Lower cumulative live birth rates in cured endometrial tuberculosis patients after one ART cycle including all subsequent frozen-thaw cycles: A matched-pair study

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A R T I C L E   I N F O

Article history:
Received 21 April 2019
Received in revised form 16 November 2019
Accepted 20 November 2019
Available online 22 November 2019

Keywords:
Infertility
Endometrial tuberculosis
Assisted reproductive therapy (ART)

A B S T R A C T

Objective: To investigate the outcomes of the first ART cycle including all subsequent frozen-thaw cycles from the same oocyte retrieval till first live birth in women with cured endometrial tuberculosis.

Study design: This is a 1:4 matched-pair study, 113 cured endometrial tuberculosis patients (TB group) and 452 patients of non-tuberculosis (Non-TB group) matched for age, basal E2, basal FSH and ovulation protocol who underwent first complete ART cycles in our institution during December 2010 and December 2015 were included in the study. The baseline characteristic, clinic data, and IVF treatment outcomes were compared and analyzed between the two groups.

Results: Compared with the Non-TB group, the cumulative clinical pregnancy rates was similar (64.6% vs 65.1%, p = 0.89) but the cumulative live birth rates (40.7% vs 52.7%, p < 0.00) were significantly lower and the spontaneous abortion rates (37.0% vs 13.2%, P < 0.05) was significantly higher in TB group. There was no significant difference in the clinical pregnancy rates, live birth rates and spontaneous abortion rates between the fresh cycles and frozen-thaw cycles in the TB group.

Conclusion: Women may have increased risk of miscarriage and decreased CLBRs after cured endometrial TB infection when undergoing IVF.

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1. Introduction

As the World health Organization (WHO) reported in 2016 [1], there were an estimated 10.4 million new tuberculosis (TB) cases worldwide, of which 3.5 million (34%) were women. The total number of TB patients in China is second only to India and Indonesia, which makes it a high incidence of TB area. Female genital tuberculosis (FGTB) is the most common extra-pulmonary TB disease [2–5], and often leads to infertility [6–8]. The incidence rate is also increasing year by year. The prevalence of FGTB is 7% to 15% in infertile women in developing countries, 26% of tertiary referral hospitals and up to 48% in tubal infertility patients [9–10]. As the course of FGTB disease is slow, atypical, and the specificity and sensitivity of examination are not high, it is still a diagnostic dilemma, making the clinical diagnosis rate to be relatively low. Guo showed that FGTB patients accounted for 5.8% of the in vitro fertilization and embryo transfer (IVF-ET) population, and accounted for 9.3% of the infertility of fallopian tube factors [11]. Thus, FGTB patients occupy a certain proportion in the IVF population, especially in infertile patients with tubal factors. The incidence of endometrial tuberculosis in FGTB are as high as 50-80% [12–14], second only to tubal tuberculosis. The uterine endometrial environment is an important cradle for embryo development, once violated, even after cured with anti-TB therapy, the damage on the endometrial blood flow and uterine cavity morphology is often irreversible, and thus affect the embryo implantation, leading to infertility. Most of the those patients have to go for assisted reproductive therapy (ART). Are cumulative pregnancy rates and cumulative live birth rates (CLBRs) similar between the cured endometrial tuberculosis patients and non-tuberculosis patients during the first one ART cycle including all subsequent frozen-thaw cycles? The aim of the present study was to analysis the outcomes of them.

2. Materials and methods

2.1. Case selection and sampling

The research was a retrospective 1:4 matched-pair study. The patients recruited were followed up at the Centre of Reproductive Medicine, Peking University Third Hospital (PUPTH). This study was approved by the Ethics Committee of PUTH (Patients diagnosed...
with endometriosis, intrauterine adhesions, uterine malformation or abnormal thyroid function were excluded). There were total of 184 women were confirmed as endometrial tuberculosis. The diagnosis were confirmed by AFB microscopy and polymerase chain reaction (PCR) for DNA analysis for detection of mycobacteria in our hospital and were given anti-tuberculosis drug treatment for 6 to 24 months [Drug-sensitive TB requires 6-12 month course of Isoniazid and Rifampin combined with Pyrazinamide and Ethambutol for the initial 2 months should be adequate. Drug-resistant TB requires 12–24 months of therapy with toxic drugs with close monitoring] between December 2010 and December 2015, and were followed for 2-7 years until December 2017. Of them, 71 patients did not return for assisted reproductive therapy after anti-tuberculosis treatment and cured, the remaining 113 cured patients detected as normal in the endometrial review and took first ART cycle including all subsequent frozen-thaw cycles from the same oocyte retrieval were included in this study. All the patients in the control group (non-TB group) had no evidence of lung tuberculosis or genital tuberculosis at the same period. Subjects were randomized in a ratio 1:4 matched for age, basal E2, basal FSH and ovulation protocol. So, 452 patients of Non-TB group were included for the next statistical analysis.

