

ORIGINAL ARTICLE

Infestation Of Endometrium By Mycobacterium Tuberculosis Bacilli-Cause Of Reproductive Failure

Rajib Gon Chowdhury¹, Suman Kalyan Paine², Basudev Bhattacharjee²
and Siddhartha Chatterjee^{1*}

¹Calcutta Fertility Mission, Pushpanjali Apartment, 102C Ballygunge Place, Kolkata- 700019 India and ² Department of Biochemistry, Institute of Post-Graduation Medical Education & Research (IPGMER), SSKM Hospital, 244, AJC Bose Road, Kolkata-700 020

Abstract: Tuberculosis is known to be one of the major diseases, causing infertility in India. The prevalence of tuberculosis causing infertility is different in different countries. Established tubercle infection may cause irreversible infertility, on many occasions as it may affect all the reproductive organs. It also produces lower pregnancy rate in Assisted Reproductive Technology program as well. Tubercular bacillary infestation of the endometrium that is mere presence of Mycobacterium Tuberculosis (MTB) bacilli on the endometrial surface has been found to affect fertility as well. In this study, we have detected *Mycobacterium Tuberculosis* (MTB) infestation of endometrium, causing implantation failure or early embryonic rejection. In many cases of unexplained infertility, tubercular bacilli infestation of endometrium, has come out to be the root cause of infertility. Association of tubercular bacillary infestation and endometriosis is another cause of concern. Recurrent abortions and ectopic pregnancy may be precipitated by the same genital pathology as well. The presence of very small number of bacilli, which escapes detection by AFB smear, culture or histology, may be detected by polymerase chain reaction techniques. These bacillary infections bring an inflammatory change in the endometrium and produces harmful cytokines which are responsible for implantation failure of micro abortion.

Keywords: MTB infestation, PCR study, unexplained infertility, recurrent pregnancy loss, endometriosis

Introduction

Infertility is the commonest symptom associated with genital tuberculosis [1, 2] and it seems to be an important under-diagnosed factor in infertility [3]. So for any measure to cure infertility, tuberculosis must be excluded. The exact incidence of female genital tuberculosis is not known partly because more often the disease remains silent and in addition, there are lack of reliable confirmatory investigations. The first reported case of gynaecological tuberculosis was described by Morgagni in 1744. On making a post-mortem examination on a woman aged 20 years, he found the uterus and both tubes filled with caseous material. Interest in the field of gynaecological tuberculosis began to build up in the early years of this century. The most important early contributions were given by Berkeley (1903), Daniel (1925), Greenberg (1924), Jameson (1935), Murphy (1903, 1904), Norris (1921) and Williams (1894) [4]. Genital tuberculosis (TB) in females, is found in 0.75 to 1% of gynaecological admissions in India with considerable variation from place to place. The disease is responsible for 5% of all female pelvic infections and occurs in 10%

