Association of sexually transmitted infection with semen quality in men from couples with primary and secondary infertility

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This study aims to compare the prevalence of sexually transmitted infections (STIs) with semen quality in men from couples with primary and secondary infertility. Semenal samples were collected from 133 men who requested fertility evaluation. Seminal tract infection with Ureaplasma spp. (UU), Mycoplasma hominis (MH), Mycoplasma genitalium (MG), Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and herpes simplex virus-2 (HSV-2) was assessed by PCR-based diagnostic assays. Among all patients, the prevalence of STIs was higher in men from couples with primary infertility than that in men from couples with secondary infertility (39.7% vs 21.7%, P = 0.03). The prevalence of UU was 28.8% and 13.3% in men from couples with primary and secondary infertility, respectively. Men from couples with primary infertility were more likely to be positive for UU than men from couples with secondary infertility (P = 0.04). Regarding the UU subtype, the prevalence of Ureaplasma urealyticum (Uuu) and Ureaplasma parvum (Uup; including Uup1, Uup3, Uup6, and Uup14) did not differ between the two groups. No associations between the prevalence rates of MH, MG, and CT were found in men from either infertility group. A lower sperm concentration was associated with STI pathogen positivity in men with primary infertility according to the crude model (P = 0.04). The crude and adjusted models showed that semen volume (both P = 0.03) and semen leukocyte count (both P = 0.02) were independently associated with secondary infertility. These findings suggest the importance of classifying the type of infertility during routine diagnosis of seminal tract infections.

Keywords: primary infertility; secondary infertility; semen parameters; sexually transmitted infections

INTRODUCTION
Collectively, primary infertility (inability to conceive after one year) and secondary infertility (infertility for one year after having conceived at least once before) affect 10%–15% of couples globally, and male factors contribute to approximately 50% of cases.1,2 Sperm quality plays an important role in fertilization and subsequent embryonic development.3,4 Both extrinsic factors (heat exposure, smoking, drinking, environmental pollutant exposure, medical interventions, and chemotherapeutic exposure) and intrinsic factors (spermatogenic arrest, cell apoptosis, varicocele, and male gland infection) negatively impact sperm quality, resulting in low semen volume, sperm concentration, and sperm motility and highly abnormal sperm morphology, and affecting both natural conception and assisted reproduction.3,4 Several studies have investigated the correlation between seminal tract infections and semen parameters5 and have indicated that the presence of pathogens in semen could be used as a parameter to predict male fertility potential.

Sexually transmitted diseases (STDs) are caused by several pathogens, including bacteria and viruses, and can induce male infertility through multiple pathophysiological mechanisms. Additionally, horizontal transmission of STD pathogens to sexual partners or vertical transmission to fetuses and neonates is possible.6 Herpes simplex virus (HSV), Chlamydia trachomatis (CT), Ureaplasma spp. (UU), Mycoplasma hominis (MH), Mycoplasma genitalium (MG), and Neisseria gonorrhoeae (NG) have been detected in the semen of symptomatic and asymptomatic men.7,8,9 These pathogens are associated with poor semen quality, which may manifest as low sperm concentration and motility.7,10,11 Genital infection and inflammation resulting from sexually transmitted infections (STIs) induce defective spermatogenesis, production of antibodies that reach with sperm and seminal tract obstruction, resulting in unfavorable semen parameters.7,12 In addition, several studies have shown that inflammation and oxidative stress occur in response to STIs, resulting in high leukocyte and sperm DNA fragmentation.13,14 Although a strong

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negative correlation between STIs and fertility in females has been identified, the link in males remains controversial.

Recently, several highly sensitive and specific PCR-based diagnostic methods have become available, enabling a better understanding of the relationship between infertility and seminal tract infection by STD-inducing pathogens. In this study, we aimed to investigate the prevalence of sexually transmitted pathogens and the effects of these pathogens on semen quality by PCR-based methods and semen samples obtained from men from couples with primary and secondary infertility.

