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Ureaplasma urealyticum induces polymorphonuclear elastase to change semen properties and reduce sperm motility: a prospective observational study

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Abstract

Objective: To elucidate the mechanism underlying how *Ureaplasma urealyticum* (UU) affects sperm quality and identify a therapeutic target.

Methods: In this prospective observational study, the differences in and relationships among semen volume, pH, viscosity, liquefaction time, sperm concentration, sperm motility [progressive motility (PR)], and seminal polymorphonuclear (PMN) elastase were analyzed in 198 normal semen samples (control group) and 198 UU-infected semen samples (observation group). The UU-infected samples were treated and the above parameters were compared between the two groups.

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Results: The semen volume, viscosity, liquefaction time, and seminal PMN elastase were significantly higher in the observation than control group, but the pH and PR were significantly lower. In the observation group, the pH and PR were significantly higher after than before treatment, whereas the semen volume, PMN elastase, viscosity, and liquefaction time were lower. UU was closely related to semen volume, pH, viscosity, liquefaction time, sperm motility (PR), and PMN elastase. PMN elastase had significant negative effects on semen pH and sperm motility (PR) but positive effects on viscosity and liquefaction time.
Conclusion: UU might induce PMN elastase to increase the liquefaction time and viscosity of semen, eventually decreasing PR. PMN elastase might be a therapeutic target of UU.

Keywords
Ureaplasma urealyticum, polymorphonuclear elastase, semen characteristics, sperm motility (progressive motility), therapeutic target, observational study

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Introduction

Ureaplasma urealyticum (UU) is a normal parasitic microorganism within the human body that does not cause serious clinical symptoms and is easily ignored.[1] It is readily concealed in the body and has a high infection rate, making it easy to become complicated with other diseases.[2] However, its potential for harm to the human body remains unclear. Some studies have shown that the rapid propagation of UU can cause pathological conditions such as genital tract infection,[3] genital tract blockage,[3] vaginitis,[4] cervicitis,[5] urethritis,[6] and prostatitis.[7] The latest research showed that UU is an important microorganism in the male reproductive tract and can affect the quality of sperm.[8] Other researchers have reported that UU can attach to the sperm tail and affect sperm motility.[9] Conversely, some studies have shown that UU does not affect the sperm and requires no treatment.[10] Therefore, whether UU affects sperm remains a hotspot of current research.

After UU infects the male reproductive tract, the seminal plasma components can become significantly unbalanced, making the sperm quality especially susceptible to the influence of various components in the seminal plasma. Human polymorphonuclear elastase (PMN elastase) is the most common spermatic elastase. It is a glycoprotein with a molecular weight of 30,000 Da, and at the ideal pH value of 7.15 to 8.50 it is evenly distributed in semen.[11] Some studies have shown that an increased PMN elastase concentration is closely related to the upregulation of tumor necrosis factor α mRNA, promoting the release of tumor necrosis factor α and causing inflammatory changes that damage the sperm concentration and motility.[12] PMN elastase can be used to detect male reproductive tract infection,[13] and it has also been considered a diagnostic and post-therapeutic indicator of static reproductive tract infection.[14]

Whether the PMN elastase concentration is abnormal after UU infection and whether the sperm parameters are accordingly affected have not been reported. To elucidate the potential mechanism underlying how UU affects sperm quality

and identify a therapeutic target of UU, we conducted a prospective clinical study to analyze the differences between UU-positive semen samples and normal semen samples. Our data show that PMN elastase might play an important mediating role in the evaluation of UU in semen.

Materials and methods

Sample sources

In this prospective observational study, all semen samples were randomly collected according to the random number table method from April 2019 to June 2019. The samples were donated by male outpatients aged 20 to 45 years who visited the Department of Andrology of Guangdong Provincial Reproductive Science Institute (Guangdong Provincial Fertility Hospital).

Grouping method

According to the results of UU culture and drug sensitivity tests, the samples were divided into a UU-positive group (observation group, $n = 198$) and normal UU-negative group (control group, $n = 198$). The mean age of the donors in the observation group was 33.38 ± 6.56 years, and 112 had primary infertility whereas 86 had secondary infertility. The mean age of the donors in the control group was 33.33 ± 7.76 years, and 101 had primary infertility whereas 97 had secondary infertility.

