# *DIAGNOSING GENITAL TUBERCULOSIS IN FEMALE INFERTILITY BY CLINICAL, HISTO-PATHOLOGICAL, CULTURE AND POLYMERASE CHAIN REACTION TECHNIQUES*

***THESIS***

*Submitted to the*
**Tamil Nadu Dr. M.G.R. Medical University**

*for the award of the degree of*

## DOCTOR OF PHILOSOPHY

*By*

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# CERTIFICATE BY THE SUPERVISOR

I, **Dr. N. Pandiyan** hereby certify that the thesis entitled, ***DIAGNOSING GENITAL TUBERCULOSIS IN FEMALE INFERTILITY BY CLINICAL, HISTO-PATHOLOGICAL, CULTURE AND POLYMERASE CHAIN*** *REACTION* ***TECHNIQUES*** by **Dr. T. Radha Bai Prabhu, Professor of Obstetrics and Gynecology, Institute of Obstetrics and Gynecology , Chennai, 600 008,** submitted for the award of Degree of Philosophy of The Tamil Nadu Dr. M.G.R. Medical University , is the result of her original work done under my supervision during the period from 2004 to 2008.This thesis fully represents an independent work conducted by the candidate and has not formed the basis for the award to the candidate of any other degree, diploma, associateship, fellowship or similar titles of this university or any other university on previous occasions. This work has been done at the Institute of Obstetrics and Gynaecology, Chennai.

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**January 2009**

**For the Award of the Degree of Philosophy of the Tamil Nadu Dr. M.G.R. Medical University**

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I, **Dr. T.Radha Bai Prabhu**, hereby declare that this thesis entitled, "***Diagnosing Genital Tuberculosis in Female Infertility by Clinical, Histo-Pathological, Culture and Polymerase Chain Reaction Techniques"*** is an original, independent work by me, and it was not used previously for the award of any other degree, diploma, associateship, fellowship or the similar titles of this university or any other university.

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***BY***

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## ABBREVIATIONS

| | | |
|---|---|---|
| AFB | - | Acid Fast Bacilli |
| AID | - | Acquired Immunodeficiency Virus |
| ATT | - | Anti Tuberculosis Treatment |
| BCG | - | Bacille Calmette Guerin |
| BMI | - | Body Mass Index |
| CDC | - | Centre for Disease Control |
| CT | - | Chlamydia Trachomatis |
| CT | - | Computerized Tomography |
| CTAB | - | Cetyl Trimethyl Ammonium Bromide |
| DAT | - | Direct Amplification Tests |
| DC | - | Differential Count |
| DNA | - | Deoxy Ribo Nucleic Acid |
| ESR | - | Erythrocyte Sedimentation Rate |
| FRC | - | Fertility Research Centre |
| GC | - | Gonococci |
| GPI | - | Guinea Pig Inoculation |
| GTB | - | Genital Tuberculosis |
| HD-TLC | - | High Performance thin Layer Chromatography |
| HIV | - | Human Immunodeficiency Virus |
| HPE | - | Histo - Pathological Examination |
| HPLC | - | High Performance Liquid Chromatography |
| H&E | - | Haematoxylin & Eosin |
| HRRC | - | Human Reproduction & Research Centre |
| HSG | - | Hysterosalpingogram |
| ICMR | - | Indian Council of Medical Research |
| IOG | - | Institute of Obstetrics & Gynaecology |
| IVF | - | In - Vitro Fertilization |
| IVF-ET | - | In - Vitro Fertilization & Embryo Transfer |
| K | - | Kirchner’s Liquid Medium |