2.2. Methods

All the patients were treated with ovulation induction. Medication was adjusted according to the development of follicular. Recombinant hCG 2500ug was injected to induce ovulation when at least three leading follicles >17 mm or two follicle >18 mm was observed. Oocyte pick-up procedure was performed in 36-38 h after hCG injection, followed by embryo transfer on the third day in the fresh transfer cycles. For frozen-thawed transfer cycles, embryo transfer were conducted on the third day of ovulation or fourth day of LH surge. The endometrial thickness was measured by ultrasound on the day of hCG trigger in fresh embryo transfer cycles, on the day of ovulation in natural cycles, or on the day before progesterone in artificial autologous cycles. Clinical pregnancy was defined as the presence of gestational sac by the transvaginal ultrasound. Live birth was defined as the birth of at least one living child. CLBRs was calculated by including the first live birth generated during the complete first IVF cycles as the molecular. The denominator was all the women of each group.

Data collected included age, duration of infertility, infertility type, basal E2, basal FSH, primary cause of infertility, ovulation protocol, total gonadotropine (Gn) dosage, No. of days of stimulation, E2 of HCG day, number of oocytes retrieved, fertilization rate, number of available embryos, number of embryos transferred, endometrial thickness. Reproductive outcomes: implantation rates, clinical pregnancy rate, spontaneous abortion rate, the live birth rate, cumulative pregnancy rates, cumulative abortion rates and cumulative live birth rates.

2.3. Statistical analysis

Descriptive statistics such as mean, median, standard deviation, and range were calculated for age, duration of infertility and so on. Assumptions of normality for continuous variables were tested using Kolmogorov-Smirnov tests. Changes in continuous variables in normal distribution were compared using student’s t-test. Similarly, changes in qualitative variables were compared by McNemar’s chi-square test. Frequency variables across categories were compared using chi-square test or Fischer’s exact test as appropriate. A P value of 0.05 is used as the cut off for significance. All statistical analysis was performed using the SPSS 24.0.

3. Results

3.1. Patients general information

Of the 184 patients who diagnosed with endometrial tuberculosis in this study, 110 cases (59.8%) had normal chest X-ray and no previous history of tuberculosis. 10 cases (5.4%) were showed bilateral tubal patency by Hystero-salpingography (HSG), 164 cases (89.2%) had side or bilateral oviduct obstruction by HSG. As shown the flowchart of the endometrial tuberculosis patients in the Fig. 1, all cases have normal intrauterine and normal endometrial pathology. Only 1 case (1.7%) was pregnant spontaneous after cured.

The baseline characteristic of the patients of the TB group and Non-TB group shown in Table 1. There was no significant difference in age, nulliparity, basal E2, basal FSH between the two groups. Compared with Non-TB group, the duration of infertility was longer (6.0 V 3.0) (year) and the proportion of primary infertility was higher (94.7% vs 40.5%), and the differences were statistically significant.

3.2. Clinical data and treatment outcome

In the case of univariate analysis, there was no significant difference in the Ovarian stimulation protocol, No. days of stimulation, fertilization rate between the two groups (P > 0.05). Total Gonadotropin(Gn) dosage was significantly lower, the number of oocytes retrieved and number of available D3 embryos was significantly higher (P < 0.05) in the TB group (shown in Table 2).

105 cycles of TB group and 417 cycles of Non-TB group included in the fresh cycles were shown in the Table 3. The E2 of the HCG day, endometrial thickness, and the number of transferred embryo was similar, but the TB group has lower implantation rate (25.5% vs 33.1%), lower clinical pregnancy rate (38.1% vs 51.8%), lower live birth rate (22.9% vs 42.4%), and with higher spontaneous abortion rate (34.7% vs 12.0%) than the Non-TB group.

In the frozen cycles (including all subsequent frozen-thaw cycles from the same oocyte retrieval) shown in Table 4. Included 96 cycles of TB group and 204 cycles of Non-TB group, the endometrial thickness, and the implantation rate, the clinical pregnancy rate and the live birth rate were similar, but with higher spontaneous abortion rate (P = 0.047) in the TB group.