Inclusion criteria

All donors were asked to provide a detailed medical history, including childhood diseases, developmental patterns, prior surgeries, allergies, systemic medical conditions (such as diabetes mellitus), family reproductive history, the use of prescription and non-prescription medications, and lifestyle factors. These data were collected and examined by special personnel. Each patient's reported medical history was confirmed by his wife. For many of the childhood or earlier diseases, we asked the patients to consult their parents for verification. Diseases that could not be completely ruled out were added to the exclusion criteria. The donors were required to have a normal sexual life, fixed sexual partners, and the ability to ejaculate *in vitro* for semen collection. None of the donors had used antibiotics 2 weeks before the test, and all were able to cooperate during a physical examination performed by special personnel. Additionally, the donors' spouses had no abnormal physical examination findings.

Exclusion criteria

The exclusion criteria were an inability to cooperate during the physical examination, an inability to ejaculate *in vitro*, sexual dysfunction, hereditary diseases or basic diseases, and a history of self-medication within 2 weeks. Color Doppler ultrasonography was performed to exclude the presence of varicoceles or testicular tumors because they may have influenced the semen analysis results.

Sample collection

All semen samples were obtained by masturbation after 2 to 7 days of abstinence. The semen samples were collected in plastic, sterile, non-toxic wide-mouth containers. Before sample collection, the donors carried out standardized disinfection of the external genitalia to avoid contamination of the samples by foreign substances.

UU detection

All semen samples underwent UU culture and drug sensitivity tests in accordance with the manufacturer's instructions [UU detection agar medium (culture method), 20 copies/box and UU identification drug

sensitivity kit (culture method), 26-well liquid type; Zhuhai Yinke Medical Engineering Co., Ltd., Zhuhai, Guangdong, China]. A research-grade universal fluorescence microscope (BX51T-12P01; Olympus Corporation, Tokyo, Japan) was used for UU settlement observation and photography.

Semen analysis

The semen volume, pH, viscosity, liquefaction time, sperm concentration, and sperm motility [progressive motility (PR)] were detected according to the semen analysis method described in the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen.[15] Semen volume was analyzed by weighing method. Precision pH test paper (pH–Fix 6.0–10.0; Macherey-Nagel, Düren, Germany) was used to detect the pH value. Semen viscosity and liquefaction time were divided into two grades: normal grade, assigned 1; and abnormal grade, assigned 2.

Seminal PMN elastase detection

Seminal PMN elastase was determined by enzyme-linked immunosorbent assay with a seminal elastase kit (96 tests/box, Guangdong Machinery registration No. 20192400687; BRED Life Science Technology Inc., Shenzhen, China).

Treatment and follow-up

The donors with positive UU results (observation group) were treated with sensitive antibiotics according to the drug sensitivity test for 2 weeks after exclusion of drug contraindications. The specific drug was doxycycline hydrochloride (Guangdong Xianqiang Pharmaceutical Co., Ltd., Guangdong, China) administered at a dosage of 0.1 g twice a day for 14 days. All donors were followed up and advised to avoid sexual activity and maintain a low frequency of ejaculation (only once or twice for the duration of treatment). One week after the last dose of doxycycline, the donors were scheduled for semen collection.

Observation indexes

The semen volume, pH value, viscosity, liquefaction time, sperm concentration, sperm motility (PR), and seminal PMN elastase were measured and compared with the reference ranges in the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen[15] and the manufacturers' kits (Table 1).

Statistical analysis

IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA) was

Table 1. Reference ranges of studied indicators.

Indicator	Reference range
Semen volume, mL	1.5 (1.4–1.7)*
pH value	$\geq$7.2*
Sperm concentration, $\times 10^6$/mL	15 (12–16)*
Sperm motility (PR), %	32 (31–34)*
Viscosity	Normal
Liquefaction time	Normal
Seminal PMN elastase, ng/mL	$\leq 600^{\Delta}$

*Lower reference limits of semen parameters according to World Health Organization (2010, 5th ed).
$^{\Delta}$Reference limits according to the manufacturer's kit. PR, progressive motility.

used for statistical analysis. Student's t test was performed for comparisons of measurement data, and the chi square test was used for comparisons of count data. Pearson's correlation analysis was used to analyze correlations between the data. Logistic regression analysis was also used to analyze and identify risk factors for mortality. A P value of <0.05 was considered statistically significant.

Ethics

The Ethics Committee of Guangdong Provincial Reproductive Science Institute (Guangdong Provincial Fertility Hospital) approved the study [approval number: [2017] (16)]. All patients provided written informed consent for publication, and the details of their information have been de-identified. This reporting of this research conforms to the STROBE guidelines,[16] and the study has been registered at https://www.researchregistry.com/ (Research Registry 5690).