cases of pulmonary tuberculosis. Although most of the affected belong to reproductive age- group, the disease has been reported in post-menopausal females as well. Lately, an increase in the trend of this disease has been reported, which may be partly due to an overall rise in tuberculosis cases. The other contributory factors may be HIV infection, as tuberculosis is the most common HIV-related opportunistic infection in India [5]. However, the average world-wide incidence of female genital tuberculosis in infertile population has been reported as 5-10% [6, 7], with the range varying between <1% in USA and about 10% in India [8]. Incidence at our clinic is 9.6%. Incidence of infertility in genital tuberculosis may vary between 40–75.6% [9, 10]. Infertility in genital tuberculosis may be due to various causes. The average incidence of genital TB in infertility clinics worldwide is 5-10%, and varies from 0.69% in Australia to 17.4% in India [11]. Tuberculosis may cause minimal damage to the tube leading to ectopic pregnancy. Extensive damage may lead to complete tubal occlusion [12]. Peri-tubal adhesions and tubo-ovarian mass have been found in 47.2% of cases [13]. Various grades of intrauterine adhesion (Asherman's syndrome) or non-receptive endometrium have been reported in association with genital tuberculosis [14]. Although pulmonary TB (PTB) remains the commonest and the most infectious type of TB, extra-pulmonary TB (EPTB) is becoming more prevalent especially in young women throughout the world [15]. Female genital TB (FGTB), being non-infectious, has been neglected by healthcare providers, but is an important cause of both significant morbidity and short- and long-term sequelae for the affected women [15, 16]. The incidence of active tuberculosis in infected individuals is only 10% [17]. Depending on the virulence of organism and immune response generated by the host, the disease remains either active or becomes asymptomatic with latent infection persisting for many years [18]. Latent infected individuals contain dormant, yet viable bacilli, which may re-activate when the host response becomes low, and as a consequence, the disease may become active again. During the process of reactivation, the bacilli induce immune modulation within the local tissues, which mimics the process seen during infection. There is release of harmful cytokines like IL2, TNF α and INF γ . The final effect will depend upon how strongly the host tissue (ovarian tissue and endometrium) can resist this trauma [19]. If unable to resist, immuno-modulatory impact will affect adversely, the endometrial receptivity. Once the adverse impact has been established on the delicate function of this reproductive organ, the consequences may continue to persist; infection subsequently remains dormant or even cured. So for any measure to cure infertility, tuberculosis must be excluded. Since physical symptoms are usually not present definitively, the disease remains undiagnosed or specific investigations are not undertaken to rule out the problem (Bateman et al, 1986) [20]. It is a pauci-bacillary form of disease of which cultures and smears are often negative (Baum et al, 2001) [21]. The primary focus is rarely found outside the genital tract (Sutherland 1985) [22]. Routine screening tests for pulmonary tuberculosis like X-ray chest, tuberculin test and sputum examination are usually negative (Schaezting, 1986) [23]. With the above mentioned background in mind this study was conducted employing a multiplex PCR, developed in our laboratory to evaluate its effectivity in diagnosing genital tuberculosis among patients attending infertility clinic and how far it can be implicated for infertility.

Materials and Methods

517 cases having C/O unexplained infertility, Recurrent Spontaneous Abortions (RSA) Ectopic Pregnancy and Mild Endometriosis were investigated by multiplex PCR screening. Out of 517 cases, in 49 cases for the detection of AFB smear by ZN stain or culture of menstrual blood in LJ media, for the detection of tubercle bacilli was performed, along with TB PCR study. In rest of the cases, only TB PCR study was undertaken. Patients were advised to attend the clinic on d2 of period. Menstrual flow was usually considerable on 2nd day of menstrual cycle and that confirms proper menstrual flow. A sterile Cusco's speculum was introduced in the vagina carefully, avoiding contamination/contact to skin. Menstrual blood was collected by a 1cc/2cc sterile syringe and transferred to a sterile vial containing normal saline. Utmost care was taken to avoid contamination with skin. On many occasions, the skin may contain commensals mycobacteria.

Selection of Cases: Studies were undertaken in 517 cases. These cases were selected from infertile patients in the following group:

Group A: 246 patients, who were apparently unexplained. These cases were either primarily normal, with tubal, ovulatory or seminal factor or after correction of the above factors, they were sub-normal. The usual waiting period for pregnancy to occur often about one year, following they were declared normal.

Group B: 218 patients with GI to II endometriosis, without any anatomical distortion of the pelvic organs, where good tubo-ovarian relation along with free Pouch of Douglas (POD) was maintained. Here also, the waiting period for pregnancy to occur was about one year, with or with out ovulation induction.

