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Patients with Cervical *Ureaplasma Urealyticum* and *Chlamydia Trachomatis* Infection Undergoing IVF/ICSI-ET: The Need for New Paradigm*

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Summary: Genital tract infections with *ureaplasma urealyticum* (UU) and *chlamydia trachomatis* (CT) are the most frequent sexually-transmitted disease worldwide. UU and CT infections are considered to be the leading cause for infertility and adverse pregnancy outcomes. However, little is known about the specific effect of cervical UU and CT infections on the etiology of female infertility, as well as the pregnancy outcomes of the patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET). In order to find the association between cervical UU and/or CT infection and pregnancy outcomes, we conducted a retrospective case-control study on the patients undergoing IVF/ICSI-ET with cervical UU and/or CT infection. A total of 2208 patients who received IVF/ICSI-ET were enrolled in this study. Data on the general conditions, pregnancy history and clinical pregnant outcomes were analyzed in terms of the cervical UU and CT detection. Our results revealed that cervical UU and CT infections were the risk factors for ectopic pregnancy and tubal factor-induced infertility. Moreover, the pregnancy rate, abortion rate, ectopic pregnancy rate and premature birth rate in patients with UU and/or CT infections showed no significant difference when compared with the control group. We recommend that cervical UU and CT detection should be an optional item for infertility patients and clinical UU detection should differentiate the subtypes of cervical UU. Positive cervical UU and CT infections should not be taken as strict contraindications for IVF/ICSI-ET.
Key words: ureaplasma urealyticum; chlamydia trachomatis; infertility; pregnancy outcomes

Approximately 10%–15% of couples suffer from infertility during their reproductive years. The comprehensive evaluation of an infertile couple requires a detailed pregnancy history, specific examination and multiple laboratory data, which are critical in establishing diagnosis, devising therapeutic regimen and assessing pregnancy outcomes[1]. In China, before undergoing *in-vitro* fertilization and embryo transfer (IVF-ET), patients are required to go through a series of medical examinations. *Ureaplasma urealyticum* (UU) and *Chlamydia trachomatis* (CT) in cervices are two necessary preoperative examination items. UU and CT are common pathogens causing sexually-transmitted diseases (STD) and are associated with the development and progression of many urogenital tract diseases[1, 2]. UU and CT infection can cause salpingitis in women, and, in severe cases, they contribute to infertility and ectopic pregnancy[3, 4]. In infertile patients, the infection rate of UU is up to 30%–50%[5, 6], and the infection rate of CT stands at 5%–25%[7, 8]. Naderi *et al*[9], by using computerized tomograhy, examined the oviduct tissues in 42 patients with ectopic pregnancy and found that the positive rate of CT was significantly higher in the patients than in control group. They concluded that CT was also an important risk factor for oviduct damage and ectopic pregnancy[9]. Because of those adverse effects of UU and CT on human reproductive capacity, they have been listed as routine preoperative examination items for IVF-ET in China. However, the high detection rates and the poor therapeutic effect of treatment on UU and CT not only are great challenges for clinicians but also impose heavy economic and mental pressure on infertile patients. Moreover, so far, researchers don't agree on the specific impact of UU and CT infection on female pregnancy outcomes during IVF/ICSI-ET[10, 11]. In this controlled study, we tried to clarify whether the UU and CT in cervical secretions are risk factors for unfavorable pregnancy outcomes during IVF/ICSI-ET, and evaluate the clinical significance of the routine detection of UU and CT in cervical secretions before IVF/ICSI-ET.

1 MATERIALS AND METHODS

1.1 Study Population and Recruitment

We conducted a retrospective case-control study on a total of 2208 female patients who received pre-operative examination for UU and CT in cervical secretions and IVF/ICSI-ET in our center from January 2010 to December 2011 (fig. 1). Among them, 1435 re-

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ceived IVF-ET, and 773 underwent ICSI-ET. The ages of the patients ranged from 20 to 44 years, with an average of 31.28±4.36 years. The causes of infertility included salpingitis, ovulation failure, endometriosis, male oligozoospermia, asthenozoospermia, azoospermia, immune and genetic factors, and unknown etiology. All the patients hadn't received any antibiotic therapy during the month prior to the UU and CT examination.