Results

The data for the semen parameters and seminal PMN elastase in all patients were checked for a normal distribution using the Kolmogorov–Smirnov test. The semen volume ($Z = 0.905$) and sperm motility (PR) ($Z = 1.187$) showed a normal distribution, but the semen pH value ($Z = 3.082$, $P < 0.001$), sperm concentration ($Z = 1.866$, $P = 0.002$), and seminal PMN elastase ($Z = 4.484$, $P < 0.001$) showed a non-normal distribution (Table 2).

Donors' baseline data

As shown in Figure 1(a) and (b), no significant differences in the baseline data of age, abstinence days, and fertility status (primary infertility/secondary infertility) were found between the observation group (33.38 ± 6.54 years, 4.20 ± 1.60 days, and 112/86, respectively) and control group (33.33 ± 7.74 years, 4.10 ± 1.61 days, and 101/97, respectively).

Comparison of parameters between the two groups

The semen volume and seminal PMN elastase concentration were significantly higher in the observation group (4.35 ± 1.23 mL and 940.29 ± 939.69 ng/mL, respectively) than in the control group (3.97 ± 1.55 mL

Table 2. Kolmogorov–Smirnov test.

		Seminal PMN elastase	Semen volume	Semen pH value	Sperm concentration	Sperm motility (PR)
N		396	396	396	396	396
Normal parameter[a,b]	Mean	756.04	4.15	7.03	32.22	40.02
	Standard deviation	899.95	1.42	0.66	10.95	10.79
Most extreme difference	Absolute value	0.23	0.05	0.16	0.09	0.09
	Maximum	0.23	0.05	0.16	0.09	0.09
	Negative	−0.21	−0.045	−0.08	−0.08	−0.08
Kolmogorov–Smirnov Z		4.484	0.905	3.082	1.866	1.187
P		0.000*	0.386$^{\Delta}$	0.000*	0.002*	0.120$^{\Delta}$

*Does not conform to a normal distribution, $P < 0.05$; $^{\Delta}$Conforms to a normal distribution, $P > 0.05$.

[a]The test distribution is normal.

[b]Calculated from the data.

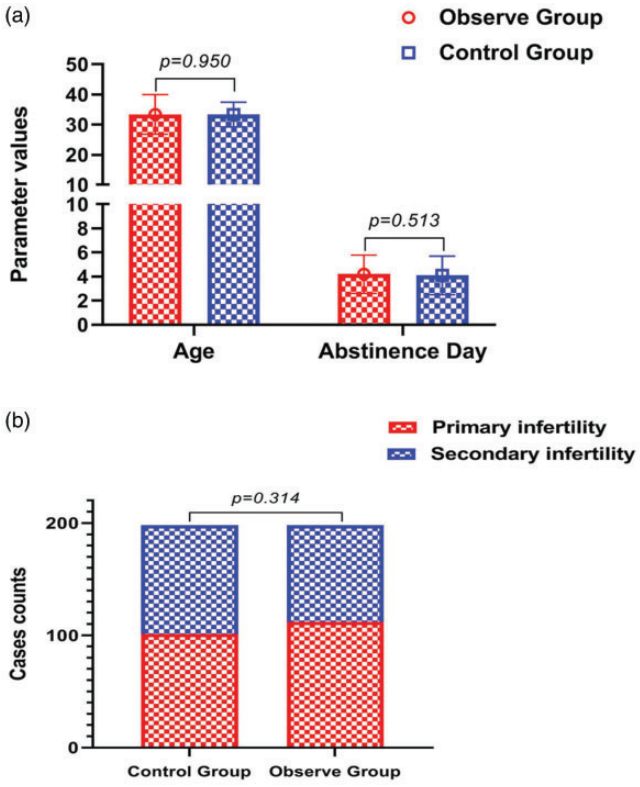

Figure 1. Comparison of baseline data. (a) Differences in age and abstinence days and (b) Differences in reproductive history.

and 571.78 ± 815.37 ng/mL, respectively) ($P_1 = 0.007$, $P_2 < 0.001$) (Figure 2(a)). The semen viscosity [abnormal rate, 56.06% (111/198)] and liquefaction time [abnormal rate, 63.13% (125/198)] were also significantly higher in the observation group than in the control group [abnormal rates of 34.85% (69/198) and 48.99% (97/198), respectively] ($P_1 < 0.001$, $P_2 = 0.005$) (Figure 2(b)). The semen pH value (6.60 ± 0.35) and sperm motility (PR) ($38.86\% \pm 11.68\%$) were significantly lower in the observation group than in the control group (7.46 ± 0.60 and $41.19\% \pm 9.66\%$, respectively) ($P_1 < 0.001$, $P_2 = 0.032$). However, there was no significant difference in the sperm concentration ($31.44 \pm 11.13 \times 10^6$/mL vs. $33.01 \pm 10.68 \times 10^6$/mL) (Figure 2(c)).

