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ORIGINAL ARTICLE

Ureaplasma urealyticum and *Mycoplasma hominis* infections and semen quality in 19,098 infertile men in China

Chuan Huang[1] · Xingyu Long[2] · Shuang Jing[1] · Liqing Fan[1,2] · Kongrong Xu[1] · Siyang Wang[1] · Wenbing Zhu[1,2]

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Abstract
Objective This study aimed to determine the incidence of *Ureaplasma urealyticum* and *Mycoplasma hominis* infections in infertile and fertile men and to investigate their effects on the semen quality. The study also aimed to analyze the drug susceptibility of *UU* and *MH* to provide guidance for reasonable antibiotic use.
Methods A total of 19,098 semen specimens were obtained from infertile men at our hospital from January to December 2014. In addition to these specimens, 3368 semen specimens of sperm were obtained from donors at the sperm bank of our hospital from January 2011 to December 2014. Semen analysis was performed using the methods outlined by the World Health Organization.
Results The prevalence of *UU* and *MH* significantly differed between infertile and fertile men. The mean progressive motility, total motility, and normal forms in the semen samples of infertile males positive for *UU* significantly differed from the corresponding values of uninfected men. However, the semen parameters did not differ between *MH*-infected and uninfected men. In the antibiotic sensitivity test, *UU*, *MH*, and *UU* mixed with *MH* were all found susceptible to doxycycline and josamycin with drug resistance rates below 6 %, but both species were highly resistant to ciprofloxacin.
Conclusions Clinical assessment revealed a significant relationship between *UU* and *MH* infections and male infertility. *UU* was found to significantly affect sperm quality, but this was not the case with *MH*. Doxycycline and josamycin should be preferred for clinically treating *UU* and *MH* infections.

Keywords Male infertility · *Ureaplasma urealyticum* · *Mycoplasma hominis* · Infection · Susceptibility

Introduction

Infertility, defined as the inability to achieve pregnancy after 1 year of regular intercourse [1], affects 15 % of couples in reproductive age. About 10–20 % of the inability to achieve pregnancy can be explained by male infertility and 30–40 % is explained by both male and female infertility [2]. Many factors can cause male infertility, and approximately 15 % of male infertility is associated with the infection in the genital tract [3]. *U. urealyticum* and *M. hominis* are commonly found in the genital tract of patients experiencing symptoms including infertility, orchitis, epididymitis, prostatitis, and nongonococcal urethritis [4, 5] as well as in asymptomatic subjects [6, 7].

For about a decade, *U. urealyticum* and *M. hominis* infections have been recognized as a common sexually transmitted disease (STD) in developed countries [8]. These microorganisms naturally inhabit the male urethra and contaminate the semen during ejaculation. However, both these microorganisms, particularly *U. urealyticum*, are potential pathogens that play etiologic roles in both genital infections and male infertility [9, 10]. Several studies have analyzed the relationship between *U. urealyticum* and *M. hominis* infections and semen quality, and some

✉ Wenbing Zhu
zhuwenbing0971@sina.com

[1] Institute of Reproductive and Stem Cell Engineering, Central South University, Changsha 410078, China

[2] The Reproductive and Genetic Hospital of CITIC-Xiangya, No. 87, Xiangya Road, Changsha 410008, Hunan, China

have demonstrated that these infections alter various characteristics of semen, such as sperm motility, density, and morphology, and that antibiotic treatment can improve the semen quality [11, 12]. On the contrary, other researchers did not find that *U. urealyticum* and *M. hominis* infections affect either semen quality or male infertility [13]. Contrary to the evidence that these microorganisms play an important role in the pathogenesis of human infertility, no convincing causal relationships between the infections and human infertility have yet been established.

In the present study, we examined the prevalence of *U. urealyticum* and *M. hominis* infections in infertile and fertile men and their effect on semen quality by analyzing the morphology, semen volume, pH value, sperm concentration, progressive motility, and total motility. Furthermore, the drug resistance of these microorganisms was evaluated.