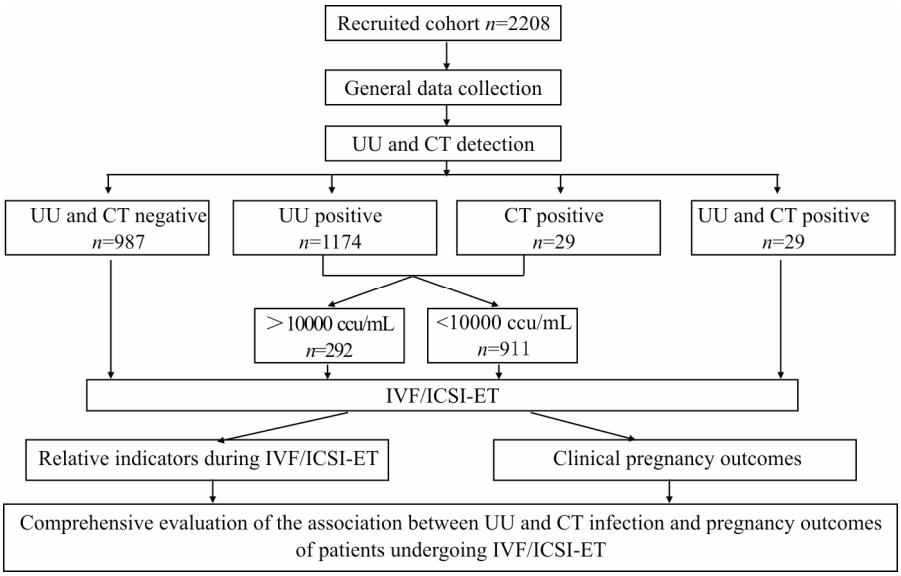

Fig. 1 Flow chart from original cohort recruitment to the final comprehensive evaluation study used in this study

1.2 Data Acquisition

Recorded data included the general condition, history of pregnancy, history of surgery, factors associated with assisted reproductive technology (such as the etiology of infertility, insemination methods, down-regulation protocols, number of implanted embryos, and luteal support methods), detection results of UU and CT in cervical secretions, and the final pregnancy outcomes of patients who had received IVF/ICSI-ER therapy.

1.3 UU and CT Detection and Treatments

The women were examined for the presence of UU by micro-liquid culture method in our clinical laboratory. Patients with UU culture count>10 000 ccu/mL or with clinical symptoms were treated with antibiotics on the basis of the results of drug sensitivity test before IVF attempt. Patients with UU culture count<10000 ccu/mL did not receive any treatment.

Cervical CT was detected by using polymerase chain reaction (PCR) in our clinical laboratory. Patients with positive result plus clinical symptoms who were treated with antibiotics according to the drug sensitivity test results were allowed to enter the IVF/ICSI-ET cycle. Women who had CT infection but were asymptomatic or only showed minimal symptoms did not receive any treatment.

The vaginal cleanliness was graded on a 3-point scale: grade Ⅰ: WBC count: 0–5/HPF, with the *Bacillus vaginalis* and epithelial cells as the main composition; grade Ⅱ: WBC count: 10–15/HPF, including a few infectious bacteria except *Bacillus vaginalis* and epithelial cells; grade Ⅲ: WBC count: 15–50/HPF, containing a lot of infectious bacteria leucorrhea indicating presence of infection.

1.4 Group Comparison

Based on the detection results of UU and CT in cervical secretions, the patients were divided into 4 groups: UU positive (UU+) group, CT-positive (CT+) group, the UU- and CT-positive (UU+CT+) group, and UU- and CT-negative (UU–CT–, or control) group. Comparison was made in terms of their general characteristics (including age, years of infertility, etiology of infertility, basal FSH level), IVF/ICSI outcomes (including the numbers of retrieved oocytes, fertilization rate, cleavage rate, high quality embryo rate, implantation rate), and pregnancy outcomes (including clinical pregnancy rate, early abortion rate, ectopic pregnancy rate, preterm birth rate) among the 4 groups.