Comparison of parameters before and after treatment

In the observation group, the semen volume (4.01 ± 1.41 mL) and PMN elastase concentration (552.55 ± 490.30 ng/mL) were significantly lower after than before treatment ($P_1 = 0.011$, $P_2 < 0.001$) (Figure 3(a)). Likewise, the semen viscosity [abnormal rate, 41.41% (82/198)] and liquefaction time [abnormal rate, 46.97% (93/198)] were significantly lower after than before treatment ($P_1 = 0.003$, $P_2 = 0.002$) (Figure 3(b)). The semen pH

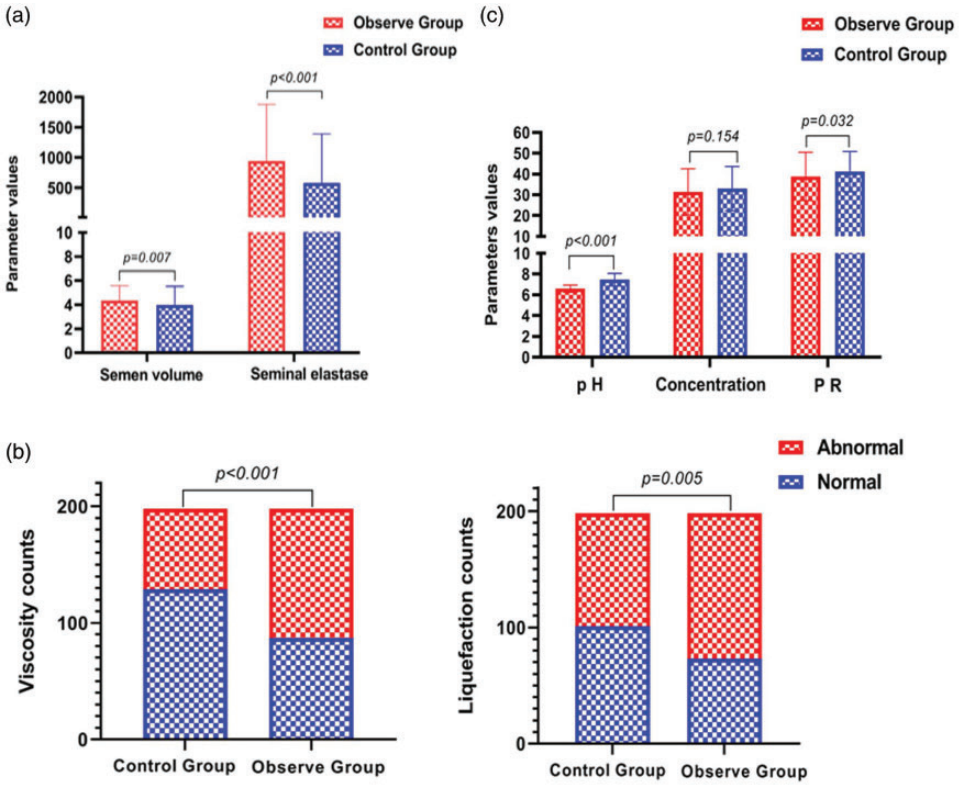

Figure 2. Comparison of parameters between observation group and control group. (a) Differences in semen volume and PMN elastase. (b) Differences in semen viscosity and liquefaction abnormalities and (c) Differences in semen pH, sperm concentration, and motility.
pH, semen pH value; Concentration, sperm concentration; PR, sperm motility (percentage of forward motile sperm).

value (7.34 ± 0.49) and sperm motility (PR) ($41.42\% \pm 9.57\%$) were significantly higher after than before treatment ($P_1 < 0.001$, $P_2 = 0.018$). However, there was no significant change in the sperm concentration after treatment ($33.36 \pm 10.97 \times 10^6$/mL) (Figure 3(c)).

Pearson correlation analysis

Pearson correlation analysis showed that UU was closely related to the semen volume ($P = 0.007$), pH value ($P < 0.001$), viscosity ($P = 0.006$), liquefaction time ($P < 0.001$), sperm motility (PR) ($P = 0.031$), and PMN elastase ($P < 0.001$). However, UU was not significantly related to age, abstinence time, or sperm concentration (Figure 4(a)).

The seminal PMN elastase concentration was positively correlated with viscosity ($r = 0.619$, $P < 0.001$) and liquefaction time ($r = 0.723$, $P < 0.001$) and was negatively correlated with pH ($r = -0.160$, $P = 0.001$) and PR ($r = -0.890$, $P < 0.001$). However, it was not significantly correlated with semen volume or sperm concentration (Figure 4(b)).