PATIENTS AND METHODS

Patients
A total of 133 men aged 20 to 55 years who attended the Reproductive Center of The First Affiliated Hospital of the University of Science and Technology of China (USTC; Hefei, China) for fertility evaluation between July 2019 and July 2020 were included in this study. Male participants were enrolled from infertile couples due to a male factor, a female factor, or a combination of them. A questionnaire that evaluated medical and reproductive history, and lifestyle factors (e.g., drinking and smoking) over the past 6 months was completed by each of the patients. The exclusion criteria were a previous diagnosis of genetic defects or chronic diseases related to male fertility, azoospermia, varicocele, testicular trauma, cryptorchidism, or postmumps orchitis.

Data collection was performed according to the principles set forth in the Declaration of Helsinki, and the collected data were used in the study only with the patients’ written informed consent. This study was approved by the Ethical Committee of The First Affiliated Hospital of USTC (Approval No. 2019P040).

Semen analysis
Semen parameters were determined according to the World Health Organization (WHO) guidelines for semen analysis (fifth edition, 2010).5 semen samples were obtained after an abstinence period of 2–7 days and evaluated after liquefaction at 37°C for 0.5 h. Sperm concentration, progressive motility, and total motility were assessed by computer-assisted sperm analysis (CASA) under a phase contrast microscope (CX43, Olympus corporation, Tokyo, Japan) equipped with a SAS-II system (SAS Medical, Beijing, China). Sperm morphology was evaluated through Diff-Quick staining (Ankebio, Hefei, China) at 100× magnification under a light microscope (UB100i, UOP, Chongqing, China). Leukocytes were stained with benzidine for a peroxidase test (Ankebio). Antisperm antibody (AsA) levels were measured by the mixed antiglobulin reaction (MAR) method (Ankebio). Sperm DNA fragmentation was evaluated by flow cytometry according to the protocol (Cellpro, Ningbo, China).

Detection of sexually transmitted pathogens
In brief, microorganismal DNA was extracted from 200 μl of each semen specimen. PCR was used to amplify the extracted pathogen DNA, including DNA from Ureaplasma urealyticum (Uuu), Ureaplasma parvum (Uup; Uup1, Uup3, Uup6, and Uup14), CT, HSV-2, MH, MG, and NG, and the DNA amplicons were then hybridized to STI pathogen-specific probes using flow-through hybridization technology followed by colorimetric detection using the enzyme immunoassay method described in the protocol furnished with the STD6 GenoArray Diagnostic Kit (Hybrbio, Guangzhou, China).

Statistical analyses
Qualitative variables are presented as frequency (percentage), and quantitative variables are presented as mean ± standard deviation (s.d.) values if the data are normally distributed and as medians (interquartile ranges [IQRs]) if they are not. The Shapiro–Wilks normality test was used to determine the normality of the variable distributions. Pearson’s Chi-squared test and Student’s t-test were used for parametric comparisons, and the Mann–Whitney U test was utilized for nonparametric comparisons. A crude model and an adjusted model were used in this study. Potential confounders included age, body mass index (BMI), duration of infertility, smoking status, and drinking status. P < 0.05 was considered to indicate statistical significance. All statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Baseline characteristics
A total of 133 men from infertile couples were recruited. Among the studied population, 73 subjects (54.9%) had primary infertility and 60 subjects (45.1%) had secondary infertility (Table 1). Men from couples with secondary infertility were more likely to be older than men from couples with primary infertility (P = 0.002). BMIs were similar between the two groups. Education level and duration of infertility did not differ between men from couples with primary and secondary infertility. Lower alcohol consumption was reported by men from couples with primary infertility, but the difference was not statistically significant. No significant difference in another lifestyle factor, smoking, was found between men from couples with primary and secondary infertility. Semen volume was slightly but nonsignificantly higher in men from couples with secondary infertility. Other semen parameters (e.g., sperm concentration, motility, morphology, DNA fragmentation index [DFI], high DNA stainability [HDS], semen leukocyte count, and AsA level) were similar in men from couples with primary and secondary infertility.