| | | |
|---|---|---|
| LJ | - | Lowenstein Jensen Medium |
| LJP | - | L-J Enriched with Sodium Pyruvate |
| MOTT | - | Mycobacteria other than Tubercle bacilli |
| MRI | - | Magnetic Resonance Imaging |
| MTB | - | Mycobacterium Tuberculosis |
| MTBC | - | Mycobacterium Complex |
| Mx | - | Mantoux test |
| NAA | - | Nucleic Acid Amplification Tests |
| NPV | - | Negative Predictive Value |
| NTM | - | Non Tuberculous Mycobacteria |
| OPD | - | Out Patient Department |
| PCR | - | Polymerase Chain Reaction |
| PID | - | Pelvic Inflammatory Disease |
| PNB | - | P - Nitro Benzoic Acid |
| POD | - | Pouch of Douglas |
| PPD - S | - | Purified Protein Derivative |
| PPV | - | Positive Predictive Value |
| r RNA | - | Ribosomal RNA |
| RNA | - | Ribo Nucleic Acid |
| RNTCP | - | Revised National Tuberculosis Control Programme |
| SDS | - | Sodium Dodecyl Sulfate |
| SK | - | Selective Kirchner’s Medium |
| TB | - | Tuberculosis |
| TC | - | Total Leucocyte Count |
| TE buffer | - | TRIS - EDTA buffer |
| TNF | - | Tumour Necrosis Factor |
| T-O Mass | - | Tubo Ovarian Mass |
| TRC | - | Tuberculosis Research Centre |
| USG | - | Ultra sonogram |
| WHO | - | World Health Organization |
| ZN | - | Ziehl Neelsen |

# CHAPTER I

# INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases as old as human civilization [1]. It is a chronic infectious disease and the morbidity associated with this condition has major health implications. The disease has a worldwide distribution and the incidence is high in developing countries. The global burden of tuberculosis remains enormous, mainly because of poor control in Southeast Asia, sub-Saharan Africa, and Eastern Europe.

Since 1980, the disease has been on the rise with its spread concentrated in South – East Asia and Sub-Saharan Africa. Much of TB's resurgence is directly connected to the HIV/ AIDS pandemic and emergence of multi – drug resistant tuberculosis [2]. In the early 1990s, approximately 3.8 million new cases of TB were reported annually to the World Health Organization (WHO) [3].

Paleopathologic findings provide presumptive evidence for the extreme antiquity of mycobacterial infections in humans [4, 5]. Tuberculosis was recognized as a clinical entity as far back as 1000 BC, when Ruffer noted Potts' disease associated with a psoas abscess in the remains of an Egyptian priest of Ammon of the twenty – first dynasty [6].

The origin of tuberculosis in humans is unknown; however Morse et al suggest that the domestication of animals that began in the Neolithic period promoted transmission of a "mutant variety" of the tubercle bacillus from livestock to humans [7].

More than a decade ago, tuberculosis was identified as a global health emergency. In 2005, WHO estimated that more than 14 million people were living with TB, and 8.8 million new TB cases were estimated worldwide. Of these, 7.4

million were estimated in Asia and sub-Saharan Africa. A total of 1.6 million people died of TB, 12% of whom were co-infected with HIV. The sequence of annual estimates suggests that all three major indicators – incidence, prevalence and mortality rates are now falling globally [8]. Cases in India alone accounted for almost a third of the worldwide burden of tuberculosis [9].The estimated burden of TB in India for the year 2000 was found to be 8.5 million (Estimated number of bacillary cases was 3.8 million (95% CI: 2.8 - 4.7), abacillary cases 3.9 million and extra-pulmonary cases 0.8 million giving a total burden of 8.5 million (95 % CI: 6.3 – 10.4) [10].

While the predilection for tuberculosis is pulmonary disease, tuberculous infection occurs with increasing frequency in extra-pulmonary locations.

**Extrapulmonary Tuberculosis:**

The WHO has defined extrapulmonary tuberculosis as tuberculosis of organs other than the lungs: pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones and meninges. The diagnosis should be based on one culture positive specimen or histological or strong clinical evidence consistent with active extra pulmonary disease, followed by a decision by a clinician to treat with a full course of anti tuberculosis chemotherapy [8].

After the initial haematogenous dissemination from pulmonary tuberculosis, the infection progresses to clinically silent granulomas. These granulomas may contain stable viable tubercle bacilli for years which may reactivate when host immune system changes occur.

In 1744, the first reported case of female genital tuberculosis was described by Morgagni – an anatomist. On a post mortem examination of a 20 year old woman, he found the uterus and the fallopian tubes filled with caseous material [1]. By

Polymerase Chain Reaction (PCR) analysis Mycobacterial DNA has been detected in the genital areas of Andean Mummies date from A.D. 140-1200 [11].