Logistic regression analysis

Logistic regression analysis showed that the seminal PMN elastase concentration had

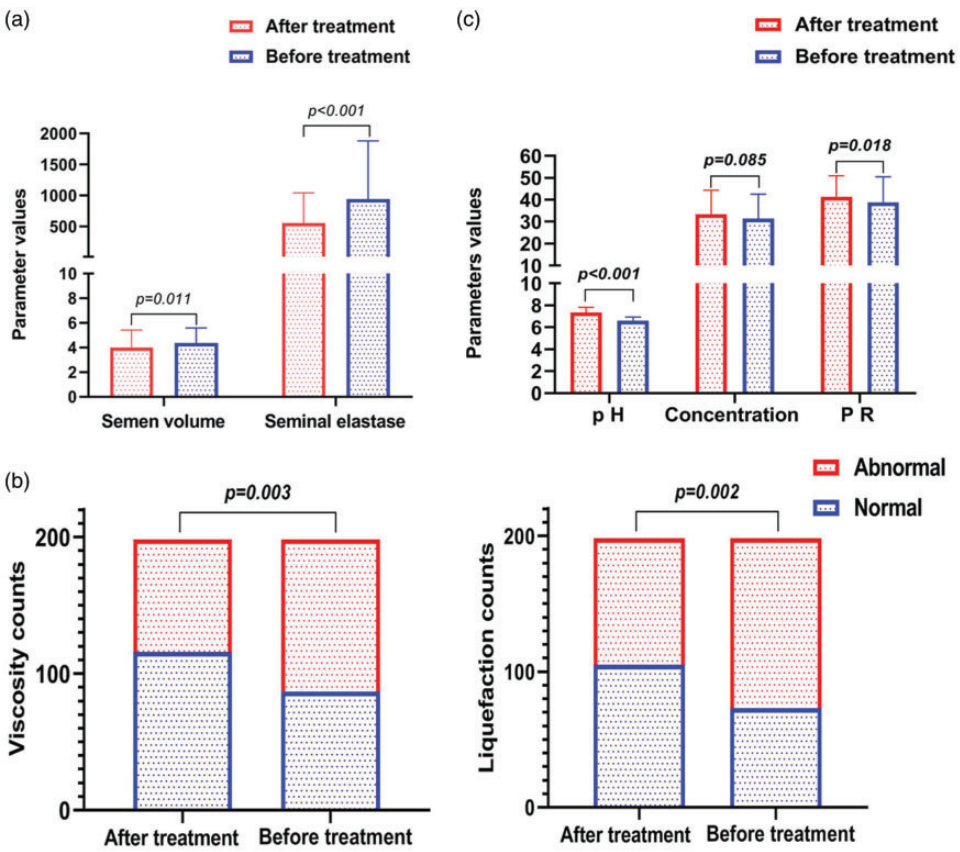

Figure 3. Comparison of parameters before and after treatment in observation group. (a) Differences in semen volume and PMN elastase. (b) Differences in semen viscosity and liquefaction abnormalities and (c) Differences in semen pH, sperm concentration, and motility.
pH, semen pH value; Concentration, sperm concentration; PR, sperm motility (percentage of forward motile sperm).

significant negative effects on semen pH ($r = -0.162$, $P = 0.001$) (Figure 5(a)) and sperm motility (PR) ($r = -0.925$, $P < 0.001$) (Figure 5(b)) but had significant positive effects on viscosity ($r = 0.538$, $P = 0.001$) (Figure 5(c)) and liquefaction time ($r = 0.485$, $P < 0.001$) (Figure 5(d)).

Discussion

Human semen is a mixed suspension containing the secretions of the accessory gonadal organs of the male reproductive tract. Its main components are sperm and seminal plasma, the latter of which accounts for more than 90% of semen.[17] Certain cytokines, protein components, or glycopeptides in seminal plasma might be useful for prediction and evaluation of the physiological and pathological functions of specific accessory gonadal organs. The content or concentration changes of these biological components also directly affect the biological characteristics of semen, thus directly or indirectly affecting the sperm parameters and male fertility.[18,19] Research has shown that semen liquefaction is regulated by coagulation

