Favorable infertility outcomes following anti-tubercular treatment prescribed on the sole basis of a positive polymerase chain reaction test for endometrial tuberculosis

U.N. Jindal*, S. Verma, and Y. Bala

Jindal IVF and Sant Memorial Hospital, 3050, Sector 20 D, Chandigarh 160020, India

*Correspondence address. Tel: +91-172-2727773, 2727774; Fax: +91-172-5086774; E-mail: drunjindal@gmail.com

Submitted on October 21, 2011; resubmitted on December 23, 2011; accepted on February 10, 2012

BACKGROUND: The endometrial tuberculosis (TB) PCR test is now commonly employed for the diagnosis of female genital TB, a common cause of infertility in India. Although treatment in the absence of demonstrable tubal damage may be of doubtful benefit to fertility, the presence of mycobacterial DNA demonstrated by a positive PCR indicates infection by tubercle bacilli causing sub-clinical or latent disease potentially responsible for future clinical manifestations. This study was undertaken to assess the outcome of infertility management following early anti-tubercular treatment (ATT) based only on a positive endometrial TB-PCR test.

METHODS: This was an intervention study conducted at an IVF center in northern India in 443 infertile women of whom 169 (38.15%) were found to have positive TB-PCR (Group I), while 274 (61.85%) had negative Mycobacterium tuberculosis (MTB)-PCR (Group II). Infertile women of <40 years of age, without any evidence of tubo-peritoneal or endometrial involvement, who underwent endometrial biopsy for the detection of MTB by PCR, were included. All the TB-PCR positive women were administered standard 6-month anti-tubercular chemotherapy. Additional treatment with assisted reproduction techniques was offered in the case of failure of spontaneous pregnancy after completion of ATT.

RESULTS: There were no statistical differences in the two groups in the overall pregnancy rate, 101 (59.8%) versus 167 (60.9%). In Group I, 48 (92.3%) spontaneous conceptions occurred within the first 12 months, i.e. during the period of ATT administration or within 6 months of treatment completion; in Group II, the occurrence of spontaneous conceptions was distributed more evenly in relation to time, i.e. 36 (53.7%) in <12 months as compared with 31 (46.3%) after first year \( P < 0.001 \).

CONCLUSION: Infertile women without tubal or endometrial damage given early anti-tuberculosis treatment based on a positive endometrial TB-PCR test had an excellent chance of early spontaneous conception.

Key words: infertility / endometrial tuberculosis / PCR / anti-tubercular therapy / assisted reproduction

Introduction

Nearly one-third of the world population is infected with *Mycobacterium tuberculosis* (MTB) of whom only 10% are known to progress to clinical disease (WHO, 2006). Depending upon the localization of the MTB in an organ, a wide spectrum of tubercular disease is encountered in clinical practice of which female genital TB (GTB) is an important manifestation (Schaefer, 1976; Parikh et al., 1997; Aliyu et al., 2004; Dannenberg and Converse, 2011). Damage to the pelvic organs after clinical GTB is well recognized both in the presence of active disease as well as during the process of healing and fibrosis. The diagnosis can be established in such cases with the help of various microbiologic, radiologic and histopathologic tests along with the clinical presentation. The diagnostic tests have high specificity but a low sensitivity even in the presence of active tuberculosis (TB). A battery of tests may be required to arrive at the diagnosis (Tripathy and Tripathy, 1990; Jindal, 2006; Rozati et al., 2006).

The fibrosis and scarring which result as a part of healing lead to the loss of function of the Fallopian tubes, and less commonly of ovaries and endometrium. It is therefore desirable to diagnose and treat
GTB as early as possible during the subclinical stage to prevent or at least to minimize the damage to the genital organs. Unfortunately, the conventional tests for the diagnosis of TB during the sub-clinical stages have poor sensitivity and specificity. However, recently the detection of MTB DNA by TB-PCR has shown high sensitivity and specificity for the diagnosis of GTB (Baum et al., 2001; Roy et al., 2003; Bhanu et al., 2005; Rana et al., 2011). We have previously shown that the maximum likelihood estimates of sensitivity and specificity for the diagnosis of GTB with a positive TB-PCR in the endometrial samples were 0.59 and 0.92, respectively (Jindal et al., 2010).