The manifestations of tuberculosis in Gynaecology were published as a monograph by Hagar in 1886 [12].

Studies have shown that genital infection is seen in 12-24% of cases with pulmonary tuberculosis [13, 14, 15].

When tuberculosis affects genital organs of young females, the disease often remains silent or may present with non- specific symptomatology. As a result, the disease is either not diagnosed at all or diagnosed at an advanced stage when irreversible damage to the fallopian tube has already occurred resulting in infertility. The prognosis for fertility is poor with both medical and surgical methods of treatment when the tubes are damaged beyond recovery [16]. With severe disease, the affected women become absolutely infertile [17].

The genital tract tuberculosis is one of the most common causes of tubal factor infertility. The incidence varies greatly from country to country, being highest in India and South African countries compared to Western countries. The prevalence of genital tuberculosis (GTB) is largely underestimated as the disease is either silent or presents with varied symptomatology.

In developed countries such as USA, Australia and Western European countries, the tuberculosis of the upper genital tract is a rare disease and the incidence is less than 1% [18]. However in other parts of the world, it is a frequent cause of chronic pelvic inflammatory disease (PID) and infertility [19]. The incidence in some African countries is as high as 21% [20]. Various Indian studies have shown that tuberculous endometritis and salpingitis account for 4-9% of all infertility cases [21, 22, 23].

In developing countries like India, genital tuberculosis is the major causative factor for severe tubal disease requiring assisted reproduction techniques [24].

**STATEMENT OF THE PROBLEM**

Unlike pulmonary tuberculosis, the clinical diagnosis of genital tuberculosis is difficult due to the asymptomatic nature and varied clinical presentation of the condition. Most cases of genital tuberculosis are asymptomatic and others have only non- specific complaints such as infertility, pelvic pain, lower abdominal pain and menstrual abnormalities.

A high degree of suspicion aided by intensive investigations is important in the diagnosis of the disease. Routine laboratory values are of little value. A positive chest X-ray for healed or active pulmonary tuberculosis, contact history, elevated ESR and positive tuberculin test may indicate the need for further investigations. Although characteristic X-ray features have been described, an absolute diagnosis of genital tuberculosis cannot be made from hysterosalpingogram (HSG) [25].

The macroscopic appearance of the tuberculous tube is not distinctive and more often the gross picture of the tuberculous salpingitis may be similar to that of chronic salpingitis of non-tuberculous conditions. Therefore, an absolute diagnosis cannot be made from characteristic features in laparoscopy. However, laparoscopy is a valuable procedure for obtaining tissue for culture and histo-pathological examination from inaccessible sites.

Definitive and rapid diagnosis of extra pulmonary tuberculosis is challenging since conventional techniques have limitations [26].

**NEED FOR THE STUDY**

A definite diagnosis can be made by positive mycobacterial culture and by demonstrating specific histo- pathological lesions in the diagnostic specimen. Due to secondary nature of genital tuberculosis; the infecting organisms are sparse in

number. Therefore, mycobacterial culture and HPE have limitations and low detection rates and the involved sites can be easily missed. In recent years, PCR technique has evolved as a useful and rapid technique for the diagnosis of pulmonary and extra-pulmonary tuberculosis. Any method that is used to diagnose genital tuberculosis should be highly sensitive to diagnose the disease reliably in its early stage, so that treatment may improve the prospects of cure before the tubes are damaged beyond recovery.

Pregnancy is possible when female genital tuberculosis is detected at an early stage and when no irreversible anatomical pathology is evident. Thus, in cases of infertility, it is important to determine the existence of tuberculosis early, and the therapy should be initiated immediately [27].

# CHAPTER II

## REVIEW OF LITERATURE

Review of literature was done with Google, Pub med and Medline search engines, using key words genital tuberculosis and / or infertility. Manual search for references from original articles was also carried out. As number of important studies was done as early as 1950, all studies linking GTB with infertility from 1950 to June 2008 were included in this review of literature.

