



Correlation Analysis between Cervical Refractory *Ureaplasma urealyticum* Infection and IVF Pregnancy Outcome

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Abstract

Objectives: To determine the effect of cervical refractory *Ureaplasma urealyticum* infection on assisted reproductive outcomes.

Patients and Methods: A total of 273 patients who received *In Vitro* Fertilization (IVF) treatment were included in this study. The ages of the female patients ranged from 20 to 35 years. All patients have normal ovarian reserve (AFC ≥ 7) and regular menstruation and were divided into Group 1 (UU-, 151 patients), Group 2 (Patients who turned negative after UU treatment, 52 patients) and Group 3 (UU after 3 cycles of sensitive antibiotic treatment has not turned negative, 70 patients).

Results: The positive rate of *Ureaplasma urealyticum* in these patients was 44.7% (122/273). The rate of negative conversion of *Ureaplasma urealyticum* positive patients after 1 to 3 time's sensitive antibiotic treatment was 42.6% (52/122). There were no significantly difference of number of eggs obtained, number of fertilizations, rate of high-quality embryos and blastocyst formation rate among the three groups. And there were no significant differences in the clinical pregnancy rates, biochemical pregnancy rates, embryo implantation rates, spontaneous abortion rates, ectopic pregnancy rates, fetal malformation rates, live birth rates, even pre-birth rate.

Conclusion: Though the positive rate of *Ureaplasma urealyticum* infection in women of childbearing age is high, there is no need for routine screening of *Ureaplasma urealyticum* in assisted reproductive women.

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Keywords: *Ureaplasma urealyticum* infection; IVF; Pregnancy outcome; Infertility

Introduction

In the past 20 years, the incidence of female *Ureaplasma urealyticum* (UU) infection has been increasing year by year [1]. UU infection is not only related to female reproductive tract infections and infertility [2], but also related to many adverse pregnancy outcomes [3] and have attracted the attention of clinical medical workers.

The recent study of Megan Cavanagh shows that *U. urealyticum* may not be directly harmful to cells; it can activate an immune response *via* its products, which ultimately contributes to the pathogenicity of infection and inflammation with possible adverse reproductive outcomes including Premature Birth (PTB) [3]. Besides some studies suggested that UU infection had also been related to an arrest of embryonic development, it might alter the microenvironment in the uterine cavity affecting the normal development of the embryo, and associate with Recurrent Spontaneous Abortion (RSA) by stimulating Anti-Cardiolipin Antibody (ACA) expression, inflammatory response, thrombogenesis, and factors associated with cell apoptosis, increasing the risk for an abortion during pregnancy [4-6].

At present, more and more infertile patients need to accept assisted reproductive technology, but the infection rate of UU in infertile patients is significantly higher than that in the control group [7]. For male patients with *Ureaplasma urealyticum* infection, it is suggested that assisted reproductive therapy should be carried out after treatment [8,9]. Recent study showed that the overall infection rates of UU of 13, 303 women who visited the gynecologic outpatient service of Taizhou First People's Hospital in Taizhou from 2013 to 2018 was as high as 62.04% [10]. However, for the female patients with *Ureaplasma urealyticum* infection, due to the low conversion rate and long treatment cycle of antibiotic treatment, the time and economic cost before assisted reproduction of infertile women

are greatly increased [11]. Some study founded reduced pregnancy rates after in vitro fertilization in women with cervical colonization with urea plasmas in comparison to women with successful embryo transfer [12]. But some researchers thought that during the infertility assessment, infertile couples should not be routinely screened for UU infection without any clinically evidence [13]. The research of Qing-zhen XIE et al. revealed that positive cervical UU and CT infections should not be taken as strict contraindications for IVF/ICSI-ET [14]. Whether to screen UU infection and antibiotic treatment is still controversial [7]. In particular, it is not clear whether drug-resistant *Ureaplasma urealyticum* infection can directly enter the IVF cycle and its impact on fertility outcome.

Considering that there are many factors influencing the outcome of assisted reproduction, this study excluded the infertility couples caused by male factors, female ovulation disorders, endocrine abnormalities and other factors, and only selected the patients with tubal factors and unexplained infertility as the research object. In order to find out the relationship between the infection of refractory *Ureaplasma urealyticum* and the pregnancy outcome of IVF.

Materials and Methods

Study population

The patients who underwent their first cycles of *In Vitro* Fertilization Embryo Transfer (IVF-ET) treatment of this study were from the Reproductive Medical Center of First Affiliated Hospital of Zhejiang University School of Medicine, between April 2013 to January 2018. This study was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Reference Number: 2020-293). The ages of the female patients ranged from 20 to 35 years. All patients have normal ovarian reserve ($AFC \geq 7$) and regular menstruation.