Though, TB group patients had thinner endometrial thickness in the frozen cycles compare with the fresh cycles, the difference is statistically significant shown in the Table 5. However, there was

![Fig. 1. Flowchart. TB: Endometrial tuberculosis](image-url)
no difference in the implantation rate, clinical pregnancy rate, spontaneous abortion rate and the live birth rate between them.

In the Table 6, of the total cycles, it is also had thinner endometrial thickness, lower clinical pregnancy rate, higher spontaneous abortion rate and lower live birth rate in the TB group, the difference is statistically significant. But we can see that they are similar in the cumulative clinical pregnancy rates, but with lower CLBRs and higher abortion rates in the TB group.

4. Discussion

Since FGTB has concealed incidence, atypical clinical symptoms, no discomfort complaints or tuberculosis history, and usually the routine chest X-ray examination were normal, so, these patients were often misdiagnosed. The morphology of genital organs infected with TB varies widely. The organs appear normal in the early stages. However, the ampullary region of the fallopian tubes could develop chronic inflammation, induration, fibrosis, narrowing of the fallopian tubes, hydrosalpinx, pyosalpinx and tubo-ovarian abscesses or mass. On the other hand, TB endometritis is often focal, and pathological changes such as ulceration, caseous necrosis and hemorrhage could be observed in advanced endometrial TB. Intrauterine adhesions can result in partial obliteration of the uterine cavity. Endometrial caseation and ulceration causing intrauterine adhesions are referred to as Asherman syndrome.

Some scholars reported that subfertility might be the only clinical symptom of genital TB [15]. In clinical work, for infertility patients, hysterosalpingography (HSG) were applied to learn whether there is tubal patency, but rarely do endometrial biopsy and oviduct mucosal examination to rule out tuberculosis infection. Through the retrospective analysis in this study, we found that more than half of the patients with endometrial tuberculosis had no clinical symptom or tuberculosis history, and had normal chest radiographs. It was consistent with the literature reports that only 9% to 49% of the pelvic tuberculosis patients had the abnormal chest X-ray [18]. It also suggested that simply by using medical history or chest X-ray for screening of female genital

Table 1
The baseline characteristic of the patients

|                         | TB group (n = 113) | Non-TB group (n = 452) | P value |
|-------------------------|-------------------|------------------------|---------|
| Age(year)               | 30.96 ± 4.03      | 31.01 ± 3.08           | 0.91    |
| Type of infertility     |                   |                        |         |
| Primary n (%)           | 107(94.7)         | 183(40.5)              | <0.00   |
| Duration of infertility | 6.0(4.0, 8.0)     | 3.0(2.0, 6.0)          | <0.00   |
| Nulliparity n (%)       | 113(100)          | 445(98.5)              | 0.18    |
| Basal FSH(U/L)          | 6.64 ± 2.46       | 6.74 ± 2.68            | 0.71    |
| Basal E2 (nmol/L)       | 146.0(117.0, 192.0) | 147.0(104.3, 191.8)  | 0.37    |

Table 2
The clinical data of TB and Non-TB group after ovarian stimulation

|                         | TB group (n = 113) | Non-TB group (n = 452) | P value |
|-------------------------|-------------------|------------------------|---------|
| Ovarian stimulation protocol |                   |                        |         |
| Long protocol, n (%)   | 60(53.1)          | 242(53.5)              | 0.99    |
| Short protocol, n (%)  | 14(12.4)          | 59(13.1)               |         |
| Antagonist protocol, n (%) | 39(34.5)       | 151(33.4)              |         |
| Total gonadotrophin dose (IU) | 2100(1587,2775) | 2350(1800,3140)        | 0.03    |
| No. days of stimulation (day) | 11.73 ± 2.46     | 11.31 ± 2.32           | 0.09    |
| Number of oocytes retrieved (n) | 14.98 ± 9.52 | 12.21 ± 6.11            | <0.00   |
| Fertilization rate (%)  | 63.6(50.0, 75.0)  | 66.7(50.0, 80.0)        | 0.39    |
| Available embryos number(n) | 6.0(2.0, 11.0) | 4.0(2.0, 8.0)           | 0.00    |