STI prevalence in men from couples with primary and secondary infertility
The prevalence of STIs (including infection with UU, MH, MG, CT, and HSV-2) was higher in men from couples with primary infertility than that in men from couples with secondary infertility (39.7% vs 21.7%, P = 0.03; Table 2). NG was not detected in any semen sample. The prevalence of UU was 21.8% among men from infertile couples and was higher than the prevalence of the other STI pathogens. Among men from couples with primary infertility, 21 of 73 were UU positive, whereas 8 of 60 men from couples with secondary infertility were UU positive (P = 0.03). Regarding the UU subtype, the prevalence of Uuu and Uup (including Uup1, Uup3, Uup6, and Uup14) did not differ between the two groups. The MH infection rate was higher in men from couples with primary infertility than that in men from couples with secondary infertility, but the difference was not statistically significant. The prevalence of MG and that of CT in semen were similar in men from couples with primary infertility and those from couples with secondary infertility. The semen samples of two men from couples with secondary infertility were positive for HSV-2, while none of the semen samples of men from couples with primary infertility were HSV-2-positive.

Men from couples with primary infertility were more likely to be positive for UU than men from couples with secondary infertility according to both the crude and adjusted models (odds ratio [OR] = 0.38, 95% confidence interval [95% CI]: 0.15–0.91; adjusted OR = 0.37, 95% CI: 0.13–0.96; Figure 1). No associations between the prevalence of MH, MG, and CT were found between men
Table 1: Characteristics and descriptive statistics of the men enrolled in the study

| Clinical characteristic                  | Total (n=133) | Primary infertility (n=73) | Secondary infertility (n=60) | P     |
|------------------------------------------|---------------|----------------------------|-----------------------------|-------|
| Age (year), mean±s.d.                    | 32.0±6.5      | 30.4±5.2                   | 33.9±7.4                    | 0.002 |
| BMI (kg m⁻²), mean±s.d.                 | 24.6±3.6      | 24.3±4.0                   | 24.9±3.0                    | 0.50  |
| Education, n (%)                         |               |                            |                             |       |
| Primary school                           | 6 (4.5)       | 2 (2.7)                    | 4 (6.7)                     | 0.26  |
| Junior high school                       | 34 (25.6)     | 17 (23.3)                  | 17 (28.3)                   |       |
| High school                              | 28 (21.1)     | 13 (17.8)                  | 15 (25.0)                   |       |
| College/university                       | 65 (48.8)     | 41 (56.2)                  | 24 (40.0)                   |       |
| Duration of infertility, n (%)           |               |                            |                             | 0.58  |
| 1–2 years                                | 53 (39.8)     | 27 (37.0)                  | 26 (43.3)                   |       |
| 2–3 years                                | 27 (20.4)     | 14 (19.2)                  | 13 (21.7)                   |       |
| ≥3 years                                 | 53 (39.8)     | 32 (43.8)                  | 21 (35.0)                   |       |
| Alcohol status, n (%)                    |               |                            |                             | 0.06  |
| Nondrinkers                              | 53 (39.8)     | 34 (46.6)                  | 19 (31.6)                   |       |
| Drinkers                                 | 80 (60.2)     | 39 (53.4)                  | 41 (68.4)                   |       |
| Smoking status, n (%)                    |               |                            |                             | 0.60  |
| Nonsmokers                               | 81 (57.4)     | 43 (58.9)                  | 38 (63.3)                   |       |
| Smokers                                  | 52 (42.6)     | 30 (41.1)                  | 22 (36.7)                   |       |
| Semen parameters                         |               |                            |                             |       |
| Abstinence time (day), mean±s.d.         | 4.4±2.0       | 4.2±1.9                    | 4.7±2.1                     | 0.12  |
| Semen volume (ml), median (Q1–Q3)        | 2.9 (2.0–4.0) | 2.6 (2.0–4.0)              | 3.3 (2.4–4.0)               | 0.09  |
| Sperm concentration (x10⁶ ml⁻¹), median (Q1–Q3) | 79.5 (28.6–118.1) | 76.5 (23.9–108.3)           | 84.3 (30.0–133.7)           | 0.36  |
| Progressive motility, % (median (Q1–Q3)  | 36.3 (15.4–51.9) | 35.8 (17.8–48.6)           | 36.5 (15.1–51.9)           | 0.91  |
| Total motility, %, median (Q1–Q3)        | 45.6 (18.7–59.4) | 45.6 (21.8–57.7)           | 45.6 (17.4–59.9)           | 0.82  |
| Normal morphology, %, median (Q1–Q3)     | 5.0 (3.0–8.0) | 5.0 (3.0–8.0)              | 4.0 (3.0–7.0)               | 0.54  |
| Leukocyte count (x10⁶ ml⁻¹), median (Q1–Q3) | 0.3 (0.1–2.0) | 0.3 (0.1–2.0)              | 0.3 (0.1–3.6)               | 0.70  |
| DFI, %, mean±s.d.                        | 18.6±13.3     | 18.7±13.3                  | 18.5±13.4                   | 0.94  |
| HDS, %, mean±s.d.                        | 8.2±6.8       | 8.3±7.1                    | 8.1±6.5                     | 0.89  |
| AsA (%), median (Q1–Q3), n               | 2.0 (0.3–4.0) | 1.0 (0.5–4.0)              | 2.0 (0.4–0.4)               | 0.93  |