Before entering the IVF cycle, the husband and wife received detailed and comprehensive examinations, including *Mycoplasma*, Chlamydia, gonococcus, endocrine, coagulation, hepatitis B, hepatitis C, syphilis, HIV, etc. All patients were divided into three groups according to the infection and treatment of *Ureaplasma urealyticum* (In our study all the UU positive patients had no clinical symptoms):

Group 1: UU- (control group);

Group 2: Patients who were positive for UU for the first time and turned negative after treatment;

Group 3: UU after 3 cycles of sensitive antibiotic treatment has not turned negative.

Exclusion criteria were as follows: 1) Women with ovarian cyst, PCOS, endometriosis; 2) women with the surgery history of ovarian malignant tumor or endocrine disorders; 3) the history of radiotherapy and chemotherapy; 4) chromosomal abnormalities (not including chromosome polymorphisms); 5) autoimmune diseases, 6) hereditary diseases; 7) smoking and alcoholism; 8) abnormal uterus, uterine malformation; 9) spontaneous abortion more than 2 times; 10) IVF contraindication; 11) various factors require ICSI patients.

UU detection and treatments

The screening for genital UU was performed in male semen and female cervical secretions by micro-liquid culture method in our clinical laboratory. In UU positive cases (UU culture count $>10\,000$ ccu/mL), couples were asked to accept sensitive antibiotic treatment on the basis of the results of drug sensitivity test before IVF attempt

and had a post-treatment microbiological test. Both of them needed to use protection (condoms) during sexual intercourse for 7 to 14 days after initiating the treatment. Patients with UU culture count <10000 ccu/mL did not receive any treatment. For men with UU infection failing antibiotic treatment were prescribed alternative antibiotics until they had negative post-therapy microbiological tests. For women with UU infection, if the third cycle of sensitive antibiotic treatment fails, we will inform the patients and let them enter the IVF cycle after the patient's signs the informed consent.

Cycle monitoring and IVF/intracytoplasmic sperm injection

The ovulation promotion protocol of all patients was long-term luteal phase protocol. The criteria for Human Chorionic Gonadotropin (HCG) administration (Livzon, 5000 to 10,000 IU, Pharmaceutical Group Inc, China,) was the presence of three or more follicles ≥ 16 mm in diameter with a consistent rise in serum estradiol concentration. Oocyte aspiration was performed using vaginal ultrasound, 34 h to 36 h after hCG injection.

According to the international morphological grading system [15], the embryos were divided into 4 grades. In grade I, blastomeres were even in appearance, their cytoplasm was transparent and even, and there was no debris. In grade II, blastomeres were even in appearance and the proportion of cytoplasmic debris was $<20\%$. In grade III, blastomeres were not irregular and the proportion of cytoplasmic debris was 20% to 50%. In grade IV, blastomeres were not irregular and the proportion of cytoplasmic debris was $>50\%$. Grade I-II embryos were regarded as high-quality, and grade I-III embryos could be used for transplantation. One or two embryos were transplanted at 3 days after egg collection and the remaining embryos were processed for blastocyst culture and cryopreservation.

Study endpoints

Clinical pregnancy was defined by the presence of a gestational sac. Biochemical pregnancy was defined by the presence of β -HCG ≥ 25 mIU/mL without ultrasound evidence of a gestational sac. Clinical pregnancy rate was defined by the ratio of the clinical pregnancy cases to the embryo transfer cases. High quality embryo rate was defined by the ratio of the number of high-quality embryos to the number of normal fertilized cleavage embryos. Live birth rate = number of live birth/numbers of transplantation $\times 100\%$.

Statistical analyses

Statistical comparisons were made with SPSS19.0 statistical software. Continuous data were expressed as the mean \pm SD, independent student t test was used to compare the Continuous data between two groups. The rate (%) was used to compare the counting data. Chi-squared test was used to compare the data between groups. $P < 0.05$ was statistically significant.

Results

General Data

A total of 273 patients who underwent their first cycles of *In Vitro* Fertilization (IVF) in the Reproductive Medical Center of First Affiliated Hospital of Zhejiang University School of Medicine between April 2013 to January 2018 met the inclusion criteria for this study. 151 women in Group 1 (control group), 52 women in Group 2 (Cervical urealyticum infection turned negative after treatment), and 70 women in Group 3 (UU after 3 cycles of sensitive antibiotic treatment has not turned negative). The positive rate of *Ureaplasma*

Table 1: Comparison of the three groups of general clinical data.