Infertility is defined as one year of unprotected intercourse without conception. Approximately 85-90% of healthy young couples will conceive within one year. Infertility therefore affects approximately 10-15% of couples and is an important part of clinical practice for many gynaecologists. The major causes of infertility include ovulatory dysfunction (15%); tubal and peritoneal pathology (30-40 %), and male factors (30-40%); uterine pathology is uncommon and unexplained infertility accounts for 10% of cases [28].

Tubal and peritoneal pathology are among the most common causes of infertility and the primary diagnosis in approximately 30-40% of infertile couples. A history of pelvic inflammatory disease (PID), septic miscarriage, ruptured appendix, tubal surgery or ectopic pregnancy suggests the possibility of tubal damage. The important causative organisms of PID and tubal infertility include Neisseria gonorrhoea, Chlamydia trachomatis and Mycobacterium tuberculosis [29, 30].

Neisseria gonorrhoea is a gram negative diplococci with rapid growth resulting in rapid and intense inflammatory response causing acute PID. The organism is sexually transmitted [19]. Chlamydia Trachomatis is also a sexually transmitted pathogen, slow growing intra cellular organism; by itself produces a mild form of salpingitis with an insidious onset. The infection may remain clinically silent or produce minimal symptoms which result in delayed diagnosis [31]. Classic studies in

women with PID diagnosed by laparoscopy have revealed that the risk of subsequent tubal infertility increases with the number and severity of pelvic infections; overall, the incidence is approximately 10-12% after one episode, 23-35% after two, and 54-75% after three episodes of acute PID [32, 33, 34].

Mycobacterial tuberculosis (*MTB*) causes chronic pelvic infection with tubal involvement resulting in infertility. Genital tuberculosis is responsible for a significant proportion of females presenting with infertility [35]. Genital tuberculosis as a cause of infertility is 10-15 times more common in developing countries [36].

Pelvic tuberculosis is commonly a reflection of systemic disease and female genital tract tuberculosis is nearly always secondary to tuberculosis focus elsewhere in the body. The clinical features develop 10-15 years after primary infection. The disease may be active, quiescent and sometimes the pulmonary focus heals with no residua and the secondary focus may remain dormant in the tube for years to flourish at a later date [37].

In a postmortem study of patients dying of pulmonary tuberculosis, 4-5% were found to have an unsuspected tubal infection [38]. Studies have looked at the genital tract involvement in women with active pulmonary tuberculosis. In one study, 57 women hospitalized for pulmonary tuberculosis were evaluated for genital involvement using menstrual blood culture, curettage and HSG. In this study 12.3% were diagnosed as genital tuberculosis [39].

In yet another study, 200 women with pulmonary tuberculosis were evaluated by histopathology studies for genital tuberculosis and this study showed 13% of genital involvement. The occurrence of genital tuberculosis was more frequent in women who had pulmonary tuberculosis of shorter duration; but genital involvement was not related to the severity of the pulmonary disease. The study concluded that pulmonary tuberculosis indirectly affects the female genital tract in almost all cases giving rise to various gynaecologic symptoms and signs [14].

In most cases genital tuberculosis occurs in the absence of an active pulmonary or other extragenital manifestation of the disease. Chest X- rays usually have no findings, or show evidence of only an old pulmonary complex. The most probable explanation for this is that a clinically undetected lung disease has undergone spontaneous resolution after there has been some type of bacteraemia to which the tubes alone are singularly responsive [40]. The majority of women with pelvic tuberculosis have not had a previous diagnosis of pulmonary tuberculosis. When there is history of pulmonary tuberculosis, the diagnosis has been made years earlier [41].

In a study conducted on 475 confirmed cases of extra genital tuberculosis, in 25.55% of women, a diagnosis of either primary or secondary infertility was made. The study suggested that in subjects presenting with extra-genital tuberculosis and infertility, investigations should be initiated to evaluate for genital tract involvement [42].