P values were derived from Pearson’s Chi-squared test and Student’s t-test for parametric comparisons and the Mann-Whitney U test for nonparametric comparisons. BMI: body mass index; AsA: antisperm antibody; DFI: DNA fragmentation index; HDS: high DNA stainability; Q1: 25th percentile; Q3: 75th percentile; s.d.: standard deviation.

Table 2: Sexually transmitted infection pathogens in the semen of men from couples with different types of infertility

| Pathogen          | Total (n=133) | Primary infertility (n=73) | Secondary infertility (n=60) | P     |
|-------------------|---------------|----------------------------|-----------------------------|-------|
| UU                | 29 (21.8)     | 21 (28.8)                  | 8 (13.3)                    | 0.03  |
| Uuu, n (%)        | 11 (8.3)      | 8 (11.0)                   | 3 (5.0)                     | 0.34  |
| Uup 1, n (%)      | 4 (2.1)       | 2 (2.7)                    | 1 (1.7)                     | 0.99  |
| Uup 3, n (%)      | 7 (5.3)       | 4 (5.5)                    | 3 (5.0)                     | 0.99  |
| Uup 6, n (%)      | 8 (6.0)       | 7 (9.5)                    | 1 (1.7)                     | 0.07  |
| Uup 14, n (%)     | 1 (0.8)       | 1 (1.4)                    | 0 (0)                       | 0.99  |
| Uup, n (%)        | 18 (13.5)     | 13 (17.8)                  | 5 (8.3)                     | 0.11  |
| MH, n (%)         | 16 (8.2)      | 9 (12.3)                   | 2 (3.3)                     | 0.11  |
| MG, n (%)         | 4 (2.1)       | 2 (2.7)                    | 1 (1.7)                     | 0.99  |
| CT, n (%)         | 7 (3.6)       | 3 (4.1)                    | 2 (3.3)                     | 0.99  |
| HSV-2, n (%)      | 2 (1.0)       | 0 (0)                      | 2 (3.3)                     | 0.20  |
| NG, n (%)         | 0 (0)         | 0 (0)                      | 0 (0)                       | -     |
| STI-positive, n (%) | 42 (31.6)    | 29 (39.7)                  | 13 (21.7)                   | 0.03  |

P values were derived from Pearson’s Chi-squared test if not otherwise indicated. *Fishler’s exact test. UU: Ureaplasma spp.; Uuu: Ureaplasma urealyticum; Uup: Ureaplasma parvum; MH: Mycoplasma hominis; MG: Mycoplasma genitalium; CT: Chlamydia trachomatis; HSV-2: herpes simplex virus 2; NG: Neisseria gonorrhoeae; STI: sexually transmitted infection; -: no value.