Group C: 46 patients with more than one spontaneous abortion (RSA)

Group D: 7 cases with previous Ectopic Pregnancy

Exclusion Criteria: Patients with previous history of anti-tubercular drug (ATD) intake were excluded from this study. For Group A, that is Unexplained Infertility, if there was ovulatory factor, previous correction of the thyroid status and control of prolactin level, followed by ovulation induction with oral agents like Clomifene or Letrozole are included in the study. Any case which required ovulation induction with Gonadotrophin, or cases where Clomifene or Letrozole failed in inducing ovulation induction, were excluded. Cases with endometriosis, having pelvic adhesion, distorting tubo-ovarian relation, or abnormal Pouch of Douglas, were excluded from this study. For recurrent spontaneous abortions, where no endocrinological, anatomical or immunological causes were found, were included in this study. Previous ectopic pregnancies, where definite tubal defects were detected, were excluded from the study.

Collection of Samples: After taking informed consent, the patients were advised to attend the clinic on 2nd day of menstrual cycle. Menstrual flow is usually considerable on 2nd day of menstrual cycle. In 49 of these cases, endometrial biopsy samples were collected by curetting the endometrial cavity by a small curette with or without dilating the cervix under deep sedation or anaesthesia, in the pre-menstrual phase of the same cycle. Menstrual fluid specimens were collected in three parts.

Endometrial specimens were aliqueted into three portions - one kept in normal saline for microscopy and culture, one in formal saline for histopathology and one in lysis buffer (50mmol/l KCl, 10 mmol/l TrisHCl pH 8.3, 1.5 mmol/l MgCl₂, 0.1% NonidetP-40, 0.5% Tween-20) for extraction of DNA s for subsequent multiplex PCR study. Similarly, three menstrual fluid specimens were treated like endometrial tissues. All the clinical samples were screened with conventional microbiological tests such as Ziehl–Neelsen acid fast staining for recording smear-positivity, identification by cultural isolation and biochemical tests. Lowenstein–Jensen medium was used for primary isolation. Tests to identify *M. tuberculosis complex* and NTM were: (i) niacin test, (ii) catalase test, (iii) heat stable catalase test, (iv) pigment production test, (v) Growth at 42–44°C, (vi) aryl sulphatase test and (vii) state-of-the-art nucleic acid amplification tools like a multiplex PCR technique. For culture, each specimen was de-contaminated by N-acetyl-L-cysteine-sodium hydroxide as per Kubica (Kubica *et al* 1963) [24]. The processed sediment was cultured for more than six weeks on two Lowenstein-Jensen medium slants at 37°C. These culture slants were inspected for growth every week. Positive cultures were further examined to confirm the presence of *M. tuberculosis*.

Processing of Positive Mycobacterial culture for extraction of Genomic DNA: One loop of cultured Mycobacterium, grown on Lowenstein-Jensen slants from menstrual fluid and endometrial specimens was suspended in 500 µl of 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). The suspension was lysed with 0.5% SDS and proteins were digested with 0.5 mg of Proteinase-K/ ml for 15 minutes at 37° C (Syun-Ichi *et al.* 1993) [25].

Processing of endometrial specimen for extraction of genomic DNA: A portion of endometrial specimen was collected in lysis buffer as described above. Sodium dodecyl sulfate (SDS) and Proteinase-K were added to final concentration of 1% and 0.1 mg/ ml respectively and incubated at 37° C for one hour. Subsequently, 5 mol/ L NaCl and 10% Cetyl trimethyl ammonium bromide (CTAB) and 0.7 mol/ L NaCl were added and kept at 65° C for 10 minutes (Herrera *et al* 1996)[26]. Mycobacterial genomic DNAs from all the mycobacterial strains grown on culture and processed endometrial tissues were extracted by addition of equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, V/V/V) solutions. The aqueous phase was transferred to another tube; DNAs were finally precipitated with 0.6 vol of isopropyl alcohol and DNA pellets were collected by centrifugation. The pellet was washed with 70% ethanol, which was then dried and dissolved in 30 µl TE buffer (pH 7.8) and stored at -20° C for future use. The DNA concentration was measured from OD at 260 nm and run in 0.8% agarose gel with λHind III DNA molecular marker.