Table 3
The outcomes of fresh cycles between TB group and Non-TB group

|                         | TB group (n = 113) | Non-TB group (n = 452) | P value |
|-------------------------|-------------------|------------------------|---------|
| Fresh ET cycles (n)     | 105               | 417                    |         |
| E2(HCG day) (pmol/ml)   | 9501(5895,14555)  | 9080(7690,11100)        | 0.42    |
| Embryos transferred number (n) | 1.98 ± 0.339 | 1.96 ± 0.312            | 0.63    |
| Endometrial thickness (mm) | 10.13 ± 1.77     | 10.46 ± 1.36           | 0.08    |
| Implantation rate n (%) | 52(25.5)          | 272(33.1)              | 0.04    |
| Clinical pregnancy rate n (%) | 40(38.1) | 216(51.8)              | 0.01    |
| Spontaneous abortion rate n (%) | 16(34.7)  | 26(12.0)               | <0.00   |
| Live birth rate n (%)   | 24(22.9)          | 177(42.4)              | <0.00   |

Table 4
The outcomes of frozen-thaw cycles between TB group and Non-TB group

|                         | TB group (n = 113) | Non-TB group (n = 452) | P value |
|-------------------------|-------------------|------------------------|---------|
| FET cycles              | 96                | 204                    |         |
| Embryos transferred number (n) | 2.01 ± 0.66 | 2.03 ± 0.58             | 0.80    |
| Endometrial thickness (mm) | 9.51 ± 2.06     | 9.91 ± 1.37            | 0.09    |
| Implantation rate n (%) | 37(12.1)          | 97(23.8)               | 0.20    |
| Clinical pregnancy rate n (%) | 33(34.4) | 79(38.7)               | 0.47    |
| Spontaneous abortion rate n (%) | 11(33.3)  | 13(16.5)               | 0.05    |
| Live birth rate n (%)   | 22(22.9)          | 61(29.9)               | 0.21    |
tuberculosis infection would be misdiagnosis. They would take ART without any standardized anti-TB treatment, which would lead to serious consequences. Wen conducted clinical analysis of 7 patients with complicated tuberculosis after IVF-ET. 4 cases were complicated with hematogenous disseminated tuberculosis and central nervous system tuberculosis. Patients with twin pregnancy were severe. 6 patients discontinued pregnancy, and 1 case of neonatal birth got congenital tuberculosis [17]. Scholars also reported that pregnancy complicated by tuberculosis will lead to tuberculosis meningitis [18], and severe cases will lead to maternal and neonatal deaths [19-20]. This even cause the outbreak of tuberculosis in the surrounding population [21]. Tuberculosis is one of the top causes of death in women of reproductive age and is a common non-obstetric cause of maternal mortality [22-25]. Untreated tuberculosis in pregnant women has a mortality risk of up to 40% [24-25]. Therefore, we need pay more attention for these patients, if necessary, a simple tuberculosis screening method such as the endometrial biopsy with pathological examination before IVF treatment. Combined endometrial biopsy with pathological diagnosis is commonly used in clinic. This operation is simple and easy to widespread, which can also help to understand the situation of endometrial situation and exclude the pathological changes, which can guided and adjusted the clinical strategies to improve pregnancy outcomes and avoid serious maternal complications.

Once the fallopian tube was infected with tuberculosis, it usually caused tubal obstruction in early stage, which causes infertility of long duration. It is accordance with the proportion of primary infertility was significantly higher and the average infertility years was longer in the TB group. The difference was statistically significant (P < 0.001). This study suggested that if the primary infertility patients combined with tubal obstruction, doctors should be more vigilant for the presence of genital tuberculosis, if necessary, endometrial biopsy and pathological diagnosis or tuberculosis screening before IVF treatment.

At present, whether FGTB infection affected ovarian function, damaged the uterine environment, and reduced the outcomes of IVF is inconclusive. Some scholars reported that FGTB might affect ovarian blood flow, ovarian reserve and function [26]. Singh carried out a prospective study showed that FGTB had no effect on endometrial blood flow and conception rate [27]. Subramani showed that the expression of LIF, LIFR and pSTAT3, which were representative of endometrial receptors, were significantly reduced in the genital tuberculosis infection group by observing the endometrium of the implantation window [28]. Only one study reported the IVF outcome of FGTB patients in China, but it was with small sample size and not special for endometrial tuberculosis. There was no significant difference in fertilization rate and numbers of high quality embryo between the FGTB group (66 cases) and the non-TB group [29]. However, the implantation rate and pregnancy rate were significantly lower and the abortion rate was significantly higher in the FGTB group. The research specifically designed for the outcomes of endometrial TB-infection patients have not yet been reported in the literature. We analyzed the outcomes of the cured endometrial tuberculosis patients for the first time. Compared with the non TB group, the CLBRs was similar, but the cumulative live birth rates was significantly lower, and the abortion rates was higher in the TB group. According to the general situation of the patients, the age, basal FSH, basal E2 and the ovulation protocol were similar. However, the total Gn dosage required was lower, the number of oocytes retrieved and available embryo numbers were more in the TB group. Thus we can conclude that cured endometrial tuberculosis does not seem to affect ovarian response. The low cumulative clinical pregnancy rates and CLBRs in TB group may be related with the endometrial receptivity or the thinner endometrial, even though all of them with the normal uterine cavity morphology.