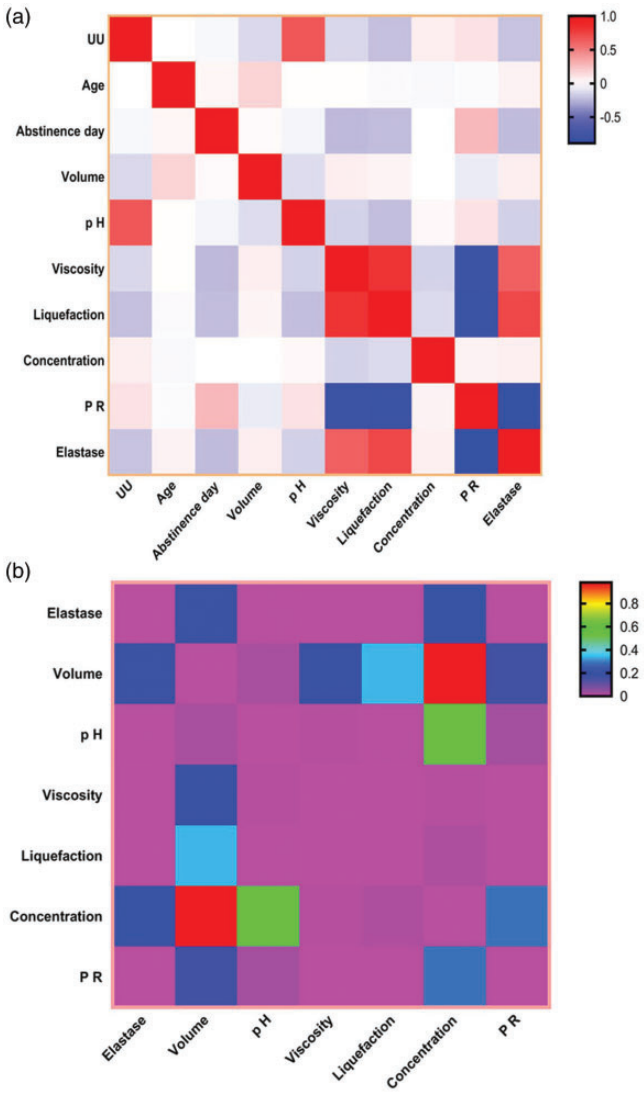

Figure 4. (a) Relationships between UU and other indexes and (b) Relationships between PMN elastase and other indexes.
UU, *Ureaplasma urealyticum*; Volume, semen volume; pH, semen pH value; Concentration, sperm concentration; PR, sperm motility (percentage of forward motile sperm); PMN elastase, polymorphonuclear elastase.

and liquefaction factors.[20] Coagulation factors, such as semen coagulating protein, collagen, or fibronectin, can maintain the gel consistency of ejaculated semen and thicken the semen. Prostate-specific antigen, fibrinolytic enzymes, and acid phosphatase can promote the development of semen liquefaction.[21,22] Studies have also suggested that the occurrence of inflammation can lead to an abnormal pH value of semen and change the physical and chemical properties of semen after genital tract