Multiplex PCR: Samples as described were screened by multiplex PCR system developed in our laboratory (Bhattacharya *et al* 2003) [27] by modifying three individual PCR (Pao *et al* 1990; Syun-Ichi *et al.* 1993; Kox *et al* 1994) [25, 28, 29] to one reaction condition. The primer pairs are selected by following manner (i) Two oligonucleotide primers derived from the sequence of gene that codes for the 65 kDa antigen of *M. tuberculosis*; a pair of 24 base synthetic oligonucleotide (primers) bracketing a 165-bp region of a gene codes for a 65 kDa antigen (Shinnick 1987).

Briefly, according to Pao *et al* 1990), from 5' to 3' ends CTA GGT CGG GAC GGT GAG GCC AGG and CAT TGC GAA GTG ATT CCT CCG GAT. (ii) In this reaction two genus-specific oligonucleotide primers (forward, 5'-AAG AGG AAG GAG AGA GGG G-3' and reverse, 5'-GTC GTT GAG GTT GAA CTC-3') were used based on nucleotide sequence of DNA gene of *M.tuberculosis* (Syun-Ichi *et al.* 1993) [25]. The amplification of the 365-bp (region between sequence position 1377–1741 of *M. tuberculosis*) band has been observed in *M.tuberculosis* and *M. avium*, but not in other species of mycobacteria.(iii)Two oligonucleotide primers within IS 6110 insertion element, designated primers Pt-8 (5'-GTG CGG AT GTC GCA GAG AT-3') and Pt-9 (5'-CTC GAT GCC CTC ACG GTT CA-3'), were used for PCR, resulting in amplification of a 541-bp DNA fragment (Kox *et al.* 1994) [29]. This *IS 6110* insertion element is almost specific for *M. tuberculosis* complex. These newly developed multiplex PCR using primers amplifying as described by Pao *et al* 1990, Syun-Ichi *et al* 1993 and Kox *et al* 1994 [25, 28, 29], with the following proposed composition of mixture and condition. The multiplex PCR was carried out in a thermal cycler (GeneAmp 2400, Perkin-Elmer Cetus, USA) by incubating 2-5 µl of chromosomal dna (approximately 0.2 – 0.4 ng with the following components (50 µl total vol): 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin (w/v), 1.5 mM MgCl₂, 0.2 mM of each of the four deoxynucleoside triphosphates (dATP, dCTP, dGTP, dTTP), 0.2 µM, 0.1 µM and 0.2 µM of primers respectively used in the PCR 1, 2 and 3 and 1 unit of Taq polymerase (Perkin-Elmer Cetus, USA). A positive amplified and negative control DNA s were run in each experiment. The reaction mixture was first pre-incubated at 85° C for 5 minutes. The amplification was performed for 40 cycles of 94° C for 1 minute, 55° C for 1 minute and 72° C for 2 minutes. After the last cycle the samples were incubated for 10 minutes at 72° C (final extension).

Analysis of PCR Products: Ten to fifteen µl of each PCR product was subjected to electrophoresis with 2% and 2.5% agarose gel respectively and the products were visualized with Ethidium bromide. The length of the PCR product was estimated by pGEM or φX174/*Hae* markers (Promega Corporation, Madison, Wisconsin, USA).

Results

Age	<30	30-40	>40	Total
Cases	221	280	16	517
Unexplained infertility	101	143	2	246
Mild Endometriosis	96	112	10	218
Recurrent Abortion	22	24	0	46
Ectopic Pregnancy	2	1	4	7

It has been observed that among 517 cases, positive PCR was found in 230 cases (%). After treatment with anti-tubercular drugs, 86 cases conceived. The previous history and clinical situations enhancing infertility

according to age distribution of patients undergoing PCR studies are mentioned (below) in Table 1.

Duration	< 5years	5-10 years	> 10 years
No. of cases	207	281	29

The duration of infertility is also of note, which is mentioned in Table 2: Positive cases were subjected to treatment by 3 drugs (Rifampicin, Ethambutal & Isoniazid) for 3 months, followed by 2 drugs like Ethambutal & Isoniazid for 3 months. No subsequent PCR study was performed as it is well known that, even dead bacteria can give +ve TB PCR. The study was also not done in patient history of previous ATD intake. The AFB smear was positive in two cases. Bactec culture was positive in 15 cases but negative in 34 patients with PCR positive for tuberculosis.