Logistic regression analyses of the association between STIs and sperm parameters in men from couples with primary and secondary infertility

The semen parameters of men from couples with primary and secondary infertility with and without STI pathogens in semen are summarized in Table 3. Among men from couples with secondary infertility, STI-positive men had lower semen volumes and higher semen leukocyte counts than STI-negative men. A lower sperm concentration was associated with primary infertility in men with STIs according to the crude model (OR = 1.01, 95% CI: 1.00–1.02), although the association was not statistically significant after adjusting for confounders (Table 4). Both the crude and the adjusted models showed that semen volume (OR = 2.02, 95% CI: 1.10–4.12; adjusted OR = 2.22, 95% CI: 1.15–4.92, both P = 0.03, respectively) and semen leukocyte count (OR = 0.80, 95% CI: 0.63–0.95; adjusted OR = 0.78, 95% CI: 0.61–0.94, both P = 0.02, respectively) were independently associated with secondary infertility (Table 4).

DISCUSSION

In this study, we showed that the prevalence of STI pathogens, especially UU, was higher in men from couples with primary infertility than that in men from couples with secondary infertility. In addition, among men with STIs, those from couples with secondary infertility exhibited poorer semen quality than those from couples with primary infertility.

The prevalence of STIs among infertile couples has been reported in numerous previous studies. A study by Abusarah et al. showed a higher prevalence of STIs, including infection with UU, MH, NG, and
### Table 3: Semen parameters of men from couples with primary and secondary infertility with sexually transmitted infection pathogens in semen

| Parameter                           | Primary infertility (n=73) | Secondary infertility (n=60) | P     |
|-------------------------------------|---------------------------|-----------------------------|-------|
|                                     | STI-positive (n=29)       | STI-negative (n=44)         |       |
|                                     | Abstinence time (day)      | 4.3±2.0                     | 4.1±1.8       | 0.67   | 4.7±2.1                     | 4.7±2.1       | 0.94   |
|                                     | Semen volume (ml)         | 2.9 (2.1–4.2)               | 2.5 (1.9–3.9)   | 0.29   | 2.5 (1.4–3.7)               | 3.4 (2.7–4.0)   | 0.04   |
|                                     | Sperm concentration (x10^6 ml^-1) | 5.5 (22.5–92.0)             | 83 (50.0–116.6)   | 0.10   | 64.6 (33.0–146.5)             | 88.5 (28.4–133.1)   | 0.93   |
|                                     | Progressive motility (%)  | 35.8 (15.1–48.6)            | 35.8 (17.5–49.9)   | 0.76   | 39.4 (14.0–49.8)            | 35.8 (15.1–51.9)   | 0.99   |
|                                     | Total motility, median (Q1–Q3) | 43.8 (20.8–57.4)            | 46.2 (21.2–61.6)   | 0.94   | 53.3 (15.6–58.6)            | 43.9 (18.4–60.7)   | 0.99   |
|                                     | Normal morphology (%)     | 5.0 (2.0–8.0)               | 5.0 (3.0–7.8)     | 0.76   | 5.0 (3.0–7.5)               | 4.0 (3.0–7.0)     | 0.89   |
|                                     | Leukocyte count (x10^6 ml^-1), median (Q1–Q3) | 0.3 (0.1–2.2)               | 0.3 (0.1–1.9)     | 0.94   | 3.7 (0.6–5.5)               | 0.2 (0.1–2.5)     | 0.002  |
|                                     | DFI (%), mean±s.d.        | 18.2±12.4                   | 19.1±14.0        | 0.91   | 14.5±10.8                   | 19.7±14.0        | 0.29   |
|                                     | HDS (%), mean±s.d.        | 8.5±5.4                     | 8.1±8.1         | 0.85   | 8.5±6.0                     | 8.0±6.7         | 0.78   |

$^P$ values were derived from Student’s t-test for parametric comparisons and the Mann-Whitney U test for nonparametric comparisons. Q1: 25th percentile; Q3: 75th percentile; s.d.: standard deviation; DFI: DNA fragmentation index; HDS: high DNA stainability.

