Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/24dbtg03

# ASSESSMENT OF THE DIAGNOSTIC ACCURACY OF ENDOMETRIAL BIOPSY AND PCR IN SUSPECTED CASES OF FEMALE GENITAL TUBERCULOSIS

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## ABSTRACT

**Background:**
Female genital tuberculosis (FGTB) is a significant but often underdiagnosed cause of infertility and chronic pelvic morbidity, particularly in developing countries. Conventional diagnostic methods, such as histopathology, often suffer from low sensitivity due to the paucibacillary nature of the disease. This study aimed to assess the diagnostic accuracy of endometrial biopsy (histopathology) and polymerase chain reaction (PCR) in the detection of FGTB, using a composite reference standard (CRS) as the benchmark.

**Methods:**
This cross-sectional study was conducted at Jinnah Post Graduate Medical Center Karachi for a period of six months from February, 2024 to July, 2024, and included 80 women presenting with symptoms suggestive of FGTB, such as infertility, abnormal uterine bleeding, chronic pelvic pain, or amenorrhea. Endometrial samples were collected during the secretory phase and processed for histopathological examination and PCR targeting the IS6110 sequence specific to *Mycobacterium tuberculosis*. The chi-square test and Fisher's exact test were applied, and $p \leq 0.05$ is considered significant.

**Results:**
Histopathology demonstrated a sensitivity of 11.5%, specificity of 92.9%, PPV of 75.0%, and diagnostic accuracy of 40.0%. In contrast, PCR showed significantly higher sensitivity (80.8%), specificity (78.6%), PPV (87.5%), NPV (68.8%), and diagnostic accuracy of 80.0%. A statistically significant association was found between PCR and CRS diagnosis ($p < 0.001$), while histopathology showed no significant association ($p = 0.531$).

**Conclusion:**
PCR markedly improves the diagnostic yield of FGTB compared to histopathology and should be incorporated into routine diagnostic algorithms, particularly in high-risk and symptomatic populations.

**Keywords:**
Tuberculosis, Female Genital Tuberculosis, Polymerase Chain Reaction, Endometrial Biopsy

## INTRODUCTION

Female genital tuberculosis (FGTB) is a significant but often underdiagnosed form of extrapulmonary tuberculosis that primarily affects women of reproductive age[1]. It poses a major public health challenge, particularly in developing countries like Pakistan, where the burden of tuberculosis remains high[2]. FGTB can involve the fallopian tubes, endometrium, ovaries, cervix, and even the vagina and vulva[3]. Among these, the endometrium is frequently affected, making it a valuable site for diagnostic investigation[4].

The clinical presentation of FGTB is often non-specific and insidious, with symptoms such as infertility, menstrual irregularities, chronic pelvic pain, or vaginal discharge[5]. Due to this subtle presentation, many cases go unrecognized or are misdiagnosed as other gynecological conditions[6]. Early and accurate diagnosis is essential, not only for initiating timely anti-tuberculous therapy but also for preserving reproductive function in affected women.

Traditional methods for diagnosing FGTB, such as histopathological examination of endometrial biopsy specimens, rely on the identification of granulomatous inflammation with or without caseation necrosis[7, 8]. However, histopathology may lack sensitivity, especially in early or paucibacillary cases. Polymerase chain reaction (PCR) and other molecular approaches have become attractive diagnostic tools over the past few decades because of their great sensitivity and speed[9]. Mycobacterium tuberculosis DNA can be directly detected from clinical samples using PCR, even in cases where the number of bacteria is minimal[10, 11]. However, despite the potential advantages of PCR, concerns about false positives, cross-contamination, and variability in diagnostic accuracy across different laboratories persist[12]. Thus, it is crucial to assess the diagnostic performance of PCR in comparison to conventional histopathology of endometrial biopsy in suspected FGTB cases.

Considering the diagnostic challenges posed by FGTB, especially in resource-limited settings, there is a pressing need to evaluate and compare the effectiveness of available diagnostic modalities. By assessing the diagnostic accuracy of endometrial biopsy and PCR, this study aims to identify the most reliable approach for diagnosing FGTB. An accurate and timely diagnosis can not only prevent long-term complications such as infertility but also reduce the burden of delayed treatment and misdiagnosis. The present study aimed to assess and compare the diagnostic accuracy of endometrial biopsy and polymerase chain reaction (PCR) in detecting female genital tuberculosis in clinically suspected cases.