	Group 1 (n=151)	Group 2 (n=52)	Group 3 (n=70)	P
Age (Maternal)	29.82 ± 3.53	28.57 ± 3.88	29.98 ± 3.46	0.061
BMI (kg/m ²)	21.39 ± 2.94	21.40 ± 2.76	21.13 ± 2.91	0.807
AFC	12.93 ± 4.42	12.92 ± 3.85	13.59 ± 4.23	0.534
bFSH (IU/L)	7.44 ± 1.91	7.76 ± 1.47	7.31 ± 1.58	0.356
bE2 (pg/ml)	48.02 ± 19.26	48.11 ± 20.74	45.86 ± 18.74	0.716
bLH (IU/L)	4.56 ± 1.86	4.78 ± 1.74	4.28 ± 1.46	0.279
FSH/LH	1.91 ± 1.07	1.86 ± .88	1.88 ± .71	0.95
E2 (HCG Day)	2816.98 ± 1633.96	3023.32 ± 1809.30	3145.09 ± 1747.09	0.377
Duration of infertility (yr)	2.98 ± 2.24	3.25 ± 2.32	3.07 ± 2.46	0.753
Cause of infertility (%)				
Primary infertility	67 (44.4%)	22 (42.3%)	24(34.3%)	0.227
Secondary infertility	84 (55.6%)	30 (57.7%)	46(65.7%)	0.227

Table 2: Outcomes after IVF/ICSI in the three groups.

	Group 1 (n=151)	Group 2 (n=52)	Group 3 (n=70)	P
Number of eggs obtained	8.87 ± 4.10	9.75 ± 5.26	9.63 ± 3.86	0.298
Number of fertilizations	5.99 ± 3.12	5.84 ± 4.34	7.00 ± 3.32	0.087
Rate of high-quality embryos (%)	63.9% (583/911)	59.5% (181/304)	59.7% (297/497)	0.094
Blastocyst formation rate (%)	36.6% (139/380)	46.8% (60/128)	35.4% (77/217)	0.996

Table 3: Comparison of clinical outcomes among the three groups.

	Group 1 (n=151)	Group 2 (n=52)	Group 3 (n=70)	P
Clinical pregnancy rate (%)	68.2 (103/151)	50.0 (26/52)	65.7 (46/70)	0.452
Biochemical pregnancy rate (%)	1.1 (6/151)	0.8 (2/52)	5.7 (4/70)	0.588
Embryo implantation rate (%)	45.0 (127/282)	33.7 (29/86)	39.7 (54/136)	0.203
Spontaneous abortion rate (%)	2.6 (4/151)	5.7 (3/52)	2.8 (2/70)	0.789
Live birth rate (%)	63.5 (96/151)	44.2 (23/52)	57.1 (40/70)	0.203
Ectopic pregnancy rate (%)	1.9 (3/151)	0 (0/52)	4.3 (3/70)	0.388
Fetal malformation rate (%)	0 (0/151)	0 (0/52)	1.4 (1/70)	0.127
Preterm birth rate (%)	11.1 (7/63)	5.9 (1/17)	3.8 (1/26)	0.242

urealyticum in these patients was 44.7% (122/273). The rate of negative conversion of *Ureaplasma urealyticum* positive patients after 1 to 3 time's sensitive antibiotic treatment was 42.6% (52/122). There were no marked differences of maternal age, AFC, bFSH, FSH/LH, duration and type of infertility, and BMI among the three groups (Table 1).

Comparison of IVF outcomes among the three groups

There were no significantly difference of number of eggs obtained, number of fertilizations, rate of high-quality embryos and blastocyst formation rate among the three groups (Table 2).

Comparison of the clinical outcomes among the three groups

Clinical pregnancy outcome is an important follow-up and monitoring indicator of assisted reproductive technology. In this study, we found there were no significant differences in the clinical pregnancy rates, biochemical pregnancy rates, embryo implantation rates, spontaneous abortion rates, ectopic pregnancy rates, fetal malformation rates, and live birth rates. In previous studies, there was more mention of the correlation between *Ureaplasma urealyticum* infection and premature delivery. Because twin pregnancies

themselves increase preterm birth rates, in this study we compared the preterm birth rates among patients with single pregnancies in the three groups. And we found that there existed no differences in the rates of preterm birth among the three groups (Table 3).