Materials and methods

Study population

Ethics approval from the regional ethics committee has been granted for the focus groups with infertile and fertile men. A total of 19,098 men of infertile couples who visited the Reproductive Center, the Reproductive and Genetic Hospital of CITIC, Xiangya, China, from January to December 2014 were enrolled in this study. The patients were enrolled for semen analysis following a failure to impregnate their wives after at least 1 year of unprotected sexual intercourse. Past history of infections and sexual history were obtained from all patients. The following exclusion criteria were applied: (1) reproductive system abnormalities, known hereditary and/or familial disorders; (2) heavy alcohol use (>60 g/day); (3) heavy smoking (>20 cigarettes/day); and (4) exposure to physical or chemical agents with known negative reproductive effects. The control group included 3368 fertile men who donated sperm at the sperm bank from January 2011 to December 2014. Men whose semen parameters were normal and/or those whose wives had nonassisted pregnancies in the past were considered fertile.

Ureaplasma urealyticum and *M. hominis* detection and antimicrobial susceptibility testing

Mycoplasma IST (Crest, China) was used for the detection and antimicrobial susceptibility testing of both microbial species. Urethral samples were fed on lyophilized medium to diagnose *U. urealyticum* and *M. hominis* infections and drug susceptibilities. The samples were homogenized by slight rotation. Then, 100 μL of the samples was transferred to microplates containing antibiotics and proliferation wells separately for the qualitative detection of *U. urealyticum* and *M. hominis*. One drop of sterile mineral oil was added to each well to generate anaerobic conditions for culture. Microplates were closed with caps and incubated at 37 °C for 24 and 48 h, respectively. For detection, *U. urealyticum* and *M. hominis* growth could be detected using the indicator phenol red in the medium. Both strains were tested for susceptibility to thiamphenicol, doxycycline, erythromycin, azithromycin, clarithromycin, josamycin, levofloxacin, ciprofloxacin, roxithromycin, and gatifloxacin.

Semen collection

Semen samples were collected by masturbation into a sterile container after 3–5 days of abstinence. The samples were liquefied at 37 °C for about 30 min in an incubator before analysis.

Semen analysis

The semen was analyzed using the methods outlined by the World Health Organization (WHO, 5th edition), and the volume, pH, progressive motility (PR), total motility (PR + NP), sperm concentration, normal forms, and total motile sperm count (TMC) were determined.

Statistical analysis

SPSS 19.0 statistical software was used for statistical analysis. The results are reported as mean ± standard deviation unless otherwise indicated. The *t* test and chi-square test were used to compare continuous variables and categorical variables, respectively. A statistically significant difference was defined when the P value was <0.05.

Results

Demographic characteristics

A total of 19,098 semen samples were taken from patients aged 23–44 years (28.6 ± 7.2), individuals in the fertile group were those who were aged 20–35 years (26.9 ± 5.5), no differences were found in the age between fertile and infertile groups ($P = 0.16$), and the groups did not differ significantly in frequency of sexual intercourse, abstinence times, BMI, alcohol drinking, and smoking (Table 1).

Prevalence of *U. urealyticum* and *M. hominis* in infertile and fertile men

The prevalence of these pathogens significantly differed between infertile and fertile men (Table 2).

Table 1 Demographic characteristics of participants

Demographic variables	Infertile man	Fertile man	*P* value
Age	28.6 ± 7.2	26.9 ± 5.5	0.16
BMI <25/BMI >25	2611/757	14,883/4215	0.60
Nonsmoker/ever smoked	2832/536	16,202/2896	0.26
Nondrinkers/irregular or regular drinker	2962/406	16,857/2241	0.59
Abstinence times	3.6 ± 0.26	3.7 ± 0.47	0.48
Frequency of sexual intercourse (a week)	2.6 ± 3.02	2.2 ± 2.79	0.18

BMI body mass index

Table 2 Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* infections in infertile and fertile men

Species	Infertile man ($n = 19{,}098$)		Fertile man ($n = 3368$)		*P* value
	N	%	*N*	%	
UU only	1951	10.22	123	3.65	<0.001**
MH only	604	3.16	37	0.89	<0.001**
UU and MH	343	1.80	16	0.48	<0.001**
Total	2898	15.17	176	5.23	<0.001**