1.5 Statistical Analysis

Data were analyzed by using the software package SPSS13.0. The inter-group comparison of mean values was made by using one-way ANOVA. The percentage values were compared by utilizing the χ^2 test and Fisher's exact test.

2 RESULTS

2.1 General Data

A total of 2208 patients with infertility were detected for UU and CT cervical secretions before IVF/ICSI-ET and 1203 were found to be UU positive (UU＋), with a positive rate of 54.48%; 47 cases were CT-positive (CT＋), with a positive rate of 2.13%; 29 cases were both UU- and CT-positive (UU＋CT＋), with a positive rate of 1.43%. The positive rates obtained in our series were consistent with previously reported data, which showed that in infertile patients, the positive rate of UU was 30%–50%[5, 6], and the rate of CT was 5%–25%[7, 8], indicating that there was no bias involved in the recruitment of subjects and the statistical data were relevant for clinical application.

To determine the impact of cervical UU and CT infections on female infertility, whether the cervical UU

and CT infections are the risk for tubal factors-induced infertility and ectopic pregnancy, we compared the related information in age, infertility duration, infertility type, previous history of pregnancy. Table 1 summarizes the general characteristics of subjects among UU+, CT+, UU+CT+ and UU–CT– group, respectively. There were no differences in the subjects' general features, such as age, infertility duration and infertility type (primary and secondary infertility). More importantly, there existed no significant differences in the rate of tubal factor-induced infertility between UU+ (65.42%), CT+ (61.11%) or UU+CT+ (58.63%) group and UU–CT– group (63.83%). Similarly, no significant difference was found in the rate of previous history of ectopic pregnancy between UU+ (18.74%), CT+ (5.55%) or UU+CT+ (20.69%) group and UU–CT– group (18.14%). However, there were significant differences in grades of the vaginal cleanliness, which reflects vaginal microorganisms, inflammatory cells and secretions, between CT+ or UU+CT+ group and UU+ group or control group, suggesting that cervical CT infection may have adverse effect on female genital tract micro-environment.

2.2 Comparison of IVF/ICSI Laboratory Indices

Next, we investigated the effect of cervical UU and CT infection on the number of retrieved oocytes, fertilization, and embryo development. Table 2 summarizes the relative indicators during IVF/ICSI-ET. The fertilization rate in UU+CT+ group and the high-quality embryo rate in UU+ group were significantly decreased when compared with control group (P<0.05). However, there were no differences in the number of follicles, retrieved oocytes and the cleavage rate between UU+, CT+ or UU+CT+ group and control group (P>0.05).

2.3 Comparison of Pregnancy Outcomes after IVF/ICSI-ET

To examine the impact of cervical UU and CT infection on pregnancy outcomes, we followed up the subjects and recorded relative pregnancy outcomes. Table 3 details the pregnancy outcomes of all subjects enrolled in this study. No significant differences were found in the clinical pregnancy rates and implantation rates between UU+ (41.50%), CT+ (50.00%) or UU+CT+ (50.00%) group and UU–CT– group (39.02%). We are more concerned with the potential role of UU and CT in adverse pregnancy outcomes. Nonetheless, there existed no differences in the rates of early abortion, ectopic pregnancy and preterm birth between UU+, CT+ or UU+CT+ and UU–CT– group. Similarly, there were no differences in the rates of single birth and twins birth among these four groups.

Table 1 The general clinical characteristics of 2208 patients recruited in this study

Indexes	UU+	P^a	CT+	P^b	UU+CT+	P^c	Control (UU–CT–)
Cases (n)	1174		18		29		987
Age (years)	31.28±4.36	0.41	31.56±3.76	0.76	31.28±3.45	0.57	31.35±4.07
Infertility duration (years)	5.29±3.51	0.82	4.94±2.98	0.28	4.90±3.35	0.29	5.18±3.45
Tubal factor-induced infertility (%)	65.42 (768/1174)	0.44	61.11 (11/18)	0.81	58.63 (17/29)	0.56	63.83 (630/987)
Basal FSH (mU/mL)	6.02±2.71	0.88	5.61±1.63	0.19	5.92±2.49	0.38	6.14±2.69
Primary infertility (%)	50.51 (593/1174)	0.49	44.44 (8/18)	0.64	62.07 (18/29)	0.35	52.08 (514/987)
Secondary infertility (%)	49.49 (581/1174)	0.49	55.56 (10/18)	0.64	37.93 (11/29)	0.35	47.92 (473/987)
Previous history of ectopic pregnancy (%)	18.74 (220/1174)	0.74	5.55(1/18)	0.22	20.69 (6/29)	0.63	18.14 (179/987)
Vaginal cleanliness							
Grade Ⅰ (%)	3.23	0.05	5.88	0.60	0	0.39	4.95
Grade Ⅱ (%)	88.48	0.69	70.59	0.60	78.57	0.16	87.93
Grade Ⅲ (%)	8.29	0.33	23.53	0.04	21.43	0.01	7.12