Discussion

UU is belonging to the *Ureaplasma* group, which are the smallest free-living microorganisms. It is considered responsible for some genital diseases, such as non-gonococcal urethritis, cervicitis, testicular inflammation, infertility, and prostatitis [16]. At present, the infection rate of *Ureaplasma urealyticum* in women of childbearing age is relatively high, but the sensitivity of antibiotic treatment is relatively low [17,18]. In the study of Young-Soo Jang, the UU positive culture rate of 200 patients was up to 47%. Some studies showed even much higher UU infection rates which were up to 60.11% to 62.04% in the women who visited the gynecologic outpatient service for various reasons [10,19]. Some women cannot turn negative in multiple cycles of treatment, which seriously increases the treatment cost and mental pressure of patients. In these UU positive patients, most of them do not have any symptoms. If there are any symptoms, the symptoms are vaginal discharge and dysuria. In our study, the UU

positive rate was up to 44.7%, and after at least three cycles of sensitive antibiotic treatment, the UU positive rate is still as high as 25.6%. All of them had no symptom, and before they entered into the IVF cycles, their leucorrhea test is normal. The conclusion of the European STI Guidelines Editorial Board in 2018 also showed that there was no adequate evidence that *M. hominis*, *U. parvum* or *U. urealyticum* caused an inflammatory vulvovaginitis, cervicitis, urethritis, PID or infertility and antimicrobial treatment resulting in eradication was difficult, and eradication was not unequivocally associated with cure [17].

Now for men with high *U. urealyticum* load, many study suggested to give active treatment [8,17]. The study published in 2019 consider that genital mycoplasma routine screening could be useful in order to increase the quality of semen which could simplify the *in vitro* fertilization procedures and raise the success rate of embryo implantation and pregnancy. For women with UU infection, especially asymptomatic non-pregnant women are discouraged to give treatment, because the evidence that they cause disease is questionable [17]. However, it is still controversial whether pregnant women should receive treatment. The large cohort study in south Asia published in 2018 showed that *Ureaplasma urealyticum* (*Ureaplasma urealyticum* and *Mycoplasma parvum*) was the second most common microorganism isolated from infants with severe bacterial infection and was more likely to be isolated from sick infants than healthy infants [20].

Women receiving assisted reproductive therapy are about to become pregnant women. It is still controversial whether this part of the population infected with *Ureaplasma urealyticum* need treatment. The study of Witkin et al. reported that cervical UU infection did not affect IVF results after embryo transfer in women treated with tetracycline after oocyte retrieval. However, some doctors still consider the potential risk of cervical *Ureaplasma* infection, and the positive patients who need assisted reproductive therapy need to turn negative before entering the cycle [21]. A study in 2016 included 2208 IVF/ICSI patients. The results showed that the fertilization rate and high-quality embryo rate of UU + CT + group decreased, but the pregnancy rate, abortion rate, ectopic pregnancy rate and premature birth rate of UU and/or CT infected patients were not significantly different from those of the control group [14]. Unfortunately, this study didn't examine the etiology of infertility and other influencing factors.

Our study only selected IVF patients who received IVF because of tubal factors and unexplained infertility. The results showed that there were no significantly difference of number of eggs obtained, number of fertilizations, rate of high-quality embryos and blastocyst formation rate among the three groups. Besides, we found there were no significant differences in the clinical pregnancy rates, biochemical pregnancy rates, embryo implantation rates, spontaneous abortion rates, ectopic pregnancy rates, fetal malformation rates, and live birth rates, even the prebirth rate. Recently, it has been reported that another species of the UU family, micro-U. parvum, may be a human cervicovaginal colonization bacterium sensitive to antibiotics, while *Ureaplasma urealyticum* may be a pathogen of human urogenital tract, which is sensitive to antibiotics [22]. In our study, the test of UU cannot distinguish *Ureaplasma urealyticum* from micro-U. parvum. And the patients in UU + group had received at least 3 cycles of sensitive antibiotic therapy. One possibility is that patients with positive UU culture may be patients carrying *U. parvum*. This is the

limitation of this study.

In conclusion, this study showed that for asymptomatic female *Ureaplasma urealyticum* positive patients, IVF outcomes including clinical pregnancy rate, live birth rate, abortion rate and premature birth rate were not affected. The positive rate of *Ureaplasma urealyticum* infection in women of childbearing age is high and the negative rate is low. Continuous antibiotic treatment is easy to increase drug resistance and increase economic pressure. There is no need for routine screening of *Ureaplasma urealyticum* in assisted reproductive women.

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Author Contributions

Lingnv Yao and Wenqin Lin contributed to the design, study execution and manuscript writing. Nan Jiang contributed to data collection. Jing Chen and Chuyan Li contributed to data analysis.

Statement of Ethics

This study was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Reference Number: 2020-293).

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