**Figure 1:** ORs (95% CIs) for STIs among men from couples with primary and secondary infertility. UU: Ureaplasma spp.; Uup: Ureaplasma urealyticum; Uup3 and Uup14 in one patient from a couple with primary infertility, CT: Chlamydia trachomatis; STI: sexually transmitted infection; OR: odds ratio; CI: confidence interval.

CT, in infertile men than in fertile men (21.5% vs 7.1%) based on semen analysis. In China, approximately 35% of infertile men are positive for at least one of pathogen (UU, MH, or CT). The findings of the study by Liu et al. are in agreement with the STI prevalence of 31.6% observed among men from infertile couples in our study. However, Liu et al. did not distinguish between primary infertility and secondary infertility when determining the prevalence of STIs. Boeri et al. reported that more than 20% of men from couples with primary infertility had asymptomatic seminal tract infections. This proportion is similar to the STI prevalence of 21.7% observed in men from couples with secondary infertility but lower than the STI prevalence of 39.7% observed in men from couples with primary infertility in this study. Our data also revealed that the semen samples of six men with primary infertility and that of one man with secondary infertility showed infection with two or more pathogens, indicating that multiple infections are more likely to be related to primary infertility than to secondary infertility in men.

With the exception of infection with UU, we did not find differences between the prevalence of any STIs between men from couples with primary infertility and men from couples with secondary infertility. Notably, a higher prevalence of MH infection was found in men from couples with primary infertility, but the difference was not statistically significant. In addition, we found a lower HSV-2 prevalence in men from couples with primary infertility than that in men from couples with secondary infertility, and this trend was also found by Dhont et al. These results indicate that UU infection is more likely than other STIs to be associated with primary infertility in men.

The exact role of UU in male infertility is still controversial. This may in part be because the two species of UU, Uuu, and Uup are more likely to be associated with infertility than other serovars. Studies have reported a higher prevalence of UU than Uup prevalence among infertile men, whereas the latter observation is consistent with the Uuu prevalence of 62.1% (18/29) observed among UU-positive infertile men in our study. Uup isolates have been reported to be more common than Uuu isolates in healthy men, indicating that Uuu is more often associated with clinical disease than Uup. Notably, a previous study demonstrated that an inflammatory response is induced when high titers of Uup are present. Some Upp serovars are more likely to be associated with urogenital disease and infertility than other serovars. We observed concomitant infection with Upp3 and Upp14 in one patient from a couple with primary infertility, but all semen parameters were normal in that sample. In addition, no differences in semen parameters were observed among UU-infected subjects from couples with primary and secondary infertility (data not shown).

Previous studies investigated the presence of pathogenic bacteria in semen based on culture or PCR assays. Whereas culture methods have limitations (they are time-consuming and have low specificity), PCR-based diagnostic methods allow rapid and sensitive detection of STI pathogens. In this study, we found a high overall prevalence (31.6%) of six pathogenic STIs using PCR-based tests in semen from subfertile men. This finding further highlights the need for routine STI pathogen screening of males attending an infertility clinic.

The current study has several limitations. Subfertile men who were undergoing infertility investigations at a single reproductive center were selected. Men from infertile couples from central China were more likely to be enrolled in this study than men from infertile couples from other regions of China. Hence, further studies across different parts of China are needed to confirm our findings. The lack of a fertile control group may in part be because the two species of UU, Uuu, and Uup are more likely to be associated with infertility than other serovars. We observed concomitant infection with Upp3 and Upp14 in one patient from a couple with primary infertility, but all semen parameters were normal in that sample. In addition, no differences in semen parameters were observed among UU-infected subjects from couples with primary and secondary infertility (data not shown).
samples of the subjects were positive due to infection of their partners. Finally, the relatively small sample size and the use of subjects from a single center might have limited the power of this study to detect weak correlations in both the crude and adjusted analyses.

This study has several strengths. First, STI pathogens were detected in men from infertile couples, especially couples with secondary infertility, and these men were found to have a high risk of low semen volume. Second, seminal tract infection was associated with increased semen leukocyte counts in men from couples with secondary infertility but not in men from couples with primary infertility, suggesting that the number of leukocytes in seminal plasma is more likely to be associated with STI pathogens in secondary infertility. Consequently, STI pathogens need to be identified in men with leukocytospermia from couples with secondary infertility.