In a recent study, the authors have shown that 57% of infertile women in whom the presence of TB was suspected on clinical grounds had a positive endo-TB-PCR test, whereas only 9.5% had a positive test where no clinical ground for suspicion were present (Thangappah et al., 2011). Endometrial TB-PCR (endo-TB-PCR) positivity in the absence of symptoms, and without any demonstrable tubal or endometrial damage, raises the possibility of a false-positive TB-PCR test in the absence of any mycobacterial infection. Empirical treatment, especially in the high-prevalence countries is also fraught with the risks of resistance and other side effects of anti-tubercular chemotherapy. On the other hand, a positive endo-TB-PCR test may also imply the presence of sub-clinical, latent or past disease, which could be managed with anti-tubercular treatment (ATT). This is supported by the limited observations made for both genital and other forms of TB in some recent studies (Cheng et al., 2004; Kulshreshtha et al., 2011; Thangappah et al., 2011).

Short course chemotherapy is effectively used to treat symptomatic GTB (Jindal et al., 1990). However, for infertile women with GTB, assisted reproduction techniques (ARTs) are also required to achieve pregnancy (Soussis et al., 1998; Jindal, 2006; Singh et al., 2008). The present study was undertaken to examine the fertility of infertile women with positive endo-TB-PCR in the absence of demonstrable damage to the endometrium or the Fallopian tubes after the early institution of ATT.

Materials and Methods

The study was undertaken from the year 2006 to 2010 at an IVF center in northern India. Women from all couples seeking treatment for infertility between 2006 and 2008 were screened for inclusion in the study. All couples were investigated and managed according to the standard protocol followed at the center. Tubal and endometrial evaluation was done either by hysterosalpingography (HSG) or laparoscopy and hysteroscopy. Endometrial samples were obtained by endometrial aspiration or curettage, done as a stand-alone test or along with laparoscopy and/or hysteroscopy. One part of the biopsy of endometrial tissue was subjected to histopathologic examination and the second part was sent to the Laboratory for TB-PCR testing. Endometrial samples were obtained by gentle curettage of the endometrium and kept in sterile containers with normal saline to avoid contamination. Histopathologic examination of endometrial biopsies did not reveal any presence of acid fast bacilli, granuloma formation or other findings suggestive of TB.

PCR testing

Endo-PCR test was arranged with Reliance Life Sciences Pvt. Ltd. Mumbai, a national laboratory providing services all over India, accredited by the American College of Pathologists, with a proficiency test score for TB of 100% in 2007 and 2008. Stringent criteria were used at the laboratory to avoid contamination of results. The test was run in duplicate. Nested PCR against the most conserved region insertion sequence 6110 gene was done employing a Fastprep® sample preparation system (BioMedics, Cambridge, UK) for mycolic acid cell wall lysis to extract DNA. The PCR assay gave a clear band of 123 base pairs (bp), indicating positivity of a sample. The assay used a cellular gene to rule out false negativity of the samples, with the cellular gene confirming no general DNA degradation in the sample and absence of PCR inhibitors in the sample. One positive and one negative clinical samples were used in every assay to validate the assay and confirm the results. A blank reagent containing no DNA was used to check contamination during PCR by the absence of any PCR fragment in the gel. The assay was also validated by direct sequencing of the PCR product indicating >95% homology with MTB, using NBLAST (www.ncbi.nlm.nih.gov/blast). The quoted sensitivity of the test is almost 100% and specificity 96–99%, with a lower detection limit of 100 TB bacilli/ml (Nolte et al., 1993; Folgueira et al., 1996; Takahashi and Nakayama, 2006).