UU+: UU-positive group; CT+: CT positive group; UU+CT+: UU and CT positive group; UU–CT–: UU and CT negative group.
P^a: UU+ *vs*. control; P^b: CT+ *vs*. control; P^c, UU+CT+ *vs*. control

2.4 Comparison of General Information and Pregnancy Outcomes between Group with the UU Culture Count >10000 ccu/mL and Group with the Count <10000 ccu/mL

To study the impact of cervical UU infection on pregnancy outcomes, we specifically compared the pregnancy outcomes between group with the UU culture count >10000 ccu/mL and group with the count <10000 ccu/mL. Table 4 lists the pregnancy outcomes of these two groups in this study. There existed no significant differences in clinical pregnancy rate and implantation rate between the group with UU >10 000 ccu/mL and the group with UU <10 000 ccu/mL (25.74 *vs*. 23.76 and 43.75 *vs*. 41.02). Similarly, there were no differences in the rates of early abortion rate and ectopic pregnancy rate between these two groups.

Table 2 The comparison of relative indicators during IVF/ICSI-ET

Indexes	UU+	$P^{\triangle}$	CT+	$P^{\#}$	UU+CT+	$P^{\blacktriangle}$	Control (UU–CT–)
Cases (*n*)	1174		18		29		987
Number of follicles	11.8±5.24	0.41	13.61±4.17	0.76	11.21±4.72	0.57	11.48±5.31
Number of retrieved oocytes[a]	12.41±6.36	0.82	12.44±6.75	0.28	12.62±6.53	0.29	12.21±6.40
Fertilization rate[b] (%)	60.79 (8809/14492)	0.13	62.41 (181/290)	0.40	53.01 (194/366)	0.009	59.72 (7158/11985)
Cleavage rate[c] (%)	96.23 (8477/8809)	0.44	97.79 (177/181)	0.33	96.91 (188/194)	0.85	96.16 (6883/7158)
High-quality embryo rate[d] (%)	77.83 (6598/8477)	<0.001	80.79 (143/177)	0.05	81.91 (154/188)	0.11	78.40 (5396/6883)
Implantation rate[e] (%)	24.25 (477/1967)	0.26	34.78 (8/23)	0.21	28.26 (13/46)	0.36	22.71 (387/1704)

$P^{\triangle}$: UU+ *vs*. control; $P^{\#}$: CT+ *vs*. control; $P^{\blacktriangle}$: UU+CT+ *vs*. control

a: Follicle number refers to the number of follicles with a diameter greater than 1.4 cm under transvaginal ultrasound on the day of intramuscular injection of human chorionic gonadotropin (hCG). b: Fertilization rate refers to fertilization of normal double-pronucleate (2PN) oocytes; the fertilization rate of normal 2PN oocytes=Number of fertilized normal 2PN oocytes/Number of retrieved oocytes. c: Oocyte cleavage rate=Number of embryos with more than 2 cells/Number of 2PN fertilized eggs. d: High quality embryos refers to grade B or higher embryos with more than 4 cells 3 days after embryo retrieval. e: Implantation rate=Number of gestational sacs with embryo and fetal heart beat as shown by B-type ultrasound 30 days after embryo transfer/Total number of transferred embryos.