## METHODOLOGY

The study was conducted in the Department of Obstetrics and Gynaecology, Jinnah Post Graduate Medical Center, Karachi in collaboration Departments 06 months from February, 2024 to July, 2024. The study population comprised women of reproductive age presenting with clinical suspicion of genital tuberculosis. These women were attending the outpatient gynecology clinic or admitted for infertility workup, chronic pelvic pain, menstrual irregularities, or unexplained infertility.

The inclusion criteria comprised women aged 18 to 35 years, those presenting with at least one symptom suggestive of FGTB (e.g., infertility, abnormal uterine bleeding, chronic pelvic pain, or amenorrhea), those with no known cause of symptoms identified after routine investigations, those willing to undergo endometrial biopsy and PCR testing and those who provided informed written consent. Patients currently on anti-tuberculous therapy, or with known cases of pulmonary or

extrapulmonary TB already under treatment, those with endometrial malignancy or uterine structural abnormalities, and those who refused to participate or were unable to provide consent.

The required sample size was calculated using the OpenEpi software, based on the diagnostic performance reported in the literature, assuming a sensitivity of approximately 60% for PCR and 10% for histopathology, with both tests displaying specificities close to 100%. At a 95% confidence level, 80% power, and allowing a 10% margin of error, the calculated minimum sample size was 80 participants.[13]

A non-probability purposive sampling was used, selecting patients who fulfilled the inclusion criteria and presented during the study period. Following informed consent, a systematic questionnaire was used to capture demographic information and a thorough clinical history. Age, parity, marital status, presenting problems, menstrual history, and pertinent medical/surgical history were all included in this. Patients had baseline tests, such as hemoglobin level, total leukocyte count, differential count, erythrocyte sedimentation rate (ESR), tuberculin skin test, chest X-ray, HIV I/II testing, and pelvic ultrasonography, after a thorough history and physical examination.

On days 21 to 23 of the menstrual cycle (secretory phase), an endometrial biopsy was obtained using a Pipelle device or curette under aseptic conditions. Two samples were collected from each patient. One sample was sent for histopathological examination, and the second sample was placed in a sterile container with phosphate-buffered saline and sent for PCR testing for *Mycobacterium tuberculosis* DNA. Laboratory processing for PCR was conducted in a certified molecular diagnostics facility. All samples were transported to the laboratory within 1 hour of collection under cold chain protocols.

The material underwent hematoxylin and eosin (H&E) staining after being fixed in 10% formalin. Epithelioid cells, Langhans giant cells, and granulomatous inflammation with or without caseation were thought to be indicative of FGTB. Using a homogenizer, the biopsy sample was thoroughly pulverized for microscopic analysis of acid-fast bacilli (AFB). The concentrated mixture was then collected for smear and stained with Ziehl-Neilsen stain. DNA extraction was performed using either a traditional phenol–chloroform method (with lysozyme and proteinase K digestion followed by phenol-chloroform cleanup) or a commercial silica-column kit such as the Qiagen QIAamp DNA Mini Kit, or a magnetic bead–based kit (e.g., PerkinElmer chemagic or similar), all proven effective for recovering *M. tuberculosis* DNA and removing inhibitors.

PCR amplification targeting the IS6110 insertion element was carried out using a real-time thermal cycler system, specifically the Applied Biosystems 7500 Real-Time PCR System (or equivalent ABI Prism platform). Each 25 µL reaction included primers and probe specific to IS6110, with 5 µL of purified DNA input. The thermal protocol comprised an initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute, enabling fluorescence detection in real time.

To ensure analytical reliability, each run included a positive control containing known *M. tuberculosis* DNA (e.g., H37Rv strain), a no-template negative control, and an extraction blank to rule out cross-contamination. Samples were considered PCR-positive upon detection of a characteristic amplification curve with a Ct value below 38, an absent signal in negative controls, and proper performance of all quality controls.

A composite reference standard (CRS) was used for final diagnosis. The CRS considered clinical features, histopathology, PCR results, response to anti-TB therapy, and radiological findings. A case was considered FGTB-positive if at least two of the following were positive: histopathology, PCR, clinical-radiological correlation, and therapeutic response. All participants were informed about the purpose and procedures of the study, and written informed consent was obtained before sample collection. Confidentiality and anonymity of patient data were strictly maintained throughout the research process.