Patients

Of 3108 infertile couples who reported between 2006 and 2008, the study included 443 (14.2%) women of <40 years of age who had no symptoms other than infertility, and without any evidence of endometrial or tubal damage on HSG or laparoscopy and hysteroscopy. The reasons for exclusion of the remaining 2665 (85.8%) cases were TB-PCR not done (1244); presence of a tubal factor (552); tubal evaluation not done (248); severe male factor (132); severe and moderate endometriosis (101); endometrial factor (76); previous history of ATT (168); age >40 or previous oophorectomy (91) and those who did not report for follow-up evaluation (53). Among 443 women who were included, 169 (38.15%) with PCR positive test constituted the study group (Group I) and 274 (61.85%) PCR negative, the control group (Group II). Follow-up assessment was continued for at least 2 years after recruitment in the study up to end of 2010.

Treatment

The study Group I, received standard short course daily ATT consisting of the intensive phase of 2 months of four drugs (isoniazid, H 300 mg; rifampicin, R 450–600 mg; ethambutol, E 800–1200 mg and pyrazinamide, Z 1200–1500 mg) followed by the maintenance phase of 4 months comprising the same doses of isoniazid and rifampicin (2HRZE, 4HR). The first-line treatment for non-tubal infertility consisted of ovulation induction along with IUI (negative Group II) and also in PCR positive Group I, after completion of ATT. IVF was undertaken for refractory infertility for 32 of Group I and 45 of Group II women; the remaining 150 women did not opt for IVF and wished to wait longer for various personal reasons.

In brief, the IVF procedure consisted of employing stimulation protocols using GnRH analogs and urinary or recombinant gonadotrophins. Final maturation trigger was given when at least three lead follicles were >16 mm. Ovum pick up was done 36 h after hCG. All metaphase-2 oocytes were injected with sperm by intracytoplasmic sperm injection. Embryo transfer was done on second/third day and a maximum of three embryos were transferred. Pregnancy was defined as the presence of viable gestational sac on ultrasound examination at 3–4 weeks after embryo transfer.

Statistical methods

Details of all cases were recorded on a structured format and analyzed with the help of registered version of SPSS version 13. Group comparisons were made using χ² test (for categorical variables) or Student t-test (for scalar variables). Statistical significance was assessed at P < 0.05. Probability of spontaneous pregnancy (without any IUI or IVF) during the follow-up
period was calculated by Kaplan–Meier method, and formal comparisons between different groups were performed using the log-rank test.

Results

The two groups were comparable in age (mean age = 32 versus 31 years; range = 20–40 years), type of infertility, menstrual history, obstetric history, etiology of infertility and previous ARTs (Table I). There were more women belonging to the lower socio-economic status (18.9 versus 8.8% \(P < 0.02\)), lower education (20.1 versus 8.4% \(P < 0.007\)) and longer duration of infertility \(>96\) months (17.2 versus 9.9% \(P < .02\)) in the study group as compared with Group II (Table I).

There were a total of 268 pregnancies in 443 (60.5%) women. The overall pregnancy rates were similar in both the groups: 101/169 (59.8%) in Group I and 167/274 (60.9%) in Group II. There was no difference in the outcome of the pregnancies, 81 (80.2%) and 134 (80.2%) were ongoing or delivered in Groups I and II, respectively.

There appeared to be more spontaneous pregnancies in the study group when compared with the controls but the difference was not statistically significant (\(P = 0.154\)); the rates were similar in the two groups when spontaneous pregnancies following COH and IUI were compared (Table II). Amongst the remaining women, 19 of 32 (59.7%) in Group I and 33 of 45 (73.3%) in Group II conceived following IVF (Table III). A significantly higher proportion of spontaneous pregnancies occurred in the first year (i.e. during or within 6 months of the completion of ATT) in Group I than in Group II, 92.3 versus 53.7% \(P < 0.001\). In Group I a higher proportion of women who achieved a pregnancy, whether spontaneous or through ARTs, had a duration of infertility \(>24\) months than in Group II, 75.2 versus 61.7% \(P < 0.05\) (Table V).