In conclusion, this study may help explain the association between STIs and semen quality in asymptomatic men from infertile couples. Therefore, we suggest that classification of the type of infertility should be part of the routine diagnosis in cases of seminal tract infections.

AUTHOR CONTRIBUTIONS
SB designed the research study. YL, LW, XHW, YXL, XCH, and SB contributed to the data acquisition. YL, MHH, LJS, QLY, LNY, KQF, XHT, XCH, and BX analyzed the data. SB wrote the paper. XCH and BX revised the manuscript and provided comments. All authors have read and approved the final manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

Table 4: Odds ratios (95% confidence intervals) for semen parameters of men from couples with primary and secondary infertility with and without sexually transmitted infection pathogens

| Parameter                  | Primary infertility (n=73) | Secondary infertility (n=60) |
|----------------------------|---------------------------|-----------------------------|
|                            | OR    | 95% CI    | P    | OR    | 95% CI    | P    |
| Semen volume (ml)          |       |           |      |       |           |      |
| Crude                      | 0.84  | 0.57–1.21 | 0.34 | 2.02  | 1.10–4.12 | 0.03 |
| Adjusted                   | 0.88  | 0.59–1.32 | 0.54 | 2.22  | 1.15–4.92 | 0.03 |
| Sperm concentration (×10^6 ml⁻¹) |       |           |      |       |           |      |
| Crude                      | 1.01  | 1.00–1.02 | 0.04 | 1.00  | 0.99–1.01 | 0.70 |
| Adjusted                   | 1.01  | 1.00–1.02 | 0.06 | 1.00  | 0.99–1.01 | 0.68 |
| Progressive motility (%)   |       |           |      |       |           |      |
| Crude                      | 1.00  | 0.97–1.02 | 0.74 | 1.00  | 0.97–1.03 | 0.92 |
| Adjusted                   | 1.00  | 0.97–1.02 | 0.91 | 1.00  | 0.97–1.04 | 0.99 |
| Total motility (%)         |       |           |      |       |           |      |
| Crude                      | 1.00  | 0.98–1.02 | 0.98 | 1.00  | 0.97–1.03 | 0.86 |
| Adjusted                   | 1.00  | 0.98–1.02 | 0.93 | 1.00  | 0.97–1.03 | 0.95 |
| Normal morphology (%)      |       |           |      |       |           |      |
| Crude                      | 0.99  | 0.83–1.17 | 9.86 | 0.99  | 0.79–1.24 | 0.90 |
| Adjusted                   | 1.00  | 0.84–1.20 | 0.97 | 0.94  | 0.74–1.21 | 0.65 |
| Leukocytes (×10^6 ml⁻¹)    |       |           |      |       |           |      |
| Crude                      | 1.04  | 0.93–1.25 | 0.55 | 0.80  | 0.63–0.95 | 0.02 |
| Adjusted                   | 1.03  | 0.92–1.23 | 0.63 | 0.78  | 0.61–0.94 | 0.02 |
| DFI (%)                    |       |           |      |       |           |      |
| Crude                      | 1.01  | 0.97–1.04 | 0.79 | 1.03  | 0.98–1.10 | 0.23 |
| Adjusted                   | 1.01  | 0.97–1.05 | 0.77 | 1.04  | 0.98–1.11 | 0.23 |
| HDS (%)                    |       |           |      |       |           |      |
| Crude                      | 0.99  | 0.93–1.07 | 0.84 | 0.99  | 0.91–1.10 | 0.78 |
| Adjusted                   | 0.99  | 0.92–1.06 | 0.74 | 1.00  | 0.91–1.12 | 0.96 |

Crude: unadjusted model; adjusted: model adjusted for age, BMI, duration of infertility, smoking status and drinking status. DFI: DNA fragmentation index; HDS: high DNA stainability; BMI: body mass index; OR: odds ratio; CI: confidence interval

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