Pregnancy Outcome: Pregnancy outcome is mentioned in following Table 3. Out of

PCR	+ve	Pregnancy
Total	230	86 (37.3%)
Unexplained Infertility	114	44 (38.5%)
Mild Endometriosis	96	33 (34.4%)
RSA	18	8 (44.4%)
Ectopic Pregnancy	2	1

230 PCR positive cases, 86 patients conceived after treatment with ATD. The completion of treatment to conception interval was 3 to 12 months. Few patients conceived during treatment. Result: 33 pregnancies were found in patients with PCR negative study.

The pregnancy rate in post-treatment of +ve TB PCR cases were similar in unexplained infertilities and mild endometriosis, and best in case of RSA.

	Cases	Abortion	Term Pregnancy
Conception by 9 months of initiation of the treatment	10	8	2
Conception after 9 months of initiation of the treatment	76	1	75

Abortion rate was higher in women conceived during or immediately after treatment with ATD. Take-home-baby rate was much high in women conceived between six

months to one year, after completion of the treatment. These are mentioned in Table 4:

Discussion

The source of bacteria is mostly the environment. Colonization of MTB may be by sexual or asexual mode. Asexual mode may be by haematogenous spread to endometrium, which ultimately remained dormant for long time, and most of bacteria were shed out from others system. The bacteria after colonizing in endometrium may proliferate or may remain dormant. They may be eliminated as well. Until and unless the bacteria enter the internal body surface, it cannot be called infection. Mere presence of bacteria on external body surface like skin, gut or endometrium, which is connected easily to the external environment, should be called infestation. The question then arises, whether infestation is pathological. The majority of the invaded bacilli are arrested by the natural defense of the human body. Bacilli reaching the endometrium are arrested by the macrophages. Several factors like the number and virulence of the infecting bacilli, host factors, including genetic susceptibility, age, immuno-competence, stress, nutrition and co-existing illness influence the outcome

of the infection. Tubercle bacilli do not contain or secrete toxin. The exact basis of their virulence is not understood, but seems to be related to their ability to survive and multiply in macrophages. Various components of the bacillus have been shown to possess different biological activities, which may influence the pathogenesis, allergy and immunity in the infection. Humans are evidently able to mount an effective defense against the invaded bacteria, as only about a tenth of the infected develop active tuberculosis. The only specific immune mechanism effective is the cell mediated type. Humoral immunity appears to be irrelevant. The key cell is the activated CD4+ helper T cell, which can develop along with two different paths – the Th1 or Th2 cells, releasing cytokines such as interferon γ (gamma) interleukins 1 and 2, tumor necrosis factor α (TNF α) and others, exerting different biological effects. Th1 dependent cytokines activate macrophages, resulting in protective immunity and containment of the infection. Th2 cytokines induce delayed type hyper-sensitivity (DTH), tissue destruction and progressive disease. The essential pathology in tuberculosis is the production in infected tissues of a characteristic lesion, the tubercle. This is an avascular granuloma, composed of a central zone containing giant cells, with or without caseation, and a peripheral zone of lymphocytes and fibroblasts. Tubercular lesions are primarily of two types – exudative and productive. The exudative type is an acute inflammatory reaction with accumulation of edema fluid, polymorphonuclear leucocytes, and later of lymphocytes and mononuclear cells. This is typically seen when the bacilli are many and virulent and the host response is more, in the nature of DTH than of protective immunity. The productive type of lesion is predominantly cellular, associated more with protective immunity than DTH. Endometrium is the normal site where embryo implants, to give rise to a healthy intrauterine pregnancy. Endometrial part of implantation involves secretion of several molecules or markers generated by hormonal, biochemical, genetic and immunomodulatory changes within the endometrium itself. Immuno-modulatory changes are brought about by cytokines and growth factors. They have a wide range of family members. Cytokines consist of different members of interleukin family, while the growth factors consist of VEGF (Vascular Endothelial Growth Factor), TNF (Tumor Necrosis Factor), LIF (Leukemia Inhibiting Factor) etc. Some of these cytokines and growth factors help in implantation, while others antagonize the procedure. If immuno-modulatory response is favorable, helpful cytokines and growth factors will appear, and successful implantation will take place. Immunologically, this is known as T helper 2 (Th2) response. On the other hand, if harmful immuno-modulators develop, the response is said to be T helper 1 (Th1) response, resulting in failure of implantation and pregnancy will not occur. In successful implantation, the Th1 sub-set of cytokines, namely TNF α , IL2 of the CD4 cell line are down-regulated, where as the cytokines of the Th2 sub-set are up-regulated. Apart from T (Thymus) lymphocyte mediated helpful or harmful cytokines and growth factors, similar type of harmful or helpful antibodies may also develop through immuno-modulation of B (Bone Marrow) lymphocytes. Helpful and harmful antibodies are known as asymmetric and symmetric antibodies respectively. Presence of MTB in the endometrium though paucibacillary, which is otherwise called MTB infestation, probably excites an inflammatory process, may be mild,