The Kaplan–Meier graph for cumulative probability of spontaneous pregnancy in women who conceived without any ART over the follow-up period revealed a highly significant difference in the time course between Groups I and II (Fig. 1). Almost 90% of spontaneous conceptions in the study group occurred in the first year and the median time to conception was 7 months (95% CI = 5.8–8.2 months). In the control group, it was nearly 50% with the median time to conception of 10 months (95% CI = 6.8–13.2 months). This difference was highly significant \(P < 0.001\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I: TB-PCR +ve ((n = 169) [(n(%)])</th>
<th>Group II: TB-PCR −ve ((n = 274) [(n(%)])</th>
<th>(P) value ((\chi^2) test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mean age (years; range: 20–40)</td>
<td>32</td>
<td>31</td>
<td>0.45 (t-test)</td>
</tr>
<tr>
<td>2. Type of subfertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>131 (77.5)</td>
<td>199 (72.6)</td>
<td>0.25</td>
</tr>
<tr>
<td>Secondary</td>
<td>38 (22.5)</td>
<td>75 (27.4)</td>
<td>0.45</td>
</tr>
<tr>
<td>3. Menstrual abnormality</td>
<td>53 (31.4)</td>
<td>74 (27.0)</td>
<td>0.45</td>
</tr>
<tr>
<td>4. Previous live issue</td>
<td>12 (7.1)</td>
<td>26 (9.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>5. Causes of subfertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>73 (43.2)</td>
<td>111 (40.5)</td>
<td>0.11</td>
</tr>
<tr>
<td>Ovulatory</td>
<td>60 (35.5)</td>
<td>81 (29.6)</td>
<td>0.11</td>
</tr>
<tr>
<td>Others</td>
<td>36 (21.3)</td>
<td>82 (29.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>6. Previous ART</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COH + IUI</td>
<td>88 (52.1)</td>
<td>155 (56.6)</td>
<td>0.11</td>
</tr>
<tr>
<td>IVF</td>
<td>31 (18.3)</td>
<td>43 (15.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>7. Duration of subfertility (months)</td>
<td>57</td>
<td>48</td>
<td>0.020 (t-test)</td>
</tr>
<tr>
<td>Mean (range: 12–216 month)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>34 (20.1)</td>
<td>23 (8.4)</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>66 (39.1)</td>
<td>44 (18.5)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>69 (40.8)</td>
<td>129 (47.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>9. Socio-economic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>32 (18.9)</td>
<td>24 (8.8)</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>77 (45.6)</td>
<td>1135 (49.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>High</td>
<td>60 (35.5)</td>
<td>115 (42.0)</td>
<td></td>
</tr>
</tbody>
</table>

COH + IUI, controlled ovarian hyper-stimulation and intrauterine insemination.

\(^a\)PCR for TB positive.

\(^b\)PCR for TB negative.
Discussion

Treatment of asymptomatic or doubtful sub-clinical TB in high-TB prevalence countries such as India can be justified only in the presence of a significant risk of progression, disease transmission or loss of function of an organ. Indications for the treatment of extra-pulmonary TB, mostly paucibacillary, have been difficult to definitely establish in the absence of definitive criteria for diagnosis, difficulties of obtaining samples and low rates of mycobacterial smear and culture positivity. Any delay in the institution of treatment especially with that of a vital organ is fraught with the risk of loss of function.

In the case of the genital tract, the initial invasion occurs with a few bacilli which slowly colonize and multiply locally without causing many symptoms. Once an active disease is established, irreversible tubal and endometrial damage can occur. However, during the pre-clinical stage, an insidious, low-grade inflammation is quite likely to alter the function of Fallopian tubes, uterus and of the endometrium which lead to subtle damage and functional loss of the uterus and the tubes. Molecular mechanisms constitute the important causes of implantation failure in gynecologic diseases (Cakmak and Taylor, 2011). Such mechanisms however have not been studied or reported in the case of GTB. Mycobacterial infection may also alter endometrial receptivity and cause implantation failure through mechanisms such as disturbed immune-modulation and cytokine over burden, endocrine disruption, activation of antiphospholipid antibodies and microthrombosis without the presence of overt clinical disease (Gurgan et al., 1996; Kumar and Rattan, 1997; Malik, 2003; Singh et al., 2011). Latent TB was also shown to be responsible for unexplained infertility and repeated IVF failures in women with apparently normal pelvic and non-endometrial tubal factors (Dam et al., 2006).