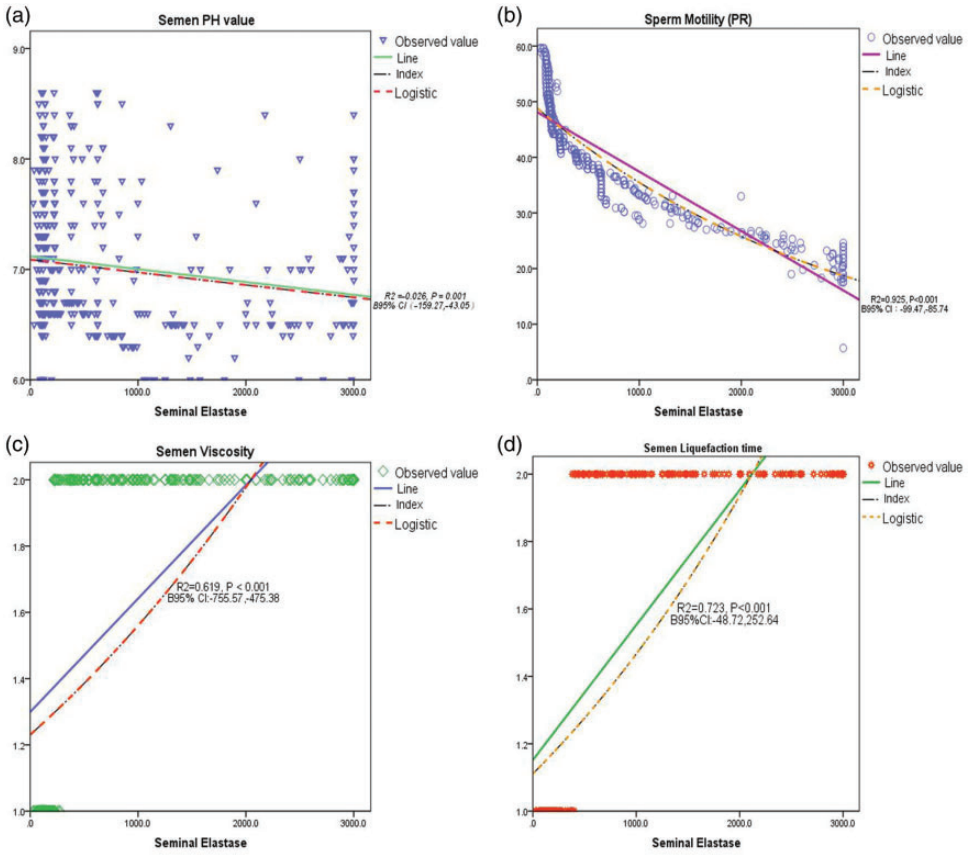

Figure 5. Logistic regression analysis. (a) Effect of PMN elastase on semen pH. (b) Effect of PMN elastase on sperm motility. (c) Effect of PMN elastase on semen viscosity and (d) Effect of PMN elastase on semen liquefaction time.

infection.[23] Therefore, studying the changes in semen parameters is helpful to judge the quality of sperm.

We herein conducted a comparative study of UU-infected and UU-uninfected semen samples. We found that the semen volume, viscosity, and liquefaction time were significantly higher in UU-infected samples. This might have been due to the increased secretion of epithelial cells in the accessory gonadal organs of the reproductive tract caused by UU infection. In particular, the secretion of inflammatory substances changes the proportion of seminal plasma components, resulting in changes in the physical and chemical properties of semen. Our previous study also showed that chlamydia infection causes abnormal semen traits.[24] To further explore the effect of inflammation on semen, we observed UU, which is the most common microbe in the male reproductive tract, using a research-grade universal fluorescence microscope (BX51T-12P01; Olympus Corporation). We found that in UU culture medium, the sperm clumped in a disorderly manner in the vicinity of the UU colonies (Figure 6(a), (b)) whereas they were arranged in a regular order when located far away from the UU

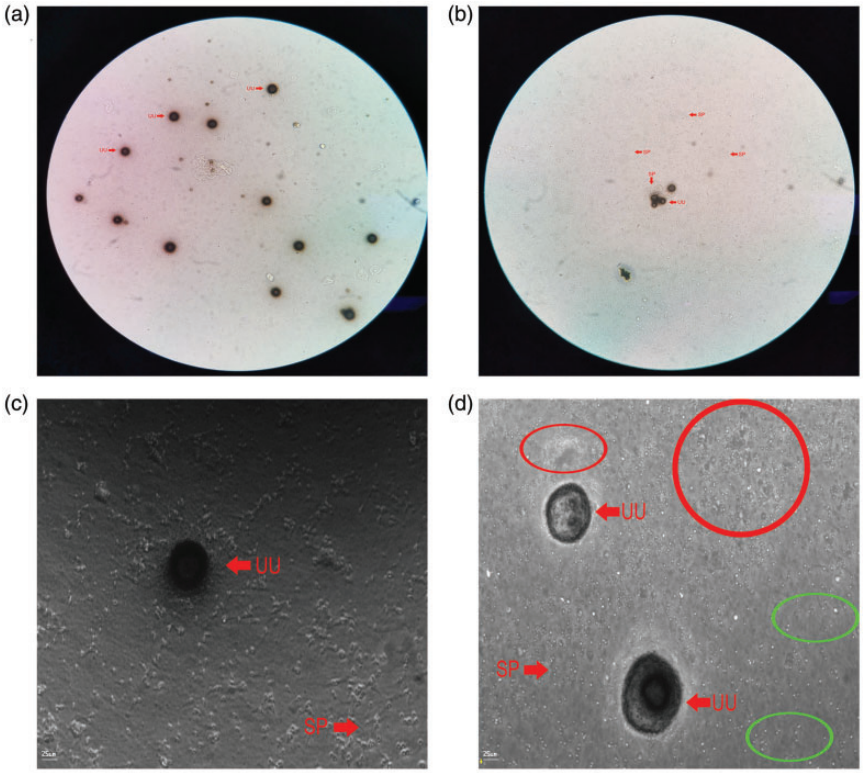

Figure 6. *Ureaplasma urealyticum* in the semen medium. UU, *Ureaplasma urealyticum*; SP, sperm. (a) UU under a 10 × 10 visual field. (b) Sperm under a 10 × 10 visual field. (c) Sperm and UU under a 40 × 10 visual field and (d) Sperm and UU colonies at different distances under a 40 × 10 visual field. Red circle, sperm near UU colonies; blue circle, sperm far from UU colonies.

colonies (Figure 6(c), (d)). This suggests that UU might influence certain components of seminal plasma, promote the production and secretion of certain substances, or play a special role in producing specific effects in seminal plasma to cause the sperm to either aggregate or clump in a disorderly way around the UU colonies. We performed further analyses to identify the potential mechanism and therapeutic target.