which induces the production of adverse cytokines and antibodies, resulting in Th1 response. It is well known that for successful implantation, Th2 bias is specifically needed. Inflammatory environment in the endometrium may inhibit down-regulation of Th1 bias, so also up-regulation of Th2 bias. This leads to pre-ponderance of harmful cytokines and antibodies of Th1 series in the endometrium, making it non-receptive to embryo, intending implantation. These cause, either so, are called unexplained infertility, recurrent implantation failure or recurrent abortion. Moreover, simple effort of Th1 response to mount up to Th2 bias may activate the dormant bacilli, which activates inflammatory process, further leading to more Th1 response, and thereby negative impact on implantation. The improvement of endometrial environment after treatment with ATD is evident by pregnancy rate in positive TB PCR cases (86 out of 230 cases - 37.4%). The period taken into account for pregnancy to occur in these cases was one and a half year, from the completion of the treatment, which is otherwise two years, from the initiation of the ATD treatment. It has been observed, that the patients who conceived during or immediately after medical treatment by anti-tubercular drugs mostly abort. Take-home-baby rate was higher in patients who conceived later. This indicates that, probably it takes longer for the inflammatory process to subside, even after the bacilli are eliminated. In many cases, probably some form of serious damage may happen, resulting in permanent non-receptive endometrium. Sub-endothelial blood flow may also be affected by the presence of MTB in endometrium, as it may down-regulate VEGF concentration. This may be other cause of implantation failure. Mild endometriosis most often is not considered to be the cause of infertility. These patients are grouped under unexplained infertility. In this study, mild to moderate endometriosis with good tubo-ovarian relations suffering from infertility showed substantial TB PCR positive cases. This raises a suspicion, whether endometriosis and tubercular bacillary invasion, either in the form of infestation or infection are different spectra of the same disorder of immuno-competence. Samples from endometrial aspirates, endometrial biopsies and the fluid from the Pouch of Douglas, from 25 women with infertility, suspected to be suffering from genital TB on laparoscopic findings were studied for the presence of the mpt64 gene of *M. tuberculosis* by Bhanu et al. [30]. Presence of *M. tuberculosis* DNA was detected in 56% of cases by PCR as compared to 1.6% smear-positive and 3.2% culture-positive cases. PCR was positive in all women with laparoscopic findings suggestive of TB, in 60% of those with probable diagnosis, in 33% of those with incidental findings and even in one case with normal laparoscopic findings [30]. Other authors have also observed PCR to be more sensitive than histopathology and culture [31, 32]. Rozati et al. [33] observed PCR to be positive in 43.1% of suspected cases of female genital tuberculosis, in contrast to 5.2%, 7.8% and 11.5% detection rates with AFB staining, culture and histopathology, respectively. Presence of at least 10,000 organisms/ml in the sample is required for microscopic detection of AFB. Culture is more sensitive, requiring as little as 100 organisms/ml, while PCR may be positive with only 1-10 organisms/ml [30]. However, PCR has its own disadvantages. It can give false-negative results, due to contamination with heparin or to a high salt concentration of the specimen, which may interfere with PCR results. As it cannot distinguish between live and dead