UU, *Ureaplasma urealyticum*; MH, *Mycoplasma hominis*

Table 3 Comparison of seminal parameters in infertile and fertile men

Variable	Infertile group Mean ± SD	Fertile group Mean ± SD	*P* value
Volume (mL)	3.26 ± 1.57	3.32 ± 1.46	0.624
PH	7.18 ± 0.87	7.17 ± 0.06	0.861
Sperm concentration ($\times 10^6$/mL)	53.66 ± 30.08	65.74 ± 5.71	<0.001**
Progressive motility, PR (%)	27.41 ± 17.50	53.65 ± 3.84	<0.001**
Total motility, PR + NP (%)	42.83 ± 19.26	71.87 ± 4.93	<0.001**
Normal forms (%)	6.13 ± 1.34	12.46 ± 1.78	<0.001**
TMC	91.27 ± 77.83	152.94 ± 32.72	<0.001**

The prevalence of *U. urealyticum*-positive specimens among urethral specimens in infertile and fertile men was 10.22 % (1951/19,098) and 3.65 % (123/3368; χ^2, 147.203; $P < 0.001$), respectively. *M. hominis* was detected in 3.16 % (604/19,098) and 0.89 % (37/3368) of infertile and fertile men, respectively (χ^2 test, 41.490; $P < 0.001$). Finally, 1.8 (343/19,098) and 0.48 % (16/3368) of fertile men were found to harbor mixed pathogens (χ^2, 31.771; $P < 0.001$).

Effects of *U. urealyticum* and *M. hominis* on semen quality

The semen variables in the infertile and fertile men are summarized in Table 3. The mean values of sperm concentration, progressive motility, total motility, normal forms, and TMC were significantly lower in infertile men than in fertile men. To identify the effects of *U. urealyticum* and *M. hominis* on semen, we compared semen parameters of the infected and uninfected subjects. Some of the parameters including sperm concentration, progressive motility, total motility, normal forms, and TMC tended to be lower in the infected group than in the uninfected group, although the differences were not significant in the case of *M. hominis*-infected and uninfected individuals ($P > 0.05$; Table 4). The progressive motility, total motility, and normal forms showed significant difference between the *U. urealyticum*-infected, mixed infection, and uninfected groups ($P < 0.05$). The TMC was significantly lower in the mixed infection group ($P = 0.017$) than in the uninfected group.

Drug susceptibility test results

Table 5 presents the antibiotic resistance profiles of each microorganism. As shown in the table, 1857 (95.2 %) and 1851 (94.9 %) of the 1951 patients with *U. urealyticum* infection were susceptible to doxycycline and josamycin, respectively. Furthermore, 1709 (87.6 %) of the 1951 patients with *U. urealyticum* infection were resistant to levofloxacin, and 1826 (93.6 %) were resistant to ciprofloxacin. A higher proportion of patients (330/343; 96.2 %) with both *U. urealyticum* and *M. hominis* showed higher resistance to ciprofloxacin at higher critical concentrations. Mixed infections were more susceptible to doxycycline and josamycin than either *U. urealyticum* or *M. hominis* infection alone. Mixed infections were more resistant to ciprofloxacin.

Discussion

The relationship between *U. urealyticum* and *M. hominis* infection and male infertility has been studied widely; however, the results remain controversial. Most patients with these infections are not aware of their infections because they do not experience any symptoms. These inconsistencies are probably because of small sample sizes and confounding factors, such as social and economic factors and sexual activity. Meanwhile, different populations have different susceptibility, which may be also one of the reasons for this controversy. China is one of the most populous countries in the world. The rapid pace of economic and social change in China over the past two decades has

Table 4 Effects (mean ± SD) of *Ureaplasma urealyticum* and *Mycoplasma hominis* infections on seminal variables in infertile men

