## Original Article

# Peritoneal Fluid from Pouch of Douglas is not a Suitable Specimen for Molecular Testing in the Diagnosis of Female Genital Tuberculosis in Women Presenting with Infertility

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**Background :** Female genital tuberculosis is a form of Extra-pulmonary tubercular disease that primarily manifests as infertility in women. It's diagnosis is challenging because of difficulty in obtaining appropriate samples for testing. Conventional diagnostic methods, such as microscopy and culture, have limited effectiveness, leading to the use of molecular techniques for accurate diagnosis. This study was conducted to evaluate the suitability of Peritoneal Fluid in the pouch of Douglas or peritoneal washings as an alternate specimen for diagnosing female Genital Tuberculosis.

**Materials and Methods :** A prospective cross-sectional study was conducted on 30 infertile women, clinically suspected of Genital Tuberculosis. Laproscopy was performed to ascertain the presence of morphological signs of Genital Tuberculosis and Peritoneal Fluid or washings were collected for molecular testing of tuberculosis.

**Results :** Among the 30 infertile women (primary infertility = 20; secondary infertility = 10), 11 were aged 21-30 years, while 19 were aged 31-40 years. Pelvic ultrasonogram showed abnormalities in only a third of the cases. In laparoscopy, definite findings of Genital Tuberculosis were noted in 13 cases (43.3%). Others had probable findings of Genital Tuberculosis, comprising of, Pelvic Adhesions, Bilateral or Unilateral Tubal Block, Tubo-ovarian Mass, Fitz Hugh Curtis Syndrome and Hydrosalpinx. Laproscopically obtained Peritoneal Fluid or washings from all the 30 women were tested using cartridge-based Nucleic Acid Amplification and Polymerase Chain Reaction for *Mycobacterium Tuberculosis*. However, all the samples tested negative.

**Conclusion :** The use of Peritoneal Fluid as a specimen for molecular detection of *Mycobacterium Tuberculosis* did not yield positive results in this study. Further research is warranted to validate the study's result and to explore better alternative approaches for the diagnosis of Female Genital Tuberculosis.

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***Key words* : Female, Genital Tuberculosis, Infertility, Peritoneal Fluid, Nucleic Acid Amplification, Laproscopy.**

Tuberculosis (TB) poses a significant Worldwide health challenge with approximately 6.4 million new TB cases documented Globally. Majority of TB cases are detected in South East Asia, Africa, and Western Pacific regions, out of which India contributes to 26 percent of all TB cases[1]. In Indian population, about 80% of TB cases are primarily of pulmonary origin and rest 20% manifests as Extra-pulmonary Tuberculosis (EPTB), which commonly affects Lymph Nodes, Meninges, Pleura, Osteo-articular System, Urogenital System, Eye, etc[2].

Female Genital Tuberculosis (FGTB) usually presents as infertility and accounts for 3-16% cases of EPTB in India[3]. Unlike Pulmonary TB, FGTB mostly presents with vague symptoms like infertility,

***Editor's Comment :***

- The present study tried to find out peritoneal fluid as an alternative sample for molecular diagnosis of female genital tuberculosis as the endometrial biopsy can be obtained through invasive procedure and has inhibitors for PCR reaction.
- The study concluded that peritoneal fluid is not a suitable alternative. Patients with features of genital tuberculosis seen through laporascopy found to have negative PCR results.
- The study recommends clinical diagnosis through gynaecological procedures can be taken as benchmark to start antitubercular therapy irrespective of results obtained through molecular methods as immediate therapy can restore fertility in some cases of female genital tuberculosis.

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menstrual disorder and chronic pelvic inflammation thereby making it difficult to diagnose clinically[4,5]. Furthermore, cases of FGTB often pose diagnostic difficulty upon lab investigations also because of inherent difficulty in obtaining specimens from deep seated organs and low bacterial load in the obtained samples.