Data were analyzed using SPSS version 26.0. Descriptive statistics (frequencies, percentages) were used for demographic and clinical variables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of histopathology and PCR were calculated using the CRS as the gold standard. Diagnostic performance was compared using 2x2 contingency

tables. The Chi-square test and for small expected frequencies, Fisher's exact test was applied to assess the statistical significance of associations between test results and CRS-confirmed diagnosis. A p-value ≤ 0.05 was considered statistically significant.

## RESULTS

Among the 80 participants, 47.5% were between 18 to 25 years of age, while 52.5% were between 26 to 35 years. The majority were married (93.8%), and 6.2% were unmarried. Regarding parity, 61.2% of the participants were nulliparous and 38.8% were multiparous. In terms of residence, 56.2% were from urban areas, while 43.8% were from rural settings. Hemoglobin levels were ≥11 g/dL in 62.5% of women, whereas 37.5% had hemoglobin levels below 11 g/dL. A normal total leukocyte count (4,000–11,000/mm³) was observed in 85.0% of the participants, with elevated counts seen in 15.0%. An elevated erythrocyte sedimentation rate (≥20 mm/hr) was present in 70.0% of cases, while 30.0% had ESR values below 20 mm/hr. The Mantoux test was positive in 45.0% of women and negative in 55.0%. Chest X-ray findings were normal in 87.5% of participants, whereas 12.5% showed features suggestive of tuberculosis. All participants tested negative for HIV I/II. On pelvic ultrasound, adnexal masses or abnormalities were detected in 31.2% of the cases, whereas 68.8% had normal findings. *(Table 1)*

**Table 1: Socio-Demographic and Baseline Clinical Characteristics of Participants (n = 80)**

| Characteristic | Category | n (%) |
|---|---|---|
| **Age Group (years)** | 18–25 | 38 (47.5%) |
| | 26–35 | 42 (52.5%) |
| **Marital Status** | Married | 75 (93.8%) |
| | Unmarried | 5 (6.2%) |
| **Parity** | Nulliparous | 49 (61.2%) |
| | Multiparous | 31 (38.8%) |
| **Residence** | Urban | 45 (56.2%) |
| | Rural | 35 (43.8%) |
| **Hemoglobin (g/dL)** | < 11 | 30 (37.5%) |
| | ≥ 11 | 50 (62.5%) |
| **Total Leukocyte Count (TLC)** | Normal (4,000–11,000/mm³) | 68 (85.0%) |
| | Raised (>11,000/mm³) | 12 (15.0%) |
| **Erythrocyte Sedimentation Rate (ESR)** | <20 mm/hr | 24 (30.0%) |
| | ≥20 mm/hr | 56 (70.0%) |
| **Mantoux Test** | Positive (≥10 mm) | 36 (45.0%) |
| | Negative (<10 mm) | 44 (55.0%) |
| **Chest X-ray** | Normal | 70 (87.5%) |
| | Suggestive of TB | 10 (12.5%) |
| **HIV I/II Status** | Negative | 80 (100.0%) |
| | Positive | 0 (0.0%) |
| **Pelvic Ultrasound Findings** | Normal | 55 (68.8%) |
| | Adnexal mass/abnormality | 25 (31.2%) |

Among the 80 study participants, the most common presenting complaint was primary infertility, reported in 47.5% of the cases. Secondary infertility was observed in 18.8% of women, followed by chronic pelvic pain in 15.0%. Abnormal uterine bleeding was noted in 12.5% of the participants, while amenorrhea was the least common presentation, seen in 6.2% of cases. *(Table 2)*

**Table 2: Frequency of the Clinical Presentations of Study Participants (n = 80)**

| Presenting Complaint | Frequency n (%) |
|---|---|
| **Primary Infertility** | 38 (47.5%) |
| **Secondary Infertility** | 15 (18.8%) |
| **Abnormal Uterine Bleeding** | 10 (12.5%) |
| **Chronic Pelvic Pain** | 12 (15.0%) |
| **Amenorrhea** | 5 (6.2%) |

Histopathological examination using hematoxylin and eosin (H&E) staining yielded a positive result for tuberculosis in 10.0% of the cases, whereas 90.0% showed no histological evidence of TB. In contrast, PCR targeting the IS6110 gene detected *Mycobacterium tuberculosis* DNA in 60.0% of participants, while 40.0% tested negative. *(Table 3; Figure 1)*

**Table 3: Frequency of Laboratory Findings of Histopathology and PCR Tests of the Study Participants (n = 80)**

| Diagnostic Tests | Laboratory Findings | |
|---|---|---|
| | **Positive n** (%) | **Negative n** (%) |
| **Histopathology (H&E)** | 8 (10%) | 72 (90.0%) |
| **PCR (IS6110 Target)** | 48 (60.0%) | 32 (40.0%) |