Variable	*U. urealyticum* Mean ± SD (*P* value)	*M. hominis* Mean ± SD (*P* value)	Mixed infection Mean ± SD (*P* value)	Uninfected Mean ± SD
Volume (mL)	3.27 ± 1.73 (0.680)	3.53 ± 1.86 (0.369)	2.95 ± 1.17 (0.629)	3.17 ± 1.67
PH	7.19 ± 0.09 (0.528)	7.19 ± 0.02 (0.593)	7.18 ± 0.23 (0.635)	7.16 ± 0.12
Sperm concentration ($\times 10^6$/mL)	52.16 ± 38.87 (0.886)	51.85 ± 38.84 (0.756)	50.80 ± 35.06 (0.184)	55.43 ± 36.05
Progressive motility, PR (%)	24.55 ± 13.91 (0.019)*	27.98 ± 12.23 (0.650)	21.82 ± 12.65 (<0.001)**	28.77 ± 13.50
Total motility, PR + NP (%)	42.91 ± 19.05 (0.022)*	1945.68 ± 16.28 (0.449)	42.07 ± 15.55 (0.013)*	47.27 ± 14.71
Normal forms (%)	4.38 ± 1.52 (<0.001)**	6.45 ± 1.41 (0.113)	4.79 ± 1.72 (0.009)*	6.79 ± 1.72
TMC	81.99 ± 68.81 (0.078)	90.38 ± 79.62 (0.500)	72.45 ± 68.29 (0.017)*	98.86 ± 79.99

TMC total motile sperm count = volume (mL) × concentration ($\times 10^6$/mL) × motility (%)

As compared with uninfected group * $P < 0.05$; ** $P < 0.001$

Table 5 Susceptibility of *U. urealyticum* and *M. hominis* to ten different antibiotics

Antibiotic	*U. urealyticum* ($n = 1951$)		*M. hominis* ($n = 604$)		Mixed infection ($n = 343$)	
	S Case (%)	R Case (%)	S Case (%)	R Case (%)	S Case (%)	R Case (%)
Azithromycin	1187 (60.8)	764 (39.2)	279 (46.2)	325 (53.8)	103 (30.0)	240 (70.0)
Levofloxacin	242 (12.4)	1709 (87.6)	92 (15.2)	512 (84.8)	31 (9.0)	312 (91.0)
Clarithromycin	1356 (69.5)	595 (30.5)	378 (62.6)	226 (37.4)	119 (44.7)	224 (65.3)
Ciprofloxacin	125 (6.4)	1826 (93.6)	67 (11.1)	537 (88.9)	13 (3.8)	330 (96.2)
Doxycycline	1857 (95.2)	94 (4.8)	573 (94.9)	26 (5.1)	335 (97.7)	8 (2.3)
Erythrocin	901 (46.2)	1050 (53.8)	209 (34.6)	395 (65.4)	113 (32.9)	230 (67.1)
Josamycin	1851 (94.9)	100 (5.1)	584 (96.7)	20 (3.3)	337 (98.3)	6 (1.7)
Roxithromycin	967 (49.6)	984 (50.4)	248 (41.1)	356 (58.9)	92 (26.8)	251 (73.2)
Thiamphenicol	502 (25.7)	1449 (74.3)	148 (24.5)	456 (75.5)	47 (13.7)	296 (86.3)
Gatifloxacin	793 (40.6)	1158 (59.4)	229 (37.9)	375 (62.1)	98 (27.6)	245 (71.4)

S susceptible, *R* resistant

been accompanied by an increased rate of infertility [14, 15]. In addition, antibiotic abuse will lead to drug resistance. As a result, the treatment options for *U. urealyticum* and *M. hominis* infections are becoming more and more limited. Our study illustrates an important link between the presence of these microorganisms and infertility in men and provides guidance for reasonable use of antibiotics in China.

Although there is considerable disagreement on the exact association of *U. urealyticum* and *M. hominis* with male infertility, it is generally agreed that the prevalence of both these infections is higher in infertile men than in fertile men [16–19]. Our study demonstrated a statistically significant higher detection rate of *U. urealyticum* in the urethral specimens from infertile men (10.22 %) compared to fertile men (3.65 %) and of *M. hominis* in the urethral specimens from infertile men (3.16 %) compared to fertile men (0.89 %). The detection rates for *U. urealyticum* and *M. hominis* in infertile men were approximately threefold higher than the corresponding rates in fertile men. *U. urealyticum* is the smallest free-living organism that most commonly inhabits the urogenital tract and has been found in male seminal fluids with a prevalence ranging from 10 to 42 % [20, 21]. The high rate of *U. urealyticum* detection in this study suggests that it is widespread among infertile men, which is consistent with previous findings [16, 17]. The prevalence of *M. hominis* varies significantly between infertile and fertile men and was low in our study; other studies have also found that *M. hominis* is uncommon in the urethra of men [22]. We were concerned that technical problems and detection method might have resulted in the low prevalence observed in this study. However, our results were in agreement with the findings reported previously, suggesting that the prevalence of *M. hominis* in the urethra of the male Chinese population is indeed low. In our study, *U. urealyticum* and *M. hominis* were often detected in both infertile and fertile men, suggesting that they may have a commensal relationship.

In addition to studying the prevalence of these microorganisms in infertile and fertile men, we also assessed the relationship of these microorganisms with sperm quality. The semen quality is considered to be one of the most significant indicators of male fertility. Previous studies on the effects of *U. urealyticum* and *M. hominis* infections or colonization on semen parameters also show conflicting results. Some studies have reported that these microorganisms have no real effect on the quality of semen [23, 24], but others have demonstrated associations between *U. urealyticum* and *M. hominis* and semen quality [10, 25]. In this study, we evaluated the correlation between progressive motility, total motility, and normal forms and *U. urealyticum* infection in infertile men. Mixed infection of *U. urealyticum* and *M. hominis* was found to be associated with lower progressive motility, total motility, normal forms, and TMC. However, although *M. hominis* infection was associated with a reduction in motility and sperm concentration, we failed to demonstrate any significant correlation between the *M. hominis* infection and semen quality ($P > 0.05$). The results of this study are consistent with previous findings [24, 26]. These microorganisms may diminish fertility by adhering to spermatozoa and directly altering sperm morphology, as well as motility and survival [24].

The susceptibilities of genital mycoplasmas to antimicrobial agents differ by geographic region [27]. The difference in the antimicrobial resistance found in reports from various countries might be due to the differences in the guidelines of antimicrobial usage. Our result showed that both *U. urealyticum* and *M. hominis* had relatively low resistance to josamycin and doxycycline in China. Similar results were found in recent studies by Yang [28], Chen [29], and Yang [30]. Therefore, josamycin and doxycycline should be the primary choice for *U. urealyticum* and *M. hominis* in China.

This study has some limitations. First, the study did not include leukocytes as a variable in the semen parameters. The presence of leukocytes in the semen may be indicative of infection or inflammation; however, there remains controversy about the significance of true leukocytospermia. The role of leukocytes in semen in predicting reproductive performance warrants further study. Second, although the semen volume, pH value, sperm concentration, and other parameters were studied, we did not study the changes in these parameters following antibiotic treatment and we also did not study the pregnancy rates before and after antibiotic treatment; studying the post-treatment changes in these parameters and pregnancy rates may better support our findings. Third, the donated sperm will be chosen for the patients with azoospermia and severe oligozoospermia, but we still included them in the study because the proportion of the patients with azoospermia and severe oligozoospermia was small, and they may still have infections.

At last, *U. urealyticum* and *M. hominis* infections may adversely affect the seminal quality in both infertile and fertile men; however, we did not study the seminal quality of fertile men because this study focused primarily on the effects of *U. urealyticum* and *M. hominis* infections on the seminal quality of infertile men. Future studies could study the seminal quality of both fertile and infertile men with these infections.

In conclusion, this study revealed that *U. urealyticum* and *M. hominis* were widely prevalent in the male population, both infertile and fertile, in China. Our results revealed that *U. urealyticum*, but not *M. hominis*, can negatively influence the seminal quality. Therefore, clinicians should pay close attention to these infections, particularly *U. urealyticum* infection, in infertile men. Our antibiotic susceptibility test results indicate that doxycycline and josamycin should be the primary choice in the empirical treatment of these infections.

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Author contributions Huang C involved in project development, data analysis, and proof reading and wrote the manuscript. Long XY wrote the manuscript and involved in data collection and analysis. Jing S, Xu KR, and Wang SY edited the manuscript and involved in proof reading. Fan LQ and Zhu WB involved in project development.

Compliance with ethical standards

Conflict of interest None.

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