UU is the most common infectious agent of the male genital tract. It causes no obvious symptoms, but it has a high rate of concealment and a great potential for bodily harm; thus, it has been listed as one of the most easily neglected agents adversely affecting male fertility.[25] In the process of infection, the UU becomes adhered to and destroys the epithelial cell membrane of the male genital tract mucosa.[26] At the same time, large numbers of ammonia substances are produced, inducing a toxic effect on the genital tract epithelium. In serious cases, adhesion and migration of inflammatory substances can readily occur, leading to genital tract obstruction.[27] One study showed that decreased sperm parameters, sperm necrosis, DNA denaturation, increased intracellular reactive oxygen species, and decreased matrix metalloproteinase were related to an increase in the leukocyte elastase concentration.[28] UU might activate the immune

response *in vivo* and enhance the chemotaxis and stress effects of inflammatory cells such as neutrophils.[29] For reasons of high cost and low patient compliance and acceptance, we did not investigate sperm DNA fragmentation. This is a major limitation of our study, and we plan to include such an investigation in our future in-depth analysis. Instead, we analyzed PMN elastase in the seminal plasma, which produced some unexpected findings.

PMN elastase is made of a group of 204 amino acid residues that can hydrolyze elastin, and it is a highly expressed inflammatory substance in the early stage of infection.[30] Studies have shown that PMN elastase is positively correlated with the degree of infection[31] and that it can be used as an evaluation index of the inflammation treatment effect.[32] PMN elastase and interleukin 6 have the same diagnostic utility in patients with genital tract inflammation, and they are significantly correlated with sperm parameters and sperm quality.[33] These studies confirmed that PMN elastase might have a special relationship with seminal plasma and sperm abnormalities, but how PMN elastase changes after UU infection has remained unclear; no studies before ours have focused on this topic. In the present study, we found that UU was closely associated with the semen volume, pH, viscosity, liquefaction time, sperm motility (PR), and PMN elastase. This suggests that PMN elastase might be a potential therapeutic target against the action of UU on abnormal sperm quality. We considered that the reasons might be as follows. First, an increase in PMN elastase changes the elastin content in seminal plasma, resulting in a change in semen viscosity and the liquefaction time. This in turn changes the resistance during sperm activity from the perspective of fluid mechanics, finally affecting sperm motility.[34] Second, an increase in PMN elastase causes the breakdown of elastin in seminal plasma, releasing more amides and esters.[35] These substances affect the pH of semen and change the acidity and alkali degree of the external environment of sperm, thus affecting the ability of sperm activity. Third, when UU attaches to the mucosal epithelium of the genital tract, it drives the release of a large number of neutrophils, which carry a high amount of PMN elastase. This dramatically changes the environment surrounding the UU colony. This might have been the real reason for our observation of the disorderly sperm without cohesion that formed around the UU colonies in the culture medium and the much more regularly arranged sperm with lower levels of PMN elastase located far away from the UU colonies. Our findings may also help to explain some of the results of similar previous studies by other researchers.[36–38]

We found that after UU infection, the PMN elastase concentration changes in seminal plasma and influences the liquefaction time and viscosity to reduce sperm motility (PR). These findings provide important inspiration for our future research. However, because of the small sample size and short observation period, our conclusions still require further verification and analysis. In particular, the therapeutic target and molecular biological mechanism of UU remain unclear. Therefore, we plan to perform more in-depth research to further explore the mechanism of UU from the perspective of molecular biology.

Conclusion

Although UU is highly concealed, it might promote the secretion of seminal PMN elastase to change the semen pH, viscosity, liquefaction time, and other physical or chemical properties, eventually leading to a decrease of PR. PMN elastase may be

used as a potential therapeutic target to reverse the effects of UU.

Data availability statement

The article already contains all relevant data. If necessary, the corresponding author can be contacted for further information.

Declaration of conflicting interests

The authors declare that there is no conflict of interests.

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Author contributions

HL designed the study, wrote the article, and collected the case data and statistics. XYS and MLH detected and analyzed the samples. HSZ and SYW collected the data and statistics. PT and SHZ took the photographs. XZZ guided the experiment. QQZ revised the article.

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