INTRODUCTION

*Mycobacterium tuberculosis* causes tuberculosis (TB), with worldwide distribution and high incidence in developing countries. It is one of the major public health problems in developing countries. As per World Health Organization (WHO), 38% of TB cases are from South East Asia region (SEAR). India is the largest country in the SEAR and has highest number of TB cases with 23% of estimated incident cases worldwide in 2013 (WHO Annual TB Report 2015). As per estimates of WHO for 2014, prevalence rate per lac population was 174 and incidence rate was 133 per lac population, showing a decreasing trend over the years (WHO Annual TB Report 2015).

Based on the clinical manifestation, TB can be classified in two types: Pulmonary and Extra pulmonary. Pulmonary TB (PTB) is the commonest form, but extra pulmonary TB (EPTB) is also a major clinical problem. Extra pulmonary TB may involve areas that are highly vascular such as meninges, kidney, lymph nodes, spine and the growing ends of bones. Some other sites which may be involved are pericardium, pleura, liver, peritoneum, gastro-intestinal tract and genito-urinary tract.

Genital tuberculosis (GTB) is a form of extra-pulmonary TB which affects the genital organs. It represents 15-20% of the extra-pulmonary TB and is the second most common site infected after pulmonary tuberculosis. Genital tuberculosis is seen in 12% of all patients with pulmonary tuberculosis and accounts for 5-10% of all pelvic infections. The incidence of GTB is high amongst the patients with infertility and it is an important cause of infertility, being an etiological factor in 1-8% of infertile cases (Arora *et al.*, 2003; Bapna *et al.*, 2005; Gatongi *et al.*, 2005). The incidence of genital tuberculosis varies widely with the socio economic status of the patients and their environment.

Female genital TB (FGTB) affects mainly the fallopian tubes (90%), endometrium (50%) and ovaries (10–30%) (Rana *et al.*, 2011).
Genital tuberculosis is reemerging as an important health problem in developed nations too because of the increasing numbers of immuno-compromised patients, large numbers of immigrants from less developed countries and due to emergence of drug–resistant TB (Bansal et al., 2012). It has been reported that in developed countries, the incidence of GTB is < 1%, but in some African as well as Asian countries, it is as high as 15-19% (Abebe et al., 2004; Thangappah et al., 2011). FGTB is the main cause of infertility in about 5–16% cases among Indian women. However, the actual incidence of FGTB is not known since most of the cases are asymptomatic and remain undiagnosed due to paucity of investigations (Malhotra et al., 2012).

As per several studies from India FGTB causes infertility in 4-9% cases and diagnosis of TB should be actively done by rapid and sensitive methods in such patients (Deshmukh et al., 1987; Deepjyoti et al., 1990; Thangappah et al., 2011; Patil et al., 2015).

Predominantly, the infertility is caused due to tubal block, adhesions in the endometrial cavity and the ovulatory dysfunction. Genital TB has now become a challenging disease from both diagnostic and therapeutic view points, as it has few characteristic symptoms. Clinical presentation of the disease is extremely variable. It may present as chronic pelvic inflammatory disease not responsive to therapy and infertility caused due to extensive tubal destruction, which is difficult to cure by reconstructive tubal surgery (Qureshi et al., 2001). The treatment of GTB consists mainly of chemotherapy and surgery. Multiple regimens of chemotherapy are used for a minimum of at least 6-9 months.

GTB has been a diagnostic dilemma for clinicians for decades due to the latency of the organism, asymptomatic and varied presentation in majority of the cases and paucity of an accurate diagnostic modality but with the advent of newer diagnostic modalities, the situation has improved. However, majority of the cases have asymptomatic presentation and hence a definitive modality is required to diagnose this condition.
Erythrocyte sedimentation rate (ESR) and Mantoux test are not specific (Puri et al., 2009). Therefore, diagnosis of GTB has to be based on imaging techniques, endoscopy, histo-pathology culture and Polymerase Chain Reaction (PCR) (Gatongi et al., 2005; Jassawala et al., 2006; Thangappah et al., 2011).