FGTB is paucibacillary in nature and hence conventional methods such as microscopic demonstration of Acid-fast Bacilli (AFB) by Ziehl Neelson staining is ineffective tool due to high false negativity[4]. Similarly, culture of tissue samples, such as endometrial curettage or biopsy usually yields inconclusive results[6]. Histopathological examination may show granuloma formation but it may not be a specific finding. It can only act as supplementary tool to clinical diagnosis and microbiological results. Therefore, detection of *Mycobacterium tuberculosis* specific genes by molecular methods is often resorted to for deriving a conclusive diagnosis. Cartridge Based Nucleic Acid Amplification (CBNAAT) or Gene expert (Cepheid, Sunnyvale, CA, USA) and Polymerase Chain Reaction (PCR) are proven to exhibit high degree of specificity but poor sensitivity in detecting FGTB in endometrial tissue specimen[2]. The limited sensitivity is attributed to presence of DNA inhibitors in blood of such specimen[2]. Therefore, this study was undertaken to test the suitability of Peritoneal Fluid present in pouch of Douglas or its washings as an alternate specimen to endometrial curettage or biopsy in infertility cases clinically suspected of FGTB presenting with infertility.

## Materials and Methods

A prospective cross-sectional study was carried out between January, 2022 to June, 2022 amongst 30 consecutive patients attending an Infertility Clinic and satisfying the inclusion criteria. Inclusion criteria were: (i) Women presenting with minimum one year of primary or secondary infertility and having minimum two failed ovulation induction attempts; (ii) Women having either definitive signs of TB by Laparoscopy such as tubercles, beaded appearance of the Fallopian Tubes, Caseous Nodules, Pelvic Adhesions, Fitz Hugh Curtis Syndrome, Bilateral or Unilateral Tubal Block, Hydrosalpinx and Tubo-ovarian Mass;[7] (iii) Normal sperm count, motility and morphology on semen analysis of husband.Women already receiving anti-TB treatment were excluded. Prior to enrollment into the study, informed written consent was obtained from all participants. Primary infertility was characterized as the inability of women to conceive after one year of unprotected intercourse, while secondary infertility was defined as inability to conceive again after a prior conception, regardless of the obstetric outcome[8]. Detailed history taking (including history of any contact with TB case), general physical examination and gynecological examination were carried out. Diagnostic procedures /interventions such as Ultrasonography of Pelvis, Laparoscopy, Hystero-salpingography and Hysteroscopy were done as a part of thorough work-up for infertility.

For diagnostic Laparoscopy and sample collection, a Laparoscope was introduced into abdomen after optimum carbon dioxide gas insufflation under General Anaesthesia. Presence of straw-colored fluid in pouch of Douglas, presence of any tubercles, adhesions or caseation were noted. Uterine tubes and ovaries were also inspected for any gross pathology like signs of endometriosis, presence of serosal fibroids or nodules or pelvic inflammatory disease. Simultaneous operative intervention was carried out to restore fertility in the same setting. Only those patients having either definite or probable signs of FGTB upon Laparoscopy, Peritoneal Fluid was collected. If there was no Peritoneal Fluid in suspected cases, 10 ml of normal saline was injected in a syringe into the peritoneal cavity through irrigation channel of the suction apparatus of Laparoscope and peritoneal washings were collected in a syringe. The specimen was transferred to two conical sterile test tubes. The tubes were properly labelled and sealed. One of the tubes was sent to a NABL accredited laboratory for CBNAAT testing and the second tube was sent to RMRC Bhubaneswar laboratory for TB-PCR. Dispatching of the sample was done within half an hour of collection and cold chain was maintained during specimen transportation. During diagnostic Hysteroscopy, uterine cavity, fundus and bilateral tubal ostia were inspected for gross pathological findings like adhesion, polyp, ostial block and if present, appropriate therapeutic procedures such as adhesiolysis, polypectomy, tubal cannulation was performed in the same sitting. Chromopertubation was done by introducing methylene blue through cervix into fallopian tubes to see the patency of the fallopian tubes.