Table 3 The comparison of pregnancy outcomes after IVF/ICSI-ET

Indexes	UU+	$P^{\triangle}$	CT+	$P^{\#}$	UU+CT+	$P^{\blacktriangle}$	Control (UU–CT–)
Cases (*n*)	1174		18		29		987
Clinical pregnancy rate[f] (%)	41.50 (383/923)	0.32	50.00 (6/12)	0.55	50.00 (11/22)	0.38	39.02 (304/779)
Early abortion rate[g] (%)	10.18 (39/383)	0.80	0.00 (0/6)	1.00	18.18 (2/11)	0.35	10.86 (33/304)
Ectopic pregnancy rate[h] (%)	3.66 (14/383)	1.00	0.00 (0/6)	1.00	0.00 (0/11)	1.00	3.62 (11/304)
Live birth							
Cases (*n*)	323		6		8		258
Percentage (%)	84.1 (323/384)	0.83	6/6[i]	0.60	8/11[i]	0.39	84.9 (258/304)
Premature birth							
Cases (*n*)	51		1		2		39
Percentage (%)	15.8 (51/323)	0.91	1/6[i]	1.00	2/8[i]	0.36	15.1 (39/258)
Full-term birth							
Cases (*n*)	272		5		6		219
Percentage (%)	84.2 (272/323)	0.91	5/6[i]	1.00	6/8[i]	0.36	84.9 (219/258)
Single birth							
Cases (*n*)	231		4		6		192
Percentage (%)	71.5 (231/323)	0.45	4/6[i]	0.65	6/8[i]	1.00	74.4 (192/258)
Twins birth							
Cases (*n*)	92		2		2		66
Percentage (%)	28.5 (92/323)	0.45	2/6[i]	0.65	2/8[i]	1.00	25.6 (66/258)

$P^{\triangle}$: UU+ *vs*. control; $P^{\#}$ CT+ *vs*. control; $P^{\blacktriangle}$: UU+CT+ *vs*. control

f: Clinical pregnancy was defined as detection of embryo and fetal heart beating by B-type ultrasound examination 30 days after implantation. Clinical pregnancy rate=Number of clinical pregnancies/Total number of implantations. g: Early abortion rate=Number abortions before 12 weeks of pregnancy/Number of weeks of clinical pregnancy. h: Ectopic pregnancy rate=Number of cases of ectopic pregnancy/Number of clinical pregnancy. i: ratio instead of percentage due to limited cases

Table 4 Comparison of general condition and pregnancy outcomes in UU+ group

Indexes	UU culture		*P*
	＞10 000 ccu/mL	＜10 000 ccu/mL	
Cases (*n*)	292	911	
Age (years)	31.26±4.48	31.21±4.33	0.87
Infertility duration (years)	5.06±3.26	5.35±3.58	0.23
Tubal factor induced infertility (%)	66.44 (194/292)	64.87 (591/911)	0.67
Basal FSH (mIu/mL)	5.76±2.51	5.97±2.52	0.31
Primary infertility (%)	47.26 (138/292)	51.92 (473/911)	0.18
Secondary infertility (%)	52.74 (154/292)	48.08 (438/911)	0.18
Previous history of ectopic pregnancy (%)	18.83 (55/292)	18.77 (171/911)	1.00
Number of follicles	13.61±5.25	11.84±5.23	0.08
Average number of retrieved oocytes	12.11±6.33	12.51±6.38	0.89
Number of retrieved oocytes	3536	11364	
Fertilization			
Number (*n*)	2193	6816	
Percentage (%)	62.02	59.98	0.09
Embryo cleavage			
Number (*n*)	2095	6573	
Percentage (%)	95.53	96.43	0.06
High quality embryo			
Number (*n*)	1601	5151	
Percentage (%)	76.42	78.37	0.07
Implantation rate (%)	25.74 (121/470)	23.76 (369/1553)	0.40
Clinical pregnancy rate (%)	43.75 (98/224)	41.02 (297/724)	0.47
Early abortion rate (%)	9.18 (9/98)	10.77 (32/297)	0.85
Ectopic pregnancy rate (%)	4.08 (4/98)	3.37 (10/297)	0.76

3 DISCUSSION

UU and CT are closely associated with female infertility[12, 13]. UU primarily resides in the human urogenital tract. Under normal circumstances, UU in the vaginal flora does not cause disease. Only when immunity is impaired or the vaginal mucosa is damaged, does UU become pathogenic. However, it is unclear whether these infections significantly contribute to female infertility and adverse pregnancy outcomes. Some studies suggested that UU and CT may exert potential adverse effects on fertility in women, while other researches indicated the otherwise.