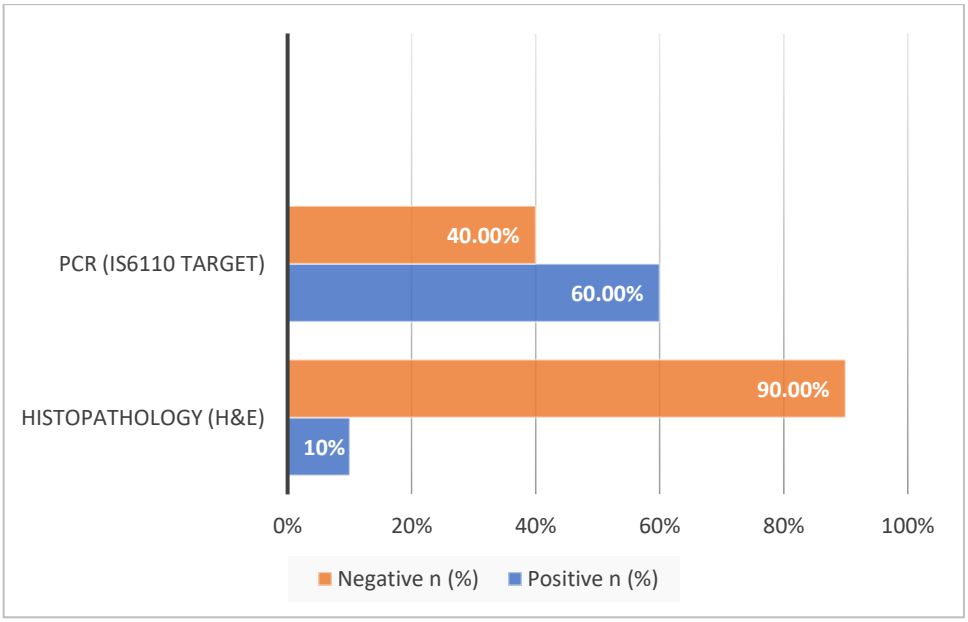


Figure 1: A Graph Showing the Frequency of Laboratory Findings of Histopathology and PCR Tests Based on the clinical and laparoscopic criteria used in this study, 65.0% of the participants were diagnosed as positive for female genital tuberculosis (FGTB), while 35.0% were classified as FGTB-negative. *(Figure 2)*

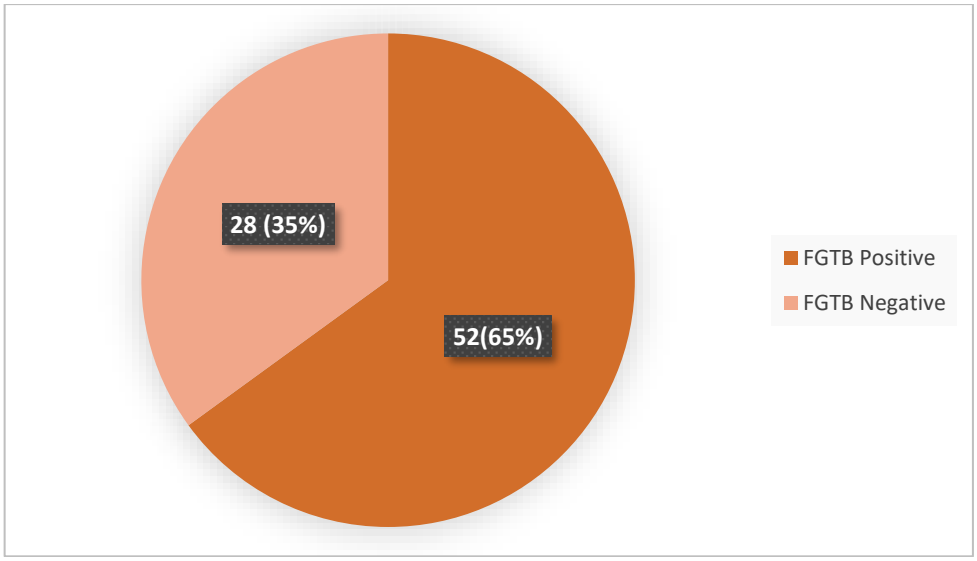


Figure 2: A pie chart showing the Frequency of the Composite Reference Standard (CRS) Outcome.