When tuberculosis affects the genital organs, infertility results as a result of

- Involvement of the fallopian tubes with tubal obstruction and dysfunction
- By affecting the endometrial receptivity
- By causing ovulatory failure from Ovarian involvement

The mechanism responsible for tubal factor infertility involves anatomic abnormalities that prevent the union of sperm and ovum. Proximal tubal obstructions prevent sperm from reaching the distal fallopian tube where fertilization normally occurs. Distal tubal occlusion prevents ovum capture from the adjacent ovary. While proximal tubal obstruction is essentially an all or none phenomenon, distal tubal occlusive disease exhibits a spectrum ranging from mild ( fimbrial agglutination ) to moderate ( varying degrees of fimbrial phimosis ) to severe ( complete obstruction ) tubal involvement [28].

When tuberculosis affects the endometrium, there is atrophy of the basal layer of the endometrium affecting the endometrial receptiveness. When the basal layer of the endometrium is affected, the patient presents with scanty periods or amenorrhoea not responding to hormone therapy. In advanced disease, the endometrium is extensively damaged resulting in intra-uterine adhesions or extensive fibrosis. Total corporeal synechiae due to tuberculosis carries a very poor prognosis [43].

**Pathogenesis of Tuberculosis**

Mycobacterium tuberculosis, Mycobacterium bovis and Mycobacterium africanum are the human pathogens that are included in the Mycobacterium tuberculosis complex (MTBC). These organisms, the “tubercle bacilli” are the etiologic agents of human tuberculosis.

Mycobacteria other than the MTBC have been called “atypical” because they differ from the tubercle bacilli. The term non tuberculous mycobacteria (NTM) and mycobacteria other than tubercle bacilli (MOTT) are preferred because these organisms are not atypical but simply have characteristics distinct from those of Mycobacterium tuberculosis [44].

Mycobacterium tuberculosis (*MTB*) is the commonest pathogen responsible for human infection. Infection caused by Mycobacterium Bovis is seen occasionally in tuberculosis of peritoneum, bowel and lymph nodes. Mycobacterium Africanum is a very rare pathogen.

Mycobacterium tuberculosis is a species of the genus Mycobacterium, family Mycobacteriaceae, belongs to the order Actinomycetales [45]. The organisms are non-motile, non-sporulating, aerobic, acid fast rods. The high lipid content in their cell walls confers upon them the distinctive property of acid fastness, their growth is slow, and special medium containing substances such as egg, potato, blood and albumin are necessary for enrichment and to support the growth [46].

The lung is most often the site of the primary lesion and Mycobacterium tuberculosis is transmitted primarily by inhalation of dried residues of small infected droplets. (1to10 μm in diameter) containing 2-3 viable bacilli. *MTB* also may be transmitted by direct inoculation of abraded skin, an event most likely to occur in laboratory personal handling infected tissues. Despite the high susceptibility to infection with MTB, most infected individuals do not develop active disease.

The usual host response to infection with *MTB* is activation of the cell mediated immune system. The genome of *MTB* has been sequenced [45A].

**Pathogenesis of Primary Infection [46]**

During the primary infection, bacilli are inhaled, and droplets containing 1-3 bacilli reach the alveolar spaces. There upon, four stages of infection have been described.

**STAGE 1**

- Scavenging resident non activated macrophages ingest the tubercle bacilli and transport them to regional tracheo-bronchial lymph nodes (hilar or mediastinal, or enter the lymphatics and blood and travel back to lungs or distal organs (Lymph nodes, kidney, bones, meninges)
- Depending upon the virulence of the organism and the microbicidal activity of macrophages, the bacilli either multiply or are destroyed.
- Infected macrophages release chemotactic factors such as complement $C5_a$ that attract additional macrophages and circulating monocytes.

**STAGE 2: Symbiotic Stage (Day 7-21)**

- Infected macrophages are still not activated, so bacilli which escape the initial intracellular destruction multiply within the macrophages. With multiplication of bacilli, there is destruction of macrophages. As a result, monocytes and other inflammatory cells are attracted to the site. During this symbiotic stage,

monocytes differentiate into macrophages, there is accumulation of macrophages in large numbers, mycobacteria continues to grow logarithmically, but there is little tissue destruction.