3.1 Cervical UU and CT Infection and Etiology of Female Infertility

UU and CT are the most common pathogens responsible for STD worldwide. Though most women infected with UU and CT are asymptomatic or only have minimal symptoms, some develop salpingitis, endometritis, pelvic inflammatory disease, ectopic pregnancy and tubal factor-related infertility. In women, the cervix is the most vulnerable site for UU infection and local inflammation tends to extend to the oviduct to cause salpingitis, thereby resulting in infertility[14, 15]. Sharma *et al* detected serum CT-specific IgG antibody in 40 hysterosalpingographically and laparoscopically confirmed cases of salpingitis-induced infertility and found that 70% patients were positive for CT IgG antibody[16].

In the present study, we conducted a large-sample survey, with an attempt to understand the impact of cervical UU and CT infection on female infertility. We compared the occurrence rate of primary and secondary infertility, tubal factor-induced infertility and previous history of ectopic pregnancy among the aforementioned four groups. Contrary to pre-study expectation, we failed to reveal any differences in the rates of primary and secondary infertility, tubal factor-induced infertility and previous history of ectopic pregnancy among these groups, indicating that cervical UU and CT infection was not a risk for ectopic pregnancy and tubal factor-induced infertility. Nevertheless, our results exhibited that the vaginal cleanliness was poor in CT+ and UU+CT+ groups than in control and UU+ groups, suggesting that CT infection may contribute to unhealthy genital environment. Females with poor vaginal cleanliness always bear high risk for genital infections, including vaginitis, salpingitis and endometritis[17]. Although there was no obvious difference in the rate of the ectopic pregnancy between patients with poor vaginal cleanliness and those with normal cleanness, which might be ascribed to the pre-treatment before IVF/ICSI-ET, we still recommend the patients with poor vaginal cleanliness should receive routine vaginal cleansing to prevent adverse outcomes.

3.2 Effect of Cervical UU and CT Infection on IVF/ICSI-ET Outcomes

Female genital tract infection is believed to be a major cause of pelvic inflammatory diseases and an adverse factor for gamete development, fertilization and embryo development[18, 19]. More importantly, women who carry UU and CT are a potential source of infection to their partners, which causes urethritis in men[20, 21]. Several studies demonstrated that genital tract infection with UU and CT in males could affect sperm function and spermatogenesis, and are intimately related to varicocele-related infertility[22, 23]. Kanakas *et al* analyzed the effect of UU infection in semen on IVF outcomes, and found the fertilization and pregnancy rate might not be affected by the presence of UU in semen on the day of oocyte retrieval, but subjects in UU-positive group showed a higher abortion rate after IVF[24]. Witkin *et al* reported that cervical UU infection did not influence IVF outcomes subsequent to embryo transfer in women treated with tetracycline after oocyte retrieval[10]. UU are not always removed from semen by a standard assisted reproductive techniques (ART) washing procedure and can remain adherent to the surface of spermatozoa[11]. In

view of the adverse effect of UU and CT infections on both male and female fertility and IVF outcomes, some clinicians suggested that the best strategy would be to cancel the IVF treatment for UU- and CT-positive couples[25].