bacilli, there is a small risk of false-positive result [30]. In our series, we get a positive PCR of 44.5% in this study, and contrast to 30.06% in culture and 4.8% in AFB smear, though the number of culture and smear performed were small. This indicates that the detection of the presence of MTB bacilli is much more with PCR study, with standard technique.

Conclusion

The influence of tubercle bacteria in fertility is well known, because tuberculosis of the genital organs has long been accepted as an important cause of sterility in this country. With increasing health consciousness, improved immunization status and easy access to diagnose and treatment of tuberculosis have diminished the incidence of frank tuberculosis. Tubercular bacterial invasion to the uterus, in the form of infestation, not infection, has been strongly recognized in the present study. It has also been found that mere presence of bacilli in the endometrial layer affects fertility status of women, as infertility or recurrent reproductive failure. Proper treatment reverses the situation to great extent. The immunological changes happening during bacterial invasion may be associated with other immuno-compromised disorders like endometriosis. This requires a further structured study, to come to a definite conclusion about the disease.

References

1. Punnonen R., Kiilholma P., Meurman L. Female genital tuberculosis and consequent Infertility. *Int J fertile* 1983; 28:235-238
2. Nogales-Ortiz F., Ildefonso T., Nogales FF: The pathology of genital tuberculosis. *Obstet Gynecol* 1979; 53: 422-428
3. Oosthuizen AP., Wessels PH., Hefer JN: Tuberculosis of the female genital tract in patients attending an infertility clinic. *S Afr Med J* 1990 ; 77(11):562-564
4. Sutherland Arthur M. Gynecological Tuberculosis, Past, Present and Future. *Arch Gynaek* 1975; 218: 261-268
5. Steinbrook Robert. Tuberculosis and HIV in India. *New Eng J Med* 2007; 356(12): 1198-1199
6. Deshmukh KK, Lopez JA, Naidu TAK, Gaurkhede MD. MV Place of laparoscopy in pelvic tuberculosis in infertile women; *Arch Gynecol* 1985; 237:197-200
7. Chhabra S. Genital tuberculosis – a baffling disease; *J Obstetr Gynecol India* 1990; 40:569-73
8. Jindal UN. Genital tuberculosis: an important cause of Asherman's syndrome in India; *International journal of tuberculosis and lung disease*; 2006
9. Tripathi SM. Infertility and pregnancy outcome in female genital tuberculosis; *Int J Gynecol. Obstet.* 2002; 76(2):159-63
10. Jahroni N, Parsanezhad ME, et. al. Female genital tuberculosis and infertility; *Int. J. Gynecol. Obstet* 2001; 75(3):269-72
11. Schaefer G. Female genital tuberculosis. *Clin Obstet Gynecol* 1976; 19:223-239
12. Malik S; Genital tuberculosis and implantation in assisted reproduction; *Reviews in Gynecological Practice* 2003;3: 160-164
13. de Vynck WE, Kruger TF, et. al; Genital tub. Associated with Female Infertility in West Cape. *S Afr Med J* 1990; 77(12): 630-1
14. Sharma JB, Roy KK, Kumar S, Malhotra N, Chawla P. Progress in Obstetrics & Gynecology; edited by John S, Scang LT, Frank AC; 2008