For CBNAAT, 5 ml of specimen was first decontaminated by mixing with an equal volume of N-acetyl-Cysteine -2% NaOH for eight minutes and then concentrated by centrifugation at 3000xg for 20 minutes. Most part of the supernatant was discarded, keeping 0.5 ml of lower part of supernatant along with the pellet. This pellet was then resuspended in 0.5 to

1 mL of Phosphate Buffered Saline (PBS). Sample reagent was added in a 2:1 ratio to the resuspended pellet, followed by vortexing the mixture twice for 10 seconds each.After incubating for 10 minutes at room temperature, 2 ml of the sample reagent treated specimen was charged into the Xpert MTB/RIF cartridge and loaded in the CBNNAT equipment.

For TB PCR, the samples were diluted three times using sterile PBS and mixed thoroughly by vortexing, followed by centrifuging at 10,000 rpm for two minutes. The supernatant thus obtained was discarded and the pellet was re- suspended with 1%, N- Acetyl L - Cystine. After incubation, the it was again re-centrifuged at 10,000 rpm for two minutes and the again the supernatant was discarded. The remaining pellet was used for DNA Extraction, which was carried out according to manufacturer's instruction using TRUPCR MTB DNA extraction kit. The extracted DNA was quantified and the purity was evaluated using Nanodrop - Lite (Thermofischer Scientific). This extracted DNA was stored at-20$^{o}$C.TRUPCR MTB nested PCR Kit was used for the subsequent PCR reaction. It is a two-step PCR where in the first step Mycobacterium genus specific DNA get detected while in the second step PCR in the same reaction amplification of *Mycobacterium tuberculosis* complex specific DNA occurs.

## Results

Out of the 30 included women, 11 patients were in the age group of 21 to 30 years while 19 were in the 31 to 40-year age group. 5 patients belonged to lower socio-economic status while rest were hailing from lower middle-class family. All the patients had received BCG vaccination and had no history of TB contact, or drank unpasteurized milk. Twenty women presented with primary infertility while secondary infertility was observed in 10 cases. Average duration of infertility was 6.5 years. Normal menstrual cycles were seen in 11 women, while menstrual disorders seen were irregular cycles (n=13), scanty menses (n=12), heavy flow (n=4), and dysmenorrhea (n=2). One patient complained of Irregular heavy menses. Weight loss, Dyspareunia, Vaginal discharge and Lower Abdominal Pain were other presenting symptoms (Table 1). None of the patient had signs of lymphadenopathy or Chest Crepitations.

The gynaecological examination findings are detailed out in Table 2. During the per speculum examination, vaginal discharge was detected in 9 (30.0%) out of 30 women. On vaginal examination, 17 patients (56.7%) showed adnexal tenderness only and one patient (3.3%) had both adnexal tenderness and adnexal mass. Ultrasonography of Pelvis did not reveal any abnormality in 20 patients (66.7%). Tubo-ovarian masses was present in 5 women (16.7%) while Polyp, Polycystic Ovaries, Fibroid, Thickened Endometrium & Hydrosalpinx were detected in one patient each. Hysterosalpingography was essentially normal in 7 women(23.3%), while others had either unilateral Tubal Block Only (n=9; 30.0%), Bilateral Tubal Block Only (n=11; 36.7%), Bilateral Tubal Block with Beaded Tubes (n=1;3.3%), Bilateral Tubal Block with Bicornuate Uterus (n=1;3.3%) and Septate Uterus (n=1;3.3%). In hysteroscopy, no abnormality could be detected in 18 women (60.0%), endometrial pallor and increased endometrial thickness was seen in one patient (3.3%) each, while pelvic adhesions were found in 9 cases (30.0 %). Among the patients with pelvic adhesions, one patient was having septate uterus and another showed bicornuate uterus which was corrected by operative procedure.

Table 1 — *Patients'clinical Symptoms*

| History/ Symptoms | | Number of Patients (n=30) |
|---|---|---|
| Menstrual patterns | Normal menses | 11 (36.7%) |
| | Heavy flow | 04 (13.3%) |
| | Scanty menses | 12 (40.0%) |
| | Irregular cycles | 13 (43.3%) |
| | Dysmenorrhoea | 02 (6.7%) |
| Weight loss | | 04(13.3%) |
| Dyspareunia | | 05 (16.7%) |
| Vaginal discharge | | 06 (20.0%) |
| Chronic pelvic pain | | 18 (60.0%) |