Traditionally diagnosis of TB is done by acid-fast bacilli (AFB) smear microscopy and culture. Microscopy has poor sensitivity ($10^4$ bacteria/ml) (Lima et al., 2008).

Histopathological examination has its own limitations (Chakrabarti et al., 1998; Rana et al., 2011), as it is only suggestive of TB and not confirmatory until AFB is demonstrated in the lesion.

Isolation of Mycobacteria from clinical specimen provides a clear and definitive diagnosis of TB (Armstrong, 2009). Culturing Mycobacteria is tough as it is a fastidious organism. Lowenstein-Jensen (LJ) medium is widely used in tuberculosis microbiology laboratories and is considered the gold standard for MTB culture. However, it presents certain challenges. Although the culture technique is very sensitive and specific, the growth rate in this medium is slow (3 to 6 weeks), resulting in an unacceptable delay in diagnosis and treatment. Traditional AFB culture on LJ medium has a low detection rate and takes longer to provide positive results (Jassawalla et al., 2006; Puri et al., 2009). When few Mycobacteria are present at the infection site, multiple cultures may be required in order to achieve a positive result. Use of rapid automated culture methods based on liquid media (BACTEC 460 and MGIT 960) have been found to be very accurate and quick for the diagnosis of even smear negative pulmonary tuberculosis. These methods are highly recommended for rapid and precise diagnosis of tuberculosis (Bohy et al., 2009). Although these rapid culture methods (BACTEC 460, BACTEC MGIT 960, MB/BacT etc) are costly, they may eventually prove cost effective for National Programs. However, besides the high cost of acquiring and maintaining the required equipment, other major drawbacks of these rapid methods include: the necessity of specially trained technical personnel and the challenges of working with a liquid medium that is highly prone to contamination.
PCR based methods are very sensitive and rapid as they can detect few copies of *MTB* DNA (less than 10 bacteria/ml), with results available within 1-2 days (Kolk *et al.*, 1992; Dar *et al.*, 1998; Bhanu *et al.*, 2005; Rana *et al.*, 2011, Chagas *et al.*, 2010). Since genital TB is paucibacillary, high sensitivity and specificity are essential for its accurate diagnosis. Being both highly sensitive and specific, PCR is an apt diagnostic tool for this condition.

Several primers have been used in PCR assays for detection of *MTB*, with varying sensitivity. Insertion and repetitive elements e.g. *IS6110, IS1081, IS1561* and *IS1552* based PCR have been extensively used for diagnosis of *MTB* in different parts of the world (Singh *et al.*, 2006; Ani *et al.*, 2009, Singh *et al.*, 2012). *IS6110* has been most commonly used for detection of *MTB* but has certain limitations as it is reported that *MTB* isolates from certain parts of India may have no copy or very low copy numbers of *IS6110* element and this may lead to false negative results (Chauhan *et al.*, 2007). Other targets like *MPB64* and *ESAT-6* have also been used with varying results (Bhanu *et al.*, 2005; Rozati *et al.*, 2006). In order to increase the sensitivity of PCR, several authors have used multiple targets simultaneously for detecting *MTB* (Kulkarni *et al.*, 2012). Singh *et al.* (2006) used *IS6110* and *MPB64* PCR for detection of *MTB* in lymph node biopsies and observed that 8.6% samples were negative by *IS6110* but positive by *MPB64* method. Hence, the use of multiple target genes can enhance the sensitivity of PCR.

In this study, we evaluated the simultaneous utility of commonly used targets *IS6110, ESAT-6* and *MPB64* to increase the sensitivity of PCR in detecting genital TB. We also compared AFB smear microscopy, culture on solid Lowenstein Jensen (LJ) and liquid Middlebrook 7H9 (MB7H9) media, and three sets of PCR assays based on *MPB64, IS6110* and *ESAT-6* genes for their efficiency to detect genital TB in infertility patients attending Sawai Maan Singh (SMS) and attached group of hospitals in Jaipur. This strategy allowed us to identify the most efficient combination of tests for improving the diagnosis of genital TB.