When evaluating the association between histopathology and the clinical diagnosis of female genital tuberculosis (FGTB), histopathology was positive in 11.5% of the CRS-positive cases and in 7.14% of the CRS-negative cases. A large proportion of CRS-positive participants (88.4%) and CRS-negative participants (92.8%) had negative histopathology findings. However, this association was not statistically significant with a p-value of 0.531, indicating limited diagnostic reliability of histopathology in this cohort. In contrast, PCR demonstrated a strong and statistically significant association with the CRS diagnosis. Among participants who were clinically diagnosed as FGTB-positive, 80.7% tested PCR-positive, while 21.4% of CRS-negative cases showed false positives. Additionally, 78.5% of CRS-negative cases were PCR-negative, and 19.2% of CRS-positive cases were missed by PCR. This association was found to be highly significant, with a p-value of 0.0001, confirming the superior diagnostic performance of PCR compared to histopathology in detecting FGTB. *(Table 4)*

**Table 4: Association of Histopathology and PCR with CRS Diagnosis of Study Participants (n = 80)**

| Test Result | CRS Positive (n = 52) | CRS Negative (n = 28) | p-value |
|---|---|---|---|
| **Histopathology** | | | 0.542 |
| Positive | 6 (11.5%) | 2 (7.14%) | |
| Negative | 46 (88.4%) | 26 (92.8%) | |
| **PCR** | | | 0.0001* |
| Positive | 42 (80.7%) | 6 (21.4%) | |
| Negative | 10 (19.2%) | 22 (78.5%) | |
| Note: *Fisher's Exact Test was applied for Histopathology; the Chi-square test was applied for PCR. A p-value less than 0.05 was considered statistically significant.* | | | |

The diagnostic performance of histopathology and PCR was assessed against the clinical diagnosis based on laparoscopy and associated criteria. Histopathology showed a low sensitivity of 11.5% but a high specificity of 92.9%, indicating that while it rarely missed false positives, it failed to identify most true cases of FGTB. The positive predictive value (PPV) of histopathology was 75.0%, while the negative predictive value (NPV) was 36.1%. The overall diagnostic accuracy of histopathology was 40.0%. On the other hand, PCR demonstrated significantly higher sensitivity (80.8%) and maintained a reasonable specificity of 78.6%. The PPV and NPV of PCR were 87.5% and 68.8%, respectively. The overall diagnostic accuracy of PCR was 80.0%, highlighting its superior performance in detecting FGTB in clinically suspected cases compared to histopathology. *(Figure 3)*

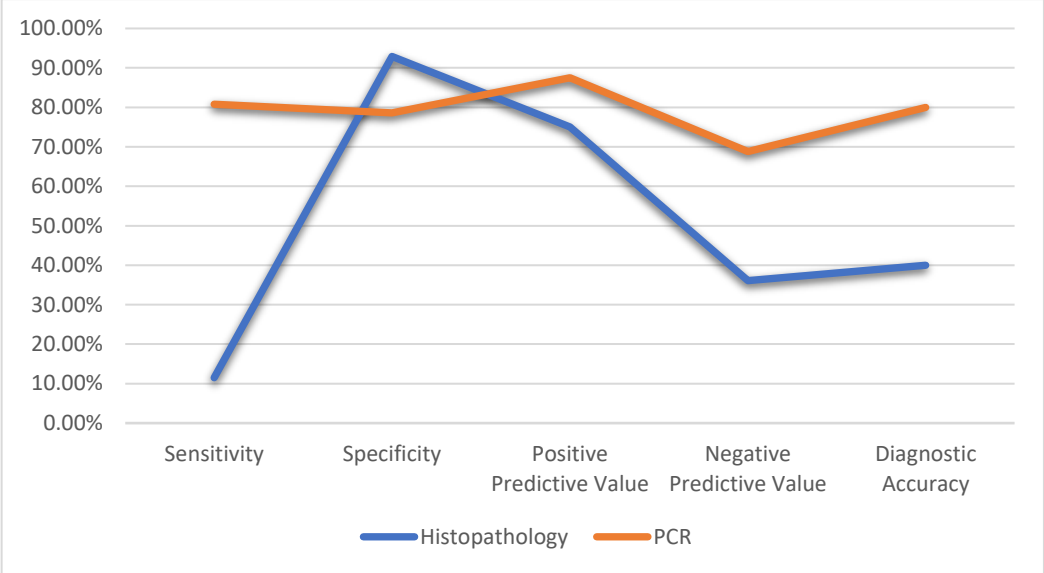


Figure 3: A Line Chart Demonstrating the Diagnostic Accuracy Parameters of Histopathology and PCR (Compared to CRS)

## DISCUSSION

Female genital tuberculosis (FGTB) remains a diagnostic challenge due to its asymptomatic nature and the paucibacillary nature of lesions, which limit the sensitivity of conventional diagnostic methods. In the present study, we assessed and compared the diagnostic accuracy of endometrial histopathology and polymerase chain reaction (PCR) in clinically suspected cases of FGTB, using laparoscopic and clinical criteria as the diagnostic reference.