Upon diagnostic laproscopy, definite findings of FGTB (that is, beaded tubes with or without tubercles) were noted in 13 cases (43.3%) (Fig 1). Probable findings of FGTB were pelvic adhesions only and bilateral tubal block only in 7cases (23.3%) each, Bilateral Tubal Block with pelvic adhesions in three patients (10.0%), Bilateral Tubal Block with Tubo-

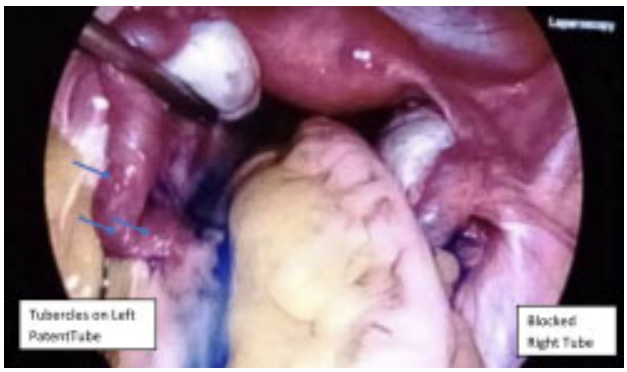


Fig 1 — Laproscopic picture of case 2 showing definitive signs of female genital tuberculosis

ovarian Mass in one patient (3.3%), Unilateral Tubal Block only in 06 cases (20.0%), Unilateral Tubal Block with Pelvic Adhesions in 3 patients(10.0%), Unilateral block with Fitz Hugh Curtis Syndrome in 1 (3.3%) case, Hydrosalpinx in 1 case (3.3%) (Fig 2). Laproscopically obtained samples, that is, peritoneal fluid/washings, from all the 30 women subjected for diagnosis of *Mycobacterium tuberculosis* by CBNAAT and nested PCR were found to be negative for *Mycobacterium tuberculosis*.

## Discussion

Since Morgagni's initial autopsy report of FGTB in a young female in 1744, its Worldwide incidence has been on the rise[9]. FGTB is one of the major causes of female infertility especially in developing countries like India where prevalence of TB is high[4]. Infertility may be caused due to tubal pathologies like tuberculous exo- or endo-salpingitis, salpingitis isthmica nodosa, interstitial tubercular salpingitis, or endometrial pathological changes such as endometrial thinning, synechiae, Asherman's syndrome or pathological changes in ovaries like reduced ovarian reserve and inferior quality of ovum. As the duration of FGTB increases the damage to genital organs become increasingly profound. Hence, early and definitive diagnosis holds paramount importance in reversing the infertility through definitive management protocols.

Microbiological tests with higher specificity such as smear microscopy or culture of peritoneal biopsy or culture of endometrial aspirate or biopsy or, molecular tests like CB-NAAT or PCR on endometrial samples have low sensitivity results for detection of *Mycobacterium tuberculosis* primarily due to paucibacillary nature of disease and due to inhibition of Polymerase Chain Reaction by inhibitory substances found in blood of such tissue samples[10]. Sharma, *et al* performed Gene Xpert on 240 endometrial samples and only seven (2.9%) cases were positive[11]. Agrawal M, *et al* found 18 positive samples (3.6%) out of 438 endometrial aspirate in infertile patients using TB-PCR[12]. Similar is the case of histopathological or radiological diagnostic methods, where the results are non-specific[7]. There is paucity of literature regarding the diagnostic utility of alternate specimen like Peritoneal Fluid accumulated at pouch of Douglas instead of endometrial tissue specimen for molecular diagnosis of FGTB, which was explored in the present study.