We believe that the detection of TB DNA by PCR in endometrium without any clinical evidence of disease reliably detects sub-clinical disease and that sub-clinical GTB leads to infertility which can be reversed by the institution of appropriate ATT before any damage to pelvic organs. There are enough studies in the literature including our studies, which have highlighted a very high diagnostic specificity, sensitivity and clinical correlation of TB-PCR with GTB (Bhanu et al., 2005; Kumar et al., 2008; Jindal et al., 2010; Thangappah et al., 2011). We had previously shown that endo-TB-PCR in infertile women could be reliably employed to diagnose endometrial TB and that laparoscopy could be avoided (Jindal et al., 2010). In addition, there are several reports in the literature to confirm the efficacy of short course standard ATT in the treatment of clinical GTB (Jindal et al., 1990; Parikh et al., 1997). In the recent literature, there is already evidence to suggest the role of PCR for diagnosis of subclinical GTB and for institution of ATT even in the presence of normal pelvic examination (Dam et al., 2006; Kulshrestha et al., 2011; Thangappah et al., 2011). For example, in one study, 2 of the 21 cases of infertility in whom the clinical criteria of GTB were negative had confirmed PCR positivity by two sets of primers (Thangappah et al., 2011). It was also shown in a different study that 31% of 29 women with GTB and infertility who received ATT solely on the basis of the PCR results had conceived after treatment (Kulshrestha et al., 2011).

We selected cases of infertility with no other demonstrable cause but for the positive endo-TB-PCR which signified the presence of the sub-clinical disease. The study and the control group were well matched for most of the confounding variables such as age, type of infertility, obstetric and menstrual history, etiology of infertility and previous infertility treatment (Table I). The statistically different baseline parameters, i.e. the longer duration of infertility as well as the lower education and socioeconomic status for the study group (Table I) in fact signified a poorer prognosis. Despite the presence of poorer prognosis factors, the study group achieved equal rate of pregnancy as the control group.

The achievement of pregnancies in a higher number of women without any ART within the first year of treatment was significant (Table IV). Moreover, these pregnancies were achieved in women with significantly longer duration of infertility of >24 months (Table V) in Group I women who failed to achieve pregnancy,
subtle tubal or endometrial damage could still be present which could be overcome with the help of ART as evidenced by overall equal pregnancy rate in both the groups.

The cumulative probability of spontaneous pregnancy, which almost reached 90% within the first year of treatment as compared with a more even distribution of occurrence of pregnancy in relation to time in the control group, confirms that ATT can result in good conception rates. The occurrence of pregnancy in the natural way without the need of any ART also ruled out any other treatment-related confounding factor which could be present in ART-related success.

Although, the study provides an adequately large database, it has important limitations for conclusive results. It did not include a ‘no-treatment arm’ for PCR-positive women. A randomized study with and without ATT could not be undertaken for ethical reasons in the presence of any ART also ruled out any other treatment-related confounding factor which could be present in ART-related success.

In conclusion, the study demonstrates that the early institution of ATT for infertility, solely on the basis of positive endo-TB-PCR in endometrium with no other demonstrable cause, restored early fertility in a significant proportion of cases. As a corollary, it can be also deduced that sub-clinical GTB present as infertility alone can be detected by the presence of TB DNA PCR in the endometrium and should be treated early.

**Ethics**

The project was approved by the Ethics Committee of the hospital. No financial assistance was received from any source including from the test laboratory. Informed consent was obtained for each patient.


Singh N, Bahadur A, Mittal S, Malhotra N, Bhatt A. Comparative analysis of endometrial blood flow on the day of hCG by 2D Doppler in two groups of women with or without genital tuberculosis undergoing IVF-ET in a developing country. Arch Gynecol Obstet 2011;283:115–120.