15. Kumar S. Female genital tuberculosis. In: Sharma SK, Mohan A. (eds) Tuberculosis, 1st edn. Delhi: Jaypee; 2001; 311:324
16. Sharma JB. Female genital tuberculosis revisited. *Obstet Gynecol Today* 2007;XII:61-63
17. Stead WW. Pathogenesis of a first episode of chronic pulmonary tuberculosis in man: recrudescence of residuals of the primary infection or exogenous reinfection; *Am Rev Resp Dis* 1967; 95:729-74
18. Stead WW, Kerby GR, et. al. The clinical spectrum of primary tuberculosis in adults: confusion with reinfection in the pathogenesis of chronic tuberculosis. *Ann Intern Med* 1968; 68:731-45
19. Raghupathy R, Makhseed M, Azizieh F; Th1 and Th2 cytokine profiles in successful pregnancy and unexplained recurrent abortions; In: Gupta SK, editor. *Reprod Immunol* 1994; 14:149-57
20. Bateman BG, Nunley WC, Kitchen RE. Genital tuberculosis in reproductive-age women. *J Reprod Med* 1986; 31: 287-290
21. Baum SE., Dooley DP., Wright J., Kost ER., Storey DF: Diagnosis of culture-negative female genital tract tuberculosis with peritoneal involvement by polymerase chain reaction. *J Reprod Med*; 2001; 46 (10): 929-32
22. Sutherland AM. Tuberculosis of the female genital tract. *Tubercle* 1985; 66:79-83
23. Schaetzing A. Infeksies van die genitaletraktus. In:OdendaalHJ, ed.Ginekologie. Kaapstad:Juta,1986:230-232
24. Kubica GP, Dye WE, Cohn.ML et al. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria; *Am Rev Respir Dis* 1964; 89: 284-286
25. Syun-Ichi T, Okuzumi K, Ishiko H, Ken-ichi N, Ohkubo A, Nagai R. Genus specific polymerase chain reaction for the Mycobacterial dnaJ gene and species-specific oligonucleotideprobes. *J Clin Microbiol* 1993; 31: 446-450
26. Herrera EA, Perez O, Segovia M. Differentiation between Mycobacterium tuberculosis and Mycobacterium bovis by a multiplex polymerase reaction; *J Appl Bacteriol* 1996; 80: 596-604
27. Bhattacharya B., Karak K, Ghosal AG, Roy A, Das S, Dandapat P, Khetawat D, Mondal DM, Bhattacharya S, Chakrabarti S. Development of a new sensitive and efficient multiplex polymerase chain reaction (PCR) for identification and differentiation of different mycobacterial species; *Tropical Medicine and International Health* 2003; 8 (2): 150-157
28. Pao CC, Yen TS, Jinn-Bang Y et al. Detection and identification of Mycobacterium tuberculosis by DNA amplification; *J Clin Microbiol* 1988; 28: 1877-1880
29. Kox LLLF, Rhienthong D, Medo Miranda N et al. A more reliable PCR for detection of Mycobacterium tuberculosis in clinical samples; *J Clin Microbiol* 1994; 32: 672-678
30. Bhanu NV, Singh UB, Chakraborty M et al. Improved diagnostic value of PCR in diagnosis of female genital tuberculosis leading to infertility. *J Med Microbiol*; 2005; 54:927-931
31. Jindal UN. An algorithmic approach to female genital tuberculosis causing infertility; *Int J Tuberc Lung Dis* 2006; 10: 1045-1050
32. Abebe M, Lakew M, Kidane D, Lakew Z, Kiros K, Harboe M. Female genital tuberculosis in Ethiopia. *Int J Gynecol Obstet* 2004; 84: 241-246
33. Rozati R, Sreenivasagari R, Rajeshwari CN. Evaluation of women with infertility and genital tuberculosis; *J Obstet Gynecol India*; 2006, 56: 423-426

*All correspondences to: Dr. Siddhartha Chatterjee, Calcutta Fertility Mission, Pushpanjali Apartment, 102C Ballygunge Place, Kolkata-700019 Phone No: 9133 2281 2813; e-mail: sidchat54@gmail.com , sid2512@vsnl.net