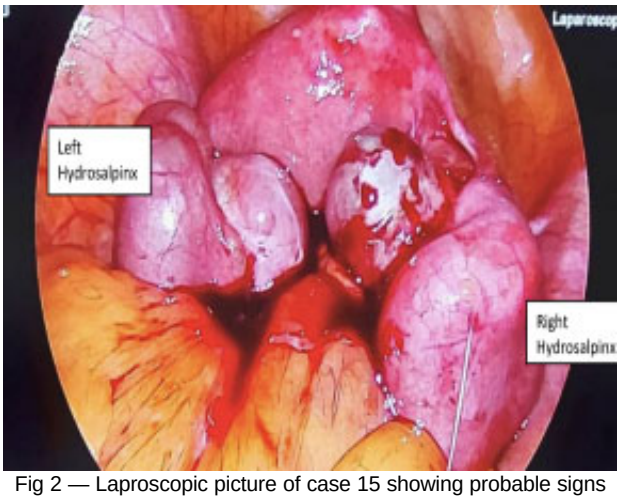

Fig 2 — Laproscopic picture of case 15 showing probable signs of female genital tuberculosis

Sharma J and colleagues evaluated the role of transvaginal and Transabdominal Ultrasound in diagnosis of FGTB and found it to be a useful adjunct tool for the diagnosis, especially in women with tubo-ovarian Masses[13]. In our study cohort, Pelvis ultrasonography revealed pathological abnormalities only in 10 cases (33.3%). Gynaecological techniques such as diagnostic Laparoscopy provide greater reliability in the diagnosis of FGTB as it allows direct visualization of the entire abdomino-pelvic cavity, enabling a direct examination of definitive and probable signs of FGTB and we opine the same. In a study of FGTB by Thangappah RBP, *et al,* Laparoscopy suggested a tubercular etiology in 59.7 per cent of cases[4]. Recently, Sharma, *et al* showed that diagnostic laparoscopy detected a greater number of FGTB cases than Gene Xpert, an indispensable diagnostic tool in pulmonary Tuberculosis, carried out on endometrial sample[7]. In the present study, primary or secondary infertility patients with ovulation induction failure with presence of definite or probable signs of FGTB on diagnostic Laparoscopic examination only were included and investigated further by molecular methods carried out in the peritoneal fluid accumulated in pouch of Douglas or in peritoneal washings.

Previously, Peritoneal Fluid or washings have also been studied by Thangappa, *et al* for *Mycobacterium tuberculosis* DNA PCR[4]. Amongst the seven aspirated peritoneal fluid samples, only two showed positivity. It was proposed by the authors that it could be attributed to paucibacillary nature of the specimen.[4] In their study cohort of 72 infertile women, smears were positive for Acid Fat Bacilli in 8.3%, culture positivity was noted in 5.6% and histopathological examination showed epithelioid granulomas in 6.9% cases where as 36.7% cases were positive for *Mycobacterium tuberculosis* DNA by PCR.

