen parameters in infected patients of the study group (infertile men) before and after the antibiotic therapy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen parameters</td>
<td>Before treatment N = 60</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Semen pH</td>
<td>7.7 ± 0.0</td>
</tr>
<tr>
<td>Sperm count (million/ml)</td>
<td>34.2 ± 3.0</td>
</tr>
<tr>
<td>Total sperm count (million)</td>
<td>100.0 ± 10.9</td>
</tr>
<tr>
<td>Total sperm motility class A + B + C (%)</td>
<td>23.5 ± 0.9</td>
</tr>
<tr>
<td>Progressive motility class A (%)</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Progressive motility class B (%)</td>
<td>12.9 ± 0.9</td>
</tr>
<tr>
<td>Non-progressive motility class C (%)</td>
<td>13.8 ± 0.9</td>
</tr>
<tr>
<td>Total progressive motility class A + B (%)</td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td>Normal sperm morphology (%)</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>WBCs (million/ml)</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>ROS (RLU/s/10⁶ sperm)</td>
<td>18.6 ± 4.3</td>
</tr>
<tr>
<td>TAC (nmol/µl)</td>
<td>65.9 ± 1.8</td>
</tr>
<tr>
<td>ROS/TAC ratio</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

ROS: reactive oxygen species; TAC: total antioxidant capacity; WBCs: white blood cells. Data are presented as mean ± standard error of the mean (SEM); p < 0.05 is considered statistically significant.

Table 3

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Rate of semen parameters reaching WHO normal ranges after antibiotic therapy in Ureaplasma positive infertile men (study group).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen parameters</td>
<td>Reach to the normal range</td>
</tr>
<tr>
<td>Sperm count*</td>
<td>14/18 (77.8%)</td>
</tr>
<tr>
<td>Total sperm count</td>
<td>16/18 (88.9%)</td>
</tr>
<tr>
<td>Total sperm motility (class A + B + C)</td>
<td>50/60 (83.3%)</td>
</tr>
<tr>
<td>Progressive motility class A</td>
<td>18/60 (30%)</td>
</tr>
<tr>
<td>Progressive motility class B</td>
<td>55/60 (91.7%)</td>
</tr>
<tr>
<td>Total progressive motility (class A + B)</td>
<td>51/60 (85%)</td>
</tr>
<tr>
<td>Non-progressive motility class C</td>
<td>43/60 (71.7%)</td>
</tr>
<tr>
<td>Normal sperm morphology</td>
<td>42/60 (70%)</td>
</tr>
</tbody>
</table>

* Values of sperm count/ml and total sperm count of only 18 Ureaplasma positive patients in the study group were lower than the WHO normal ranges before antibiotic therapy.

CI = 2.5 – 7.8. The detection rate for Ureaplasma species in infertile men was more than three-fold higher than that in the healthy fertile men which is in accordance with Lee et al. (2013) who reported that the detection rate for Ureaplasma spp. in infertile men was approximately two-fold higher than that in fertile ones. Moreover, in real-time PCR, we found that the mean Ct value for detection of these bacteria was significantly higher in infected healthy fertile men compared with infected infertile ones, indicating that the copy number of Ureaplasma DNA in semen samples of infected infertile men was higher in comparison to that of infected healthy fertile men; these findings suggest a correlation between these bacteria and male infertility.
of the present study. However, others did not find any correlation between the presence of these bacteria and alterations in semen parameters (Andrade-Rocha 2003; Bornman et al., 1990; Gdoura et al., 2008; Wang et al., 2006b). The controversies and differences in results may be due probably to a variation in sample sizes, study settings, detection methods, studied population, and/or presence of some interventional factors not being excluded. Other possible reasons for the debates may be attributed to the diversity in pathogenicity of the infecting strains and/or differences in immunity and microbiome of the various studied populations (Citti and Blanchard 2013).