In our study, we divided the UU positive patients into a >10 000 ccu/mL group and a <10 000 ccu/mL group in terms of UU culture count, and the patients in the >10 000 ccu/mL group received antibiotics treatment for 7–10 days based on the results of drug sensitivity test before entering the cycle. However, the conversion to negative in some patients was not satisfactory, and a small number of the patients still had mild cervical UU infection before entering IVF-ET treatment. Although the fertilization rate was significantly lower in UU+CT+ group than in other groups, the number of follicles and retrieved oocytes, the embryo cleavage rate and implantation rate were not affected by UU or CT infection. Presumably, the semen preparation for IVF eliminated the adverse effect of UU and CT infection on sperms and fertilization. Epidemiological studies confirmed that 40%–80% of sexually mature women normally carried UU in the cervix and vagina, indicating that a positive result of UU culture of cervical secretions is not necessarily an indication of pathological status[26]. In addition, women with cervical UU and CT infection often respond poorly to antibiotics treatments. Recently, reports found that *U. parvum*, the other species of the UU family, might be a human cervicovaginal colonizer that is insensitive to antibiotics, while the *U. urealyticum* might be a pathogen in human urogenital tract and was sensitive to antibiotics[27]. It is difficult to evaluate the curative effect of antibiotics on cervical UU infection in our retrospective study since the micro-liquid culture method couldn't differentiate the subtypes of cervical UU. Our results showed the fertilization rate in UU+CT+ group was decreased, and the high quality embryo rate declined in UU+ group when compared with other groups. Studies at molecular level revealed that UU infection might work with abnormal reactive oxygen species and inflammatory cells, and subsequently induce sperm apoptosis[28, 29]. The semen of infertile men positive for CT and UU showed lower mean semen count and lower count of spermatozoa with rapid progressive motility, and an increased caspase 3 activity compared to their uninfected counterparts, with the differences being statistically significant[30] and these findings suggested that CT and UU infection might directly exert negative impact on thefertilizing ability of sperms[30]. Our comparison showed that more precise laboratory methods should be used to distinguish the subtypes of UU infection, because asymptomatic patients with cervicovaginal colonial subtype of UU infection might receive IVF-ET without receiving antibiotics treatment.

3.3 Correlation between Cervical UU and CT Infection and Pregnancy Outcomes

Most studies linked pathogens UU and CT with genital tract infections, infertility and adverse pregnancy outcomes[31, 32]. Over past decades, clinicians believed UU and CT infections were also major causes of ectopic pregnancy, premature rupture of the membrane, and premature birth[33, 34]. However, Karaer *et al* detected serum UU-IgG and UU-IgM in 125 patients with ectopic pregnancy and found no statistically difference in the detection rate of the patients and controls, and they suggested that the ectopic pregnancy was not associated with current mycoplasma infection[35]. Due to the limited sample size, we didn't examine the etiology of infertility and other influencing factors, as a result, the relationship between UU and CT infection and pregnancy outcomes was very preliminary and further comprehensive investigations are needed.

The inclusion of subjects into this study completely excluded such influencing factors as, male infertility, fertilization and embryo development, with focus being directed on the effect of cervical UU/CT infection on pregnancy outcomes. We followed all the subjects by visiting them and recorded pregnancy outcomes. Our study showed that cervical UU and/or CT infection did not adversely impact on pregnancy outcomes in terms of abortion, preterm birth and ectopic pregnancy. The detection rate of UU in healthy women during physical check-ups could be as high as 60%[36], indicating that UU could be a natural parasitic microorganism in female cervicovaginal tract. Cervical CT infection rate is relatively low and, therefore, the impact of CT on pregnancy outcomes needs to be further studied.

The data in this study were relatively inclusive, covering causes of infertility, basic information, IVF-ET data and clinical pregnancy data *etc*.

This study also had some limitations. First, this was a retrospective case-control study and there were some drawbacks in data analysis and experimental design. For example, *U. urealyticum* was subdivided into two species as *U. parvum* and *U. urealyticum* recently, in which *U. parvum* is considered to be a human cervicovaginal colonizer, while *U. urealyticum* is thought to be a pathogen of human urogenital tract[27]. However, the micro-liquid culture method used in our center is incapable of differentiating the subtype of UU family. As a consequence, we could not specifically establish the connection between antibiotic treatment used for cervical UU infection and IVF-ET outcomes. Second, we performed this study primarily on infertility patients and no comparison was made with fertility couple in terms of related measures. Second, the grouping was not comprehensive. The subjects were grouped in terms of tubal factor-induced infertility and ectopic pregnancy history to assess the pathogenic effects of UU and CT. Other causes, including ovulation failure, endometrosis, immune and genetic factors, and factors of male parteners were ignored. Third, we didn't analyze the status of the babies.

In general, our results suggest that cervical UU and CT infection might not be a risk for tubal factor-induced infertility or adverse pregnancy outcomes of IVF-ET. Further confirmation is needed by conducting bettr-designed multi-center, large sample and control study, with more possible affecting factors covered.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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