Table 2 — *Gynecological examination findings*

| Patient | Age (Years) | Parity | Type of infertility | Duration of infertility (Years) | Number of failed ovulation induction | Per Speculum examination | Vaginal examination | Ultrasound Pelvis | Hysterosalpingography | Hysteroscopy | Definite Laparoscopic findings | Probable Laparoscopic finding | Chromopertubation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case 1 | 32 | P1 | SY | 11 | 04 | N | N | Polyp in Uterus | UTB | PA | Nil | UTB | N |
| Case 2 | 29 | P0 | PY | 04 | 03 | N | N | N | BTB | N | BFT | BTB | N |
| Case 3 | 29 | P0 | PY | 06 | 02 | N | ADM | TOM | BTB | N | Nil | BTB+TOM | BTB |
| Case 4 | 38 | P1 | SY | 05 | 02 | N | N | N | UTB | TNE | Nil | UTB | UTB |
| Case 5 | 25 | P0A1 | SY | 05 | 02 | N | N | N | UTB | N | Nil | UTB | UTB |
| Case 6 | 31 | P2L0 | SY | 05 | 02 | N | N | N | UTB | N | Nil | UTB+PA | UTB |
| Case 7 | 21 | P0 | PY | 04 | 06 | N | N | PCOD | N | PD | Nil | PD | DP |
| Case 8 | 29 | P0A1 | SY | 07 | 04 | N | N | TKE | UTB | TKE | Nil | UTB | UTB |
| Case 9 | 29 | P0 | PY | 11 | 02 | N | AT+ADM | TOM | BTB | PAE+PA | Nil | PA | BTB+Frozen Pelvis |
| Case 10 | 27 | P0 | PY | 04 | 04 | N | N | N | BTB | N | Nil | PA | BTB |
| Case 11 | 30 | P0 | PY | 03 | 02 | VD | N | N | UTB | N | Nil | UTB | N |
| Case 12 | 34 | P0 | PY | 05 | 03 | VD | AT | N | BTB | N | Nil | BTB | DP |
| Case 13 | 37 | P0 | PY | 17 | 02 | VD | AT | Fibroids | Septate Uterus | PD+ Septations | BFT | BTB | DP |
| Case 14 | 37 | P0 | PY | 12 | 04 | VD | AT | N | N | AD | BFT | Nil | N |
| Case 15 | 38 | P0 | PY | 17 | 02 | N | AT | TOM | Bicornuate Uterus +BTB | Bicornuate Uterus | Nil | BTB + Hydrosalpinx | BTB |
| Case 16 | 25 | P0 | PY | 03 | 03 | VD | AT | N | BTB | N | BFT | BTB | BTB |
| Case 17 | 26 | P0 | PY | 05 | 03 | VD | N | N | BTB | N | Nil | BTB | DP |
| Case 18 | 30 | P0 | PY | 04 | 06 | VD | AT | TOM+BTB | BTB | N | BFT | BTB+PA | BTB |
| Case 19 | 37 | P1 | SY | 09 | 02 | VD | AT | N | BTB | N | BFT | BTB+PA | BTB |
| Case 20 | 35 | P0 | PY | 06 | 03 | VD | AT | TOM | BTB | PA | BFD+ Fibroid Uterus | BTB+PA | BTB |
| Case 21 | 32 | P0 | PY | 05 | 06 | N | AT | N | N | N | BFT | UTB+ Fitz Hugh Curtis Syndrome | DP |
| Case 22 | 33 | A6P0 | SY | 05 | 02 | N | N | N | N | PA, Synechia | Nil | PA | N |
| Case 23 | 39 | P0 | PY | 02 | 02 | N | AT | N | N | N | Nil | PA | N |
| Case 24 | 31 | P0 | PY | 04 | 02 | N | AT | N | UTB | N | BFT+ Tubercles | UTB | UTB |
| Case 25 | 32 | P1L0 | SY | 08 | 03 | N | AT | N | BTB | N | BFT | PA | BTB |
| Case 26 | 25 | A1P0 | SY | 03 | 02 | N | AT | N | UTB | PA | Nil | PA+UTB | UTB |
| Case 27 | 32 | A1P0 | SY | 10 | 03 | N | AT | Hydrosalpinx | BTB | N | BFT+ Hydrosalpinx | PA+BTB | BTB |
| Case 28 | 36 | P0 | PY | 10 | 03 | N | AT | N | UTB | N | Nil | PA+UTB | UTB |
| Case 29 | 24 | P0 | PY | 02 | 02 | N | N | N | N | N | BFT | Nil | N |
| Case 30 | 34 | P0 | PY | 02 | 02 | N | AT | N | N | PA | BFT | PA | N |

Abbreviations: N: Normal, PY: Primary, SY: Secondary, UTB: Unilateral Tubal Block, BTB: Bilateral Tubal Block, PA: Pelvic Adhesions, BFT: Beaded Fallopian Tubes, AT: Adnexal Tenderness, ADM: Adnexal mass, TOM: Tubo-ovarian mass, TNE: Thin Endometrium, TKE: Thick Endometrium PCOD: Polycystic Ovarian Disease, DP: Delayed Patency, PAE: Pale Endometrium, VD: Vaginal Discharge

In the present study, Peritoneal Fluid/ Washings collected from 30 infertile women with laparoscopic findings suggestive of TB (43.3% of those with definitive findings of FGTB and rest with probable findings), were tested negative by PCR and CBNAAT for *Mycobacterium tuberculosis*. Similar to our findings, other investigators have also observed poor diagnostic yield with Peritoneal fluid[2,14,15]. Bhanu, *et al* performed DNA PCR on Peritoneal Fluid of clinically suspected FGTB cases and found positivity rate of 16% only, whereas higher positivity rates were observed with endometrial aspirates (47.6 %), and endometrial biopsies (53.3 %)[14]. Rana, *et al* conducted a similar study utilising Peritoneal Fluid obtained from 200 infertile women, revealing that DNA PCR showed a positive result in only 9.6% cases[15]. On the contrary, they documented 44.85% positivity for *Mycobacterium tuberculosis* in endometrial specimens by PCR.