Pajovic et al. (2013) reported that 30 days after antibiotic treatment completion of asymptomatic Ureaplasma-related pyospermia, sperm concentration significantly increased and progressive motility greatly improved. Our results indicate that all of the semen parameters, except volume, pH, and viscosity, have significantly improved after antibiotic treatment. In addition, most of the improved parameters (≥70%) reached their normal range, according to the WHO criteria, after antibiotic therapy as well. The only exception was the progressive motility class A, only 30% of which reached its normal range. Other investigators reported similar results in terms of Ureaplasma species influences on the semen parameters of infertile men, including decrease in sperm count (Al-Sweih et al., 2012; Lee et al., 2013; Liu et al., 2014), total/progressive motility (Al-Daghistani and Abdel-Dayem, 2010; Al-Sweih et al., 2012; Lee et al., 2013; Nunez-Calonge et al., 1998), and/or normal morphology (Zhang et al., 2011).

However, we found no significant differences in semen volume, pH, and/or viscosity neither between the infertile and healthy fertile men, nor before and after the therapy. These findings are in agreement with Gdoura et al. (2007). In contrast, some researchers reported a high semen viscosity (Al-Sweih et al., 2012), and a decline in semen pH (Abusarah et al., 2013) and/or volume (Zeighami et al., 2009) in infertile men who were positive for Ureaplasma species.

Al-Sweih et al. (2012) reported that the presence of Ureaplasma was associated, in infected compared with uninfected individuals, with a higher leukocyte count. In our study, the number of WBCs was higher in the infected infertile men rather than the infected fertile ones. WBCs count decreased and the leukocytes were eliminated from semen specimens after the antibiotic therapy.

Some investigators have demonstrated and confirmed the adherence of Ureaplasma spp. to spermatozoa through the head and midpiece of these cells (Nunez-Calonge et al., 1998; Shalika et al., 1996; Xu et al., 1997). This phenomenon may help to explain the associated motion abnormalities in infected individuals that may impair sperm function (Potts et al., 2000) and lead to male infertility.

Moreover, these bacteria produce reactive oxygen species, which can induce lipid peroxidation, thereby reduce membrane fluidity and sperm fertilization capability, that may be another mechanism by which Ureaplasma impairs sperm function (Potts et al., 2000) and causes male infertility. Moreover, an inverse relationship has been observed between TAC and lipid peroxidation due to the ROS production and the imbalance between ROS production and TAC in seminal fluid has been hypothesized as a cause of oxidative stress and subsequent injury to the sperm membrane and is correlated with male infertility (Sharma et al., 1999; Smith et al., 1996).

In the present study, seminal ROS level significantly reduced, whereas the level of seminal TAC elevated after the antibiotic therapy in the infected infertile men. In addition, ROS/TAC ratio reduced post-treatment as well. Consequently, female partners of 37 infected patients (61.7%) became pregnant after an average of six months of antibiotic treatment completion and resolving from the infection.

Conclusions

The results of the present study indicate that asymptomatic infections caused by Ureaplasma species are correlated with male infertility, and that antibiotic therapy can improve the semen parameters and fairly treat Ureaplasma-induced infertility. Moreover, our findings denote that a considerable amount of urogenital infections caused by Ureaplasma spp. is asymptomatic and therefore remains undetected and consequently untreated; while these infections may be the cause of some cases of unexplained infertility in males/couples. Because most asymptomatic infected people do not seek medical care and treatment, the infection may persist and be transmitted to the sexual partner(s). This highlights the necessity of planning national programs for adequate diagnosis of and screening for asymptomatic genitourinary tract infections due to these bacteria and for treatment of infected individuals (including sexual partners) to control sexually transmitted infections (STIs) and prevent consequent complications, reduce the carrier rate, and maintain reproductive health and the potential for fertility.

Conflict of interest statement

The authors have no conflicts of interests to declare.

Acknowledgments

This research has been supported by Tehran University of Medical Sciences & Health Services (grant No. 93-03-30/26350) and Royan Institute for Reproductive Biomedicine (grant No. 93/19/80024).

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