**STAGE 3: (After 3 weeks)**

- This stage is characterized by the onset of cell-mediated immunity and delayed hypersensitivity.
- In this stage T-cell immunity develops, antigen specific T lymphocytes arrive, proliferate in early lesions and activate macrophages.
- Now the lymphokine activated alveolar macrophages destroy the intracellular bacilli.
- At this stage, the logarithmic increase in the number of organisms decreases. For an unknown reason, macrophage death also increases.
- The resulting pathological lesion in this primary infection is a tubercle. Tubercle is a granuloma, characterized by collection of epithelioid cells which are modified plump macrophages. These epithelioid cells are large in size, acquire an acidophilic cytoplasm, the nucleus becomes pale with lose chromatin. The macrophage some times divides atypically; coalesce to form multinucleated giant cells of the Langhans type.

With these cellular reactions, capillaries, lymphocytes, fibroblasts and collagenous tissue surround the entire area. The Cytokines (Interleukin -1, interferon - δ and Tumour Necrosis Factor - TNF) and lytic enzymes released by the macrophages contribute to the local tissue destruction. Due to the tissue destruction, granulomas often have central caseation. Caseum is amorphous debris with dead bacilli, is soft with acidic environment and low $O_2$ tension. These granulomas later calcify.

Histologically, a tuberculous granuloma is characterized by the caseous necrotic core, surrounded by a layer of epithelioid macrophages, which are in turn

surrounded by concentric layers of lymphocytes and fibrosis and multi-nucleated Langhan's giant cells.

The extent and the nature of granulomatous reaction vary with the host response to eliminate intracellular bacilli. In immune compromised individuals, the granulomatous reaction is weak and the granuloma present with extensive caseous necrosis and large number of mycobacteria. In individuals with uncompromised host response, the granulomas are non-caseating and contain few organisms. When the mycobacterial growth continues, the lesions grow and reach macroscopic sizes forming tubercles. Presence of macroscopic tubercles is typical of advanced disease. If tubercles occur in large numbers, the morphological picture resembles tumour formation and is called productive tuberculosis.

The presence of caseous tubercle indicates that

- There are large numbers of free viable bacilli within the granuloma and widespread dissemination is possible.
- There has already been considerable damage to the organs which is irreversible.

In approximately 90% of the infected individuals, once the cellular immune system is activated, the infection becomes quiescent and the symptoms resolve. The Caseum remains solid and gets localized. Subsequently there is resorption of the caseum and the lesion heals by fibrosis / calcification and even ossification can occur.

**STAGE 4: Stage of secondary infection (Reactivation)**

In very young or immuno compromised hosts who are incapable of mounting the necessary immune response, the primary infection may disseminate, producing miliary disease or progress and spread to the bronchi. In these cases with larger areas of caseation and polymorpho nuclear invasion, the caseum softens and liquefy and there is intense multiplication of bacilli. The liquefied caseum empties into the

bronchus and spreads to other parts of the lung. A miliary disease may occur by lymphatics and blood stream to distant sites.

Despite containment of mycobacterial multiplication in primary and metastatic foci by the activated macrophages, a residual nidus of infection remains indefinitely in the lungs and less often at distant sites. During periods of immuno suppression, there is potential for reactivation of quiescent foci in localized extra-peritoneal sites such as bones, fallopian tubes, meninges etc. This reactivation can occur at any time after the primary infection.

The disease can progress and disseminate to pulmonary and extra-pulmonary sites by various mechanisms.

**1. Direct Extension**

If bacterial multiplication and dissection through the tissue is rapid, the exudative phase progresses rapidly by direct extension.

**2. Ductal and Intracanalicular dissection:**

With liquefication of caseum, sloughing causes spread into broncho pulmonary segments and spread may occur to larynx, mouth and pharynx.

**3. Lymphatic Spread:**

As there is more number of lymphatic channels in the lung, lymphatic spread is more common. Through the lymphatics, the bacilli enter the blood stream easily and spread to distant sites.

**4. Haematogenous Spread:**

Haematogenous dissemination results from rupture of liquefied caseous material into the pulmonary vein, often from tubercles in the walls of the vein. Other sources are caseous mediastinal lymph nodes.