## Conclusion

To conclude, we found that Peritoneal Fluid from pouch of Douglas or peritoneal washings is not a suitable specimen for *Mycobacterium tuberculosis* detection by molecular methods in the diagnostic work-up of clinically or laproscopically suspected cases of FGTB. However, since the major limiting factor of the study is low sample size, further larger validation studies are needed.

**Conflict of Interest :** None declared.

## References


1 World Health Organization. WHO global tuberculosis report 2021: Geneva: WHO; 2021.https://www.who.int/publications/i/item/9789240037021accessed on 1st May 2023.

2 Agrawal S, Kant S, Das V, Jain A, Mishra S — Limited role of GeneXpert in peritoneal fluid in the diagnosis of genital tuberculosis in infertile women. *J Family Med Prim Care* 2021; **10:** 1656-60

3 Grace GA, Devaleenal DB, Natrajan M — Genital tuberculosis in females. *Indian J Med Res* 2017; **145(4):** 425-36.

4 Thangappah RBP, Paramasivan CN, Narayanan S — Evaluating PCR, culture & histopathology in the diagnosis of female genital tuberculosis. *Indian J Med Res* 2011; **134:** 40-6.

5 Wang Y, Shao R, He C, Chen L — Emerging progress on diagnosis and treatment of female genital tuberculosis. *Journal of International Medical Research* 2021; **49(5):** 1-8.

6 Dahiya B, Kamra E, Alam D, Chauhan M, Mehta PK — Insight into diagnosis of female genital tuberculosis. *Expert review of Molecular Diagnostics* 2022; **22(6):** 625-42.

7 Sharma JB, Jain S, Dharmendra S, Singh UB — An evaluation of Composite Reference Standard (CRS) for diagnosis of Female Genital Tuberculosis. *Indian J Tuberc* 2023; **70(1):** 70-6.

8 Naik SN, Chandanwalea A, Kadama D, Sambareya PW, *et al* — Detection of genital tuberculosis among women with infertility using best clinical practices in India: An implementation study. *Indian J Tuberc* 2021; **68(1):** 85-91.

9 Sharma JB, Sharma E, Sharma S, Dharmendra S — Female genital tuberculosis: Revisited. *Indian J Med Res* 2018; **148 (Suppl):** 71-83.

10 Bhanothu V, Theophilus JP, Rozati R — Use of Endo-Ovarian Tissue Biopsy and Pelvic Aspirated Fluid for the Diagnosis of Female Genital Tuberculosis by Conventional versus Molecular Methods. *PLoS One* 2014; **9(5):** 5.

11 Sharma JB, Kriplani A, Dharmendra S, Chaubey J, Kumar S, Sharma SK — Role of Gene Xpert in diagnosis of female genital tuberculosis: A preliminary report. *Eur J ObstetGynecolReprodBiol* 2016; **207:** 237e238.

12 Agrawal M, Roy P, Bhatia V, Dutt S, Gaur R — Role of microbiological tests in diagnosis of genital tuberculosis of women with infertility: A view. *Indian J Tuberc* 2019; **66:** 234e239.

13 Sharma J, Khurana A, Hari S, Singh U, Yadav R, Dharmendra S — Evaluation of role of transabdominal and transvaginal ultrasound in diagnosis of female genital tuberculosis. *Journal of Human Reproductive Sciences* 2021; **14(3):** 250-9.

14 Bhanu NV, Singh UB, Chakraborty M, Suresh N, Arora J, Rana T, *et al* — Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. *J Med Microbiol* 2005; **54:** 927-31.

15 Rana T, Singh UB, Kulshrestha V, Kaushik A, Porwal C,Agarwal N, *et al* — Utility of reverse transcriptase PCR andDNA PCR in the diagnosis of female genital tuberculosis. *J Med Microbiol* 2011; **60:** 486-91.