The results of fresh cycles and frozen-thaw cycles of TB group in our study showed that in the case of no difference in the number of embryos transferred, the clinical pregnancy rate, the abortion rate and the live birth rate were not with statistically significant difference. It shows that the same successful rate of fresh cycles can achieved during frozen-thaw cycles. Prompted us for ovarian hyper-stimulation syndrome patients can consider whole embryo freezing. Similarly, for patients with poor ovarian function, we can also choose frozen embryo transfer cycles for the accumulation of more embryos. With the continuous improvement of the success rate in ART, the cumulative clinical pregnancy rates were greatly improved in the TB group (64.6% VS 65.1%, P = 0.89, Table 6). However, the CLBRs were still lower and the abortion rates were still higher compared with the non-TB group.

One limitation of this study, however, is that it is a retrospective study, and therefore susceptible to certain types of bias. Moreover this study were only conducted in one center which may also lead to bias. Perspective, multiple center, randomized clinical trial could be applied further to demonstrate the results. Another limitation for this study is that we do not quantify the grading of TB or tubal or endometrial damage in different patients. At this moment there

Table 5
The outcomes of fresh and frozen-thaw cycles in TB group

|                        | Fresh cycles (n = 105) | Frozen cycles (n = 96) | P value |
|------------------------|-----------------------|------------------------|---------|
| Embryos transferred number (n) | 1.98 ± 0.34          | 2.01 ± 0.66            | 0.69    |
| Endometrial thickness (mm)       | 10.13 ± 1.75         | 9.51 ± 2.06            | 0.02    |
| Implantation rate n (%)           | 52(25.5)             | 37(19.2)               | 0.13    |
| Clinical pregnancy rate n (%)     | 40(38.1)             | 33(34.4)               | 0.58    |
| Spontaneous abortion rate n (%)   | 16(15.2)             | 11(11.5)               | 0.73    |
| Live birth rate n (%)             | 24(22.9)             | 22(22.9)               | 0.99    |

Table 6
The outcomes of total cycles between TB group and Non-TB group

|                        | TB group (n = 113) | Non-TB group (n = 452) | P value |
|------------------------|-------------------|------------------------|---------|
| Total cycles           | 261               | 621                    |         |
| Embryos transferred number (n) | 2.0 ± 0.51     | 1.99 ± 0.42            | 0.81    |
| Endometrial thickness (mm)       | 9.84 ± 1.92       | 10.28 ± 1.39           | 0.00    |
| Clinical pregnancy rate n (%)    | 73(36.3)          | 295(57.5)              | <0.00   |
| Spontaneous abortion rate n (%)  | 27(13.4)          | 39(6.3)                | <0.00   |
| Live birth rate n (%)           | 46(22.9)          | 238(38.3)              | <0.00   |
| Total patients            | 113               | 452                    |         |
| Cumulative clinical pregnancy rates n (%) | 73(64.6)  | 295(65.1)              | 0.90    |
| Cumulative abortion rates n (%) | 27(30.7)          | 39(13.2)               | <0.00   |
| Cumulative live birth rates n (%) | 46(40.7)         | 238(52.7)              | 0.02    |
are lack of uniform standards for the grading of tubal or endometrial damaged caused by TB. This may also lead a new research area in the grading of pelvic status in TB patients.

In conclusion, in this retrospective matched-pair study, we found that the pregnancy outcomes of women of TB group were still impaired when compared with non-TB group. Our results indicate that women may have increased risk of miscarriage and decreased CLBRs after cured endometrial TB infection when undergoing IVF. But, this study with a small sample size, we should continue to accumulate and expand the sample size in future, if possible, prospective randomized controlled studies are needed to confirm the conclusion.

Conflict of interest

The authors have no conflicts of interest to declare.

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