In our study, the majority of participants were between 26 to 35 years of age (52.5%), which is consistent with the reproductive age group most commonly affected by genital TB. This age distribution aligns with findings from Kesharwani et al. (2022), Sandhyasri et al. (2024), and Sharma et al. (2021) who reported the highest incidence of FGTB among women aged 20–40 years[1, 3, 14].

The most common clinical presentation in our cohort was primary infertility (47.5%), followed by secondary infertility (18.8%) and chronic pelvic pain (15.0%). These findings are consistent with previous reports from Tjahyadi et al. (2022) and Vijay et al. (2023), who identified infertility as the leading clinical manifestation of FGTB[15, 16]. The high prevalence of menstrual irregularities, including amenorrhea and abnormal uterine bleeding, reflects the endometrial involvement seen in most cases.

Histopathology in our study demonstrated low sensitivity (11.5%) and high specificity (92.9%). These findings are similar to the results reported by Trosen et al. (2022), who observed that histopathology often lacks sensitivity due to the patchy and intermittent distribution of granulomatous inflammation[17]. Rammeh et al. (2022), McLaughlin et al. (2022) and Sinha et al. (2022) also reported comparable results, highlighting that histopathology, though specific, frequently fails to detect early or focal disease due to the paucibacillary nature of genital TB[18-20]. Our study confirms that while a positive histological finding is highly suggestive of TB, a negative result does not rule out the disease.

In contrast, PCR showed significantly superior diagnostic performance, with a sensitivity of 80.8%, specificity of 78.6%, positive predictive value (87.5%), and diagnostic accuracy of 80.0%. These findings are in agreement with previous literature. A study by Shallalet al. (2021) found PCR to have a high sensitivity and specificity in endometrial samples[21]. Similarly, Moureen et al. (2023) and Sethi et al.(2022) demonstrated high PCR sensitivity for IS6110 and TRC4 primers, indicating PCR's value as a rapid and sensitive test for detecting *Mycobacterium tuberculosis* DNA in endometrial tissue[22, 23].

Our results also revealed a strong statistical association between PCR and CRS diagnosis ($p = 0.0001$), reinforcing the clinical utility of PCR in suspected FGTB cases. Conversely, histopathology did not show a statistically significant association ($p = 0.531$) with the clinical diagnosis, further supporting

its limited standalone diagnostic value. These results corroborate the position of the WHO and various gynecological societies, which emphasize the need for combining molecular diagnostics with clinical and laparoscopic evaluation to improve diagnostic yield in genital TB[4, 24, 25]. While PCR is not without limitations, including the risk of contamination and detection of non-viable organisms, its advantages in sensitivity and turnaround time make it a valuable addition to the diagnostic algorithm.

One notable observation in our study is the moderate number of false negatives (19.2%) and false positives (21.4%) with PCR, which highlights the importance of interpreting PCR results in the context of clinical and laparoscopic findings. A single test should not be relied upon in isolation. Our study's limitations include a relatively small sample size and a lack of cultural confirmation due to resource constraints. Moreover, the use of a composite diagnostic standard, while practical, may introduce subjectivity, especially in interpreting clinical and laparoscopic findings.

## CONCLUSION

This study demonstrates that while endometrial histopathology remains highly specific, its low sensitivity limits its utility as a standalone diagnostic tool for female genital tuberculosis. In contrast, PCR offers significantly higher sensitivity and diagnostic accuracy, making it a valuable molecular tool in the early detection of FGTB, especially in paucibacillary cases. The strong association between PCR results and clinical-laparoscopic findings underscores its relevance in the diagnostic workup. Therefore, integrating PCR with clinical, radiological, and laparoscopic evaluation enhances the accuracy of FGTB diagnosis and can lead to timely and appropriate management, particularly in women presenting with infertility and other subtle symptoms.

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