**5. Dissemination in serous Cavities:**

In the pleural, peritoneal and peri cardial cavities, tubercle bacilli may be seeded from the liquefying caseous foci on the surface of an organ.

**Pelvic Tuberculosis**

Pelvic tuberculosis is commonly a reflection of systemic disease and the female genital tuberculosis is nearly always secondary to tuberculous focus elsewhere in the body. The clinical features develop 10-15 years after the primary infection.

The tubercle (TB) bacilli reach the genital tract by one of several routes. In 90% of cases, the female genital tract is usually infected by haematogenous miliary spread from primary pulmonary lesion or from a secondary miliary sites, such as lymph nodes, urinary tract, bone and joints. Direct extension from adjacent abdominal organs such as small intestine, appendix, rectum and bladder can also occur. Lymphatic spread from a primary pulmonary site, from intestinal lymph nodes and then to pelvis is another route of infection.

Primary infection of the female genital tract is extremely rare unless the husband has active genito-urinary tuberculosis [47]. The squamous epithelium of the cervix is not penetrated by the TB bacilli; therefore the cervix is immune to tuberculosis. Bobhate et al reported that the tuberculosis of cervix was mostly observed in endocervical region, seen in 84.6% of cases [48]. When tuberculosis of the cervix is diagnosed, it is almost certain that TB spreads to the cervix from the Endometrium [49]. However, a venereal transmission of the disease to the lower genital tract has been reported with a primary genital infection in the woman occurring after coitus with sexual partners who had TB of the genito – urinary tract. When TB of the Vulva, Vagina and Cervix is present without evidence of tuberculosis elsewhere in the body, venereal transmission should be suspected [50].

The fallopian tubes are believed to be the initial and most frequently affected genital organ in pelvic mycobacterial infection [51]. The cause of the predilection of *MTB* for the fallopian tube is unknown. It has been reported that both fallopian tubes are involved in almost all the patients. From the fallopian tubes secondary spread can occur to the peritoneum in 45% or to the ovaries in10 to 30% of cases. The

Endometrium is involved in 50 to 80% cases. Cervix is involved in 5 to 10% of cases, vulva and vagina in <0.5 to 1% of cases [52].

The infection spreads from the fallopian tube to the adjacent structures involving the surface of the ovary and peritoneum. Ovaries can also be infected by direct spread of infection from the neighbouring structures such as bowel. Tubal disease also spreads to the Endometrium by discharging tubercle bacilli through the osier into the uterine cavity. Rarely, the ovary, cervix and endometrium can be infected primarily from the blood stream [53]. Few reports have found endometrium to be the most commonly involved site [54, 55].

**Tuberculous Salpingitis:**

When the tubercle bacilli reach the tube, infection begins in the mucosa and then spreads through the tubal wall to the peritoneal surface. In a small number of cases secondary to an abdominal lesion the tubes, ovaries and the uterine serosa are involved initially and then spread towards the mucosa takes place [56].

The macroscopic appearance of the tuberculous tube is not distinctive and more often the gross picture of the tuberculous salpingitis may be similar to that of chronic salpingitis of non-tuberculous pathology. The tubal pathology present as a chronic interstitial salpingitis, a pyosalpinx, a follicular hydrosalpinx or a salpingitis isthmic nodosa. When tuberculous pyosalpinx exists; a cross section of the tube may reveal a cheesy necrotic exudates and caseation of the tubal wall.

There is one characteristic which may point to the possibility of tuberculosis. In chronic gonococcal salpingitis, the fimbrial end of the tube is closed and bulbous with inversion and obliteration of the fimbriae. In the tuberculous tube, there is a tendency for the tube to remain everted, with patency of the orifice even when the tube is enlarged and distended, thus producing the characteristic “tobacco pouch” appearance. When this finding is noted, the probability of tuberculosis should be

suspected. In these cases, as the tubal orifice remain open; there is periodic expulsion of exudates into the peritoneal cavity resulting in frequent exacerbation of symptoms.

Although, the tube may be anatomically patent, in such cases, the mucosal folds have flattened with loss of muco-ciliary apparatus, the muscularis is replaced by fibrous tissue and the tube becomes rigid, narrow and non-functional. This collaