Do *Ureaplasma urealyticum* infections in the genital tract affect semen quality?

Yan Wang¹,², Cui-Ling Liang²,³, Jun-Qing Wu², Chen Xu⁴, Shi-Xiao Qin⁴, Er-Sheng Gao²

¹Shanghai Institute of Planned Parenthood Research, Fudan University, Shanghai 200032, China
²Department of Epidemiology, Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China
³Institute of Population Studies, East China Normal University, Shanghai 200062, China
⁴Department of Histology and Embryology, School of Medicine, Shanghai Jiaotong University, Shanghai 200025, China

Abstract

**Aim:** To investigate the relationship between *Ureaplasma urealyticum* (UU) infection and semen quality. **Methods:** From 2001 to 2003, 346 eligible patients aged 20–45 years were invited from two hospitals in Shanghai, China, to participate in an investigation which included questionnaires about general and reproductive health, an external genital tract examination, UU culture and semen analysis. Multiple linear regression models were used to examine whether UU had a significant effect on semen quality after adjustment for confounding factors. **Results:** Findings suggested that UU infection was associated with higher semen viscosity and lower semen pH value. Sperm concentration was lower in UU positive subjects than that in UU negative subjects (54.04 × 10⁶/mL vs 70.58 × 10⁶/mL). However, UU did not significantly affect other semen quality indexes. **Conclusion:** UU infection of the male genital tract could negatively influence semen quality. (Asian J Androl 2006 Sep; 8: 562–568)

Keywords: *Ureaplasma urealyticum*; semen quality; sperm concentration; seminal plasma; questionnaire; infection

1 Introduction

It is estimated that 15% of male infertility is related to genital tract infection [1]. Among infection microorganisms, *Ureaplasma urealyticum* (UU) is one of the most common species [2]. Since 1972, when Gnarpe and Friberg [3] discovered UU in the semen of patients with infertility of uncertain etiology, many researchers have tried to discover whether semen quality and male infertility were associated with UU infection. Contradictory findings have been reported in a series of studies [4–7]. Some investigators found that UU infection altered various characteristics of semen, such as sperm motility, density and morphology, and antibiotic treatment resulted in semen quality improvement. However, other investigators failed to show that UU had any influence on semen quality or male infertility. These inconsistencies were probably the result of small sample sizes and confounding factors, such as social and economic factors, sexual activity, etc.

In the present study, we examined the effect of UU infection on the parameters of semen under the control of other confounding factors, such as alcohol consumption and spermatorrhea, thus attempting to determine the
association between UU infection and the decreasing semen quality.

2 Materials and methods

2.1 Populations

The sample content was calculated based on sperm concentration. All together, 346 male patients aged 20–45 years who consecutively attended the andrology clinics in Shanghai Tongji Hospital and Shanghai Renji Hospital from March 1, 2001 to March 1, 2003 were invited to participate in the present study. Men who had reproductive system abnormalities (e.g. varicocele), who had been exposed to known toxic agents (e.g. benzene) and who had received antibiotics within the preceding 1 year or infertility therapy were excluded from the study. Consent information was signed before patients entered into the study.

2.2 Questionnaire investigation

The questionnaires contained information on demographic characteristics, previous diseases and reproductive conditions. In addition, the men were asked on their lifestyle, living environment, matters concerning sex and genital tract infection.

2.3 External genital examination

Each subject had a routine external genital examination and the testis volume was measured using a standard measuring model by clinical physicians.

2.4 Semen analysis

Semen samples were obtained from subjects by masturbation after 3–6 days of sexual abstinence and were evaluated for the following parameters: semen appearance, pH value, liquefaction, semen volume, sperm concentration, motility and morphology.

To measure semen viscosity, a glass bar was put into the semen and then pulled out to observe the length of the filament. An abnormal viscosity was more than 2 cm. Acidity was measured using pH paper (from pH 6.1–10.0). Liquefaction, how long seminal plasma took to liquefy, was recorded in 10 minute intervals.

Sperm counts were measured using a hemacytometer. Semen was diluted and one drop (about 10–20 µL) was placed on a microscope slide. Sperms were counted in 5–10 large sections. Sperm concentration per mL was calculated according to the ratio of dilution.

Sperm motility and forward movement were measured by each sample being placed on a microscope slide and being counted in 4–6 fields. The quality of sperm motility was graded on a scale of A–D. Sperms that moved at the speed of 25 µm/s or more were considered to show rapid forward movement. Sperms that moved at the speed of less than 25 µm/s and more than 15 µm/s were considered to show slow forward movement. Sperms that moved at the speed of less than 15 µm/s and more than 4 µm/s were considered to show no movement.

At least 100 sperms were observed after Papanicolaou stain. Sperms were considered abnormal if they included any of the following characteristics: head, body, tail or any combination abnormality.

The viability was the proportion of the living sperms in 100 sperms with eosin stain.

For UU cultivation, 100 µL of the semen specimen was inoculated in UU culture medium available from Shanghai Enkang Corporation (Shanghai, China) for 1–3 days at 37ºC. UU positive was defined as the color of the culture medium changing from light yellow to red, and UU negative was defined as the color of the culture medium not changing or being obviously cloudy.

2.5 Statistical analysis

The following semen variables were used: semen volume, sperm concentration, total sperm count, sperm viability, sperm motility and the percentage of spermatozoa with normal morphology. Data on these variables were not normally distributed, so some transformation was carried out to obtain normality of distribution. The formulae were as follows:

Semen volume' = SQRT (semen volume)
Sperm concentration' = ln (sperm concentration + 25)
Sperm counts' = ln (sperm counts + 60)
Sperm viability' = arcsin \sqrt{\text{sperm viability}}
Normal morphology' = arcsin \sqrt{\text{normal morphology (％)}}
Sperm forward motility' = arcsin \sqrt{\text{sperm forward motility}}

First, the means of the semen variables were compared between the UU positive and negative groups. Then, the adjusted analysis was carried out in multiple linear regression models, taking into account confounding factors, such as age, study center, abstinence time and alcohol consumption. Confounders were excluded stepwise if they were not statistically significant. We also used principal component analysis to analyze the sperm motility parameters.

All the statistical analyses were carried out using SAS.
software version 8.0 (SAS institute Inc. Cary, NC, USA) 

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\( P \) values were two-sided. When multiple regression models were carried out, a 10% level of significance was used, whereas a 5% level was used in other models.

3 Results

3.1 Comparison of general characteristics of subjects between UU positive group and UU negative group

In the present study, 136 of 346 subjects had UU in their genital tracts (39.31%). Table 1 summarizes the general characteristics of subjects between the UU positive group and UU negative group. Apart from residence and alcohol consumption, there were no differences in the subjects’ characteristics, such as age, education and income, between the two groups.

3.2 Influence of UU on semen quality

The semen of men infected with UU was more viscous than that of non-infected men. The proportion of viscosity was 12.50% and 4.76%, respectively, with a significant difference \( (P < 0.01) \). Significantly lower pH values were found in UU positive men in comparison with UU negative men \( (P < 0.05) \), Table 2).

Table 3 summarizes the relationship between UU infection and semen quality. The mean sperm concentrations and sperm counts of UU positive subjects were significantly lower than those of UU negative subjects \( (P < 0.01) \), whereas the means of other indexes, such as volume, viability and morphology in UU negative and positive subjects were almost identical.

3.3 UU effects after adjustment for confounding factors

To further analyze the relationship between UU infection and semen quality, multiple linear regression models were used to adjust the confounding factors and findings suggested UU still had a significant effect on sperm concentration \( (P < 0.05) \). All the risk factors that had statistical effects on semen quality, such as number of years living in Shanghai, alcohol consumption, testis volume, playing Mah-jang, external genitals and so on, are listed in Table 4.

4 Discussion

The presence of UU in the genital tract of men was thought to be chronic and asymptomatic [8], however, accumulated evidence has suggested that asymptomatic UU infection could cause dysfunction of accessory sex glands [9]. The abnormality of their secretion can lead to a change of seminal characteristics. In the present study, high viscosity an the decrease in pH value was found in the UU positive group. This suggested that seminal vesicles blockage or chronic prostatitis was occurring [10, 11]. Consistently thick semen does have an effect on the ability of the sperm to move, so this would also be important if a couple was trying to conceive.

The sperm concentration in the group harboring UU was found to be significantly lower \( (P < 0.01) \). To avoid confounding factors, the multiple linear regression analysis was used to analyze the adjusted mean of sperm concentration in the UU positive and negative group. The result was still significant, as a result, the significance still existed. The present results are in agreement with previous researchers. Upadhyaya et al. [12] observed a significantly lower sperm concentration in 280 men in their UU group, but no change with sperm morphology and viability, and this finding had also been confirmed by other researchers [13].

The reason why UU infection can reduce sperm concentration might be explained by glutamic oxalacetic transaminase (GOT) in the prostate. Because GOT activity has a positive correlation with sperm activity [14] and UU presence could cause prostatitis, there might be certain relationship between the reduction of the sperm concentration and the decrease of GOT activity. However, this finding needs further substantiation.

Animal models have shown that UU infection could block sperm formation. Xu et al. [15] found that germ cell apoptosis of rats was induced by UU infection. This was also the case in humans. Shang et al. [16] found the germ cell apoptosis in 35 infertile men as the result of UU infection was higher than that in the control group.

Despite the fact that UU infection can lead to the reduction of sperm concentration, UU is an organism with weak pathogenicity and is not serious enough to cause oligospermia. It is possible that UU can reduce the carriers’ reproduction levels and delay conception [17].

The present study failed to find whether UU infection has any influence on semen volume, sperm forward motility, viability and sperm with normal morphology, regardless of the presence or absence of other risk factors. Conflicting results regarding the role of UU infection on sperm characteristics have been reported previously.
Table 1. Comparison of general characteristics of subjects between *Ureaplasma urealyticum* (UU) positive group and UU negative group. \(^bP < 0.05\).

| General characteristics          | UU positive group | UU negative group | \(\chi^2\) | P     |
|----------------------------------|-------------------|-------------------|-----------|-------|
| Study center                     |                   |                   |           |       |
| Tongji hospital                  | 20                | 33                | 0.06      | 0.80  |
| Renji hospital                   | 116               | 177               |           |       |
| Age (year)                       |                   |                   |           |       |
| 20 ~                             | 74                | 100               | 1.69      | 0.43  |
| 30 ~                             | 57                | 99                | 47.62     | 47.14 |
| 40 ~ 45                          | 5                 | 11                | 5.24      |       |
| Education                        |                   |                   |           |       |
| Less than high school            | 51                | 63                | 2.28      | 0.32  |
| High school                      | 30                | 56                | 26.67     |       |
| More than high school            | 55                | 91                | 43.33     |       |
| Residence                        |                   |                   |           |       |
| Urban                            | 84                | 154               | 5.15      | 0.02  |
| Rural                            | 52                | 56                |           |       |
| Marriage status                  |                   |                   |           |       |
| Married                          | 133               | 201               | 1.07      | 0.30  |
| Single                           | 3                 | 9                 | 4.29      |       |
| Income (RMB/person/year)         |                   |                   |           |       |
| < 10 000                         | 43                | 57                | 0.83      | 0.66  |
| 10 000 ~ 40 000                  | 52                | 87                | 41.43     |       |
| > 40 000                         | 41                | 66                | 31.43     |       |
| Years living in Shanghai         |                   |                   |           |       |
| < 5                              | 29                | 39                | 0.40      | 0.53  |
| ≥ 5                              | 107               | 171               | 81.43     |       |
| Smoking                          |                   |                   |           |       |
| Yes                              | 67                | 104               | 0.002     | 0.96  |
| No                               | 69                | 106               | 50.48     |       |
| Alcohol consumption              |                   |                   |           |       |
| Yes                              | 60                | 70                | 4.09      | 0.04  |
| No                               | 76                | 140               | 66.67     |       |
| Enuresis                         |                   |                   |           |       |
| Yes                              | 12                | 29                | 1.94      | 0.16  |
| No                               | 123               | 180               | 8.61      |       |
| External genitals                |                   |                   |           |       |
| Normal                           | 118               | 166               | 3.34      | 0.07  |
| Abnormal                         | 18                | 44                | 20.95     |       |
| Infertility within one year      |                   |                   |           |       |
| Yes                              | 87                | 145               | 1.72      | 0.19  |
| No                               | 49                | 60                | 29.27     |       |
| Spermatorrhea                    |                   |                   |           |       |
| Yes                              | 125               | 200               | 2.16      | 0.14  |
| No                               | 11                | 9                 | 4.31      |       |
| Often wearing tight pants        |                   |                   |           |       |
| Yes                              | 27                | 34                | 0.41      | 0.52  |
| No                               | 109               | 176               | 83.81     |       |

(to be continued)
Table 1 (continued)

| General characteristics                  | UU positive group | UU negative group | \( \chi^2 \) | \( P \) |
|------------------------------------------|------------------|------------------|-------------|-------|
| Knowledge on STD prevention              |                  |                  |             |       |
| Correct                                  | 96 (70.59)       | 138 (65.71)      | 0.90        | 0.34  |
| Incorrect                                | 40 (29.41)       | 72 (34.29)       |             |       |
| Sexual activity in latest three months (times/month) |                  |                  |             |       |
| < 4                                      | 21 (15.56)       | 47 (23.62)       | 3.63        | 0.16  |
| 4 – 8                                    | 80 (59.26)       | 112 (56.28)      |             |       |
| > 8                                      | 34 (25.18)       | 40 (20.10)       |             |       |
| Using mobile phone                       |                  |                  |             |       |
| Yes                                      | 108 (80.00)      | 182 (86.67)      | 2.73        | 0.10  |
| No                                       | 27 (20.00)       | 28 (13.33)       |             |       |
| Reproductive system infection            |                  |                  |             |       |
| Yes                                      | 13 (9.56)        | 21 (10.00)       | 0.02        | 0.89  |
| No                                       | 123 (90.44)      | 189 (90.00)      |             |       |

Table 2. Relationship between *Ureaplasma urealyticum* (UU) and seminal plasma characteristics. \( ^*P < 0.05 \).

| Semen characteristics                  | UU positive group | UU negative group | \( \chi^2 \) | \( P \) |
|----------------------------------------|------------------|------------------|-------------|-------|
| Liquescence time (min)                 |                  |                  |             |       |
| \( \leq 60 \)                          | 120 (88.24)      | 192 (91.43)      | 0.95        | 0.33  |
| > 60                                    | 16 (11.76)       | 18 (8.57)        |             |       |
| Viscosity (cm)                         |                  |                  |             |       |
| \( \leq 2 \)                           | 119 (87.50)      | 200 (95.24)      | 6.87        | 0.01* |
| > 2                                     | 17 (12.50)       | 10 (4.76)        |             |       |
| Cohesion                               |                  |                  |             |       |
| No                                      | 123 (90.44)      | 190 (60.48)      | 0.0001      | 0.99  |
| Yes                                     | 13 (9.56)        | 20 (9.52)        |             |       |
| pH value                                |                  |                  |             |       |
| Abnormal (pH < 7.2)                    | 57 (41.91)       | 58 (27.88)       | 7.27        | 0.01* |
| Normal                                  | 79 (58.01)       | 150 (72.12)      |             |       |

Table 3. Relationship between *Ureaplasma urealyticum* (UU) and different characteristics of seminal plasma. \(^{#}\), General Liner Model. –, UU negative group; +, UU positive group.

| Semen characteristics                  | UU              | n  | Mean | SD  | \( F \) | \( P^{#} \) |
|----------------------------------------|-----------------|----|------|-----|--------|------------|
| Volume (mL)                            |                 | 209| 3.42 | 0.15| 2.83   | 0.09       |
| Sperm concentration (10^6/mL)          |                 | 210| 70.58| 23.01| 7.07   | <0.01      |
| Sperm count (10^6)                     |                 | 209| 230.03| 57.77| 10.22  | <0.01      |
| Viability (%)                          |                 | 199| 86.19| 2.23| 0.23   | 0.63       |
| Sperm forward motility (%)             |                 | 136| 28.22| 4.76| 1.12   | 0.29       |
| Morphology (% normal forms)            |                 | 189| 55.45| 2.23| 1.50   | 0.22       |

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Table 4. Multiple linear regression analysis of risk factors for semen quality. UU, Ureaplasma urealyticum; STD, sex transmitted disease. –, UU negative group; +, UU positive group.

| Variable                          | Group (code)                  | β ± SE  | P    |
|-----------------------------------|-------------------------------|---------|------|
| **Semen volume**                  |                               |         |      |
| UU                                | – (0) + (1)                   | -0.06 ± 0.04 | 0.16 |
| Abstinence (day)                  | Scale variable                | 0.11 ± 0.03 | < 0.01 |
| Center                            | Tongji (1) Renji (2)          | 0.07 ± 0.03 | 0.04 |
| Spermatorrhea                     | Yes (1) No (2)                | -0.20 ± 0.09 | 0.03 |
| Body Mass Index                   | Scale variable                | -0.01 ± 0.01 | 0.07 |
| Washing external genitals         | Yes (1) No (2)                | -0.12 ± 0.07 | 0.10 |
| **Sperm concentration**           |                               |         |      |
| UU                                | – (0) + (1)                   | -0.15 ± 0.07 | 0.04 |
| Abstinence (day)                  | Continuous variable           | 0.04 ± 0.01 | < 0.01 |
| Center                            | Tongji (1) Renji (2)          | 0.14 ± 0.06 | 0.01 |
| Living years in Shanghai          | Long time (0) Short time (1)  | 0.28 ± 0.09 | < 0.01 |
| Alcohol drinking                  | Yes (1) No (2)                | 0.16 ± 0.07 | < 0.01 |
| Testis volume (cm³)               | Continuous variable           | 0.02 ± 0.01 | 0.01 |
| Playing Majiang game              | Yes (2) No (1)                | -0.27 ± 0.10 | 0.02 |
| **Sperm forward motility**        |                               |         |      |
| UU                                | – (0) + (1)                   | -0.02 ± 0.02 | 0.34 |
| Center                            | Tongji (1) Renji (2)          | -0.07 ± 0.02 | < 0.01 |
| Abstinence (day)                  | Continuous variable           | -0.01 ± 0.01 | < 0.01 |
| Living years in Shanghai          | Long time (0) Short time (1)  | 0.05 ± 0.03 | 0.09 |
| Using mobile phone                | Yes (1) No (2)                | -0.08 ± 0.03 | 0.01 |
| Age (years)                       | Scale variable                | 0.005 ± 0.003 | 0.07 |
| External genitals                 | Normal(0) Abnormal (1)        | -0.09 ± 0.04 | 0.01 |
| Enuresis                          | Yes (1) No (0)                | 0.08 ± 0.04 | 0.05 |
| Playing Majiang game              | Seldom(1) Usually(2)          | -0.09 ± 0.04 | 0.03 |
| **Sperm with normal morphology**  |                               |         |      |
| UU                                | – (0) + (1)                   | -0.02 ± 0.02 | 0.22 |
| Center                            | Tongji (1) Renji (2)          | 0.11 ± 0.01 | < 0.01 |
| Sexual activity (times)           | Scale variable                | 0.03 ± 0.01 | 0.04 |
| Spouse pregnancy                  | Yes (1) No (2)                | -0.05 ± 0.02 | < 0.01 |
| **Sperm viability**               |                               |         |      |
| UU                                | – (0) + (1)                   | -1.14 ± 1.46 | 0.44 |
| Study center                      | Tongji (1) Renji (2)          | 2.72 ± 1.03 | 0.01 |
| Often wearing tight pants         | Yes (1) No (2)                | 4.04 ± 1.88 | 0.03 |
| Reproductive system Infection     | Yes (1) No (0)                | -8.41 ± 3.61 | 0.02 |
| Knowledge on STD prevention       | Correct (0) Incorrect (1)     | -4.36 ± 1.66 | 0.01 |

Xu et al. [4] reported that UU infection reduced the sperm motility and increased the sperm abnormality rate. Recently, a study found that deteriorated semen density, sperm progressive motility and sperm vitality were associated with UU infection [18]. Conversely, a relationship between UU and sperm morphology, motility and viability was not observed in some literature [5, 6]. The study by Kjaergaard et al. [7] indicated that the presence of UU in the genital tract of men did not impair semen quality. This contradiction could be partly due to the following factors: 1) selection of patients; 2) small sample size; 3) variation in the geographic distribution of the
population; and 4) the presence of confounding factors. Genital UU has been found in a large proportion of infertile people and, therefore, many researchers have proposed that UU might be causing infertility. In the present study, UU infection was associated with higher semen viscosity, higher acid pH of semen and low sperm concentration, whereas UU did not significantly affect other semen quality indexes. However, the sample size in the present study was calculated based on the sperm concentration, so it could not be adequate enough to conclude whether UU infection has any effect on these semen characteristics.

The results of the present study were substantial, as we collected information about a wide range of present exposures probably related to semen quality and controlled them in the multiple regressions. However, there were some limitations in this study. First, the subjects were outpatients of male clinics and they always went to see doctors because their spouses had failed to conceive for a long period of time. About 68.5% of subjects had been infertile for 1 year, so their semen quality might have differed from the general population. In addition, we could not ascertain how long and why they were infected with UU, so the results were not suitable for the general male. Second, according to some reports, various organisms were found to cohabit with UU in the genital tract [19], but the cooperation between UU and other microorganisms was not separated in the present study. However, the subjects of the present study came from clinics and most of them had no knowledge of their genital tract infection, so it was impossible that they were infected with organisms which could cause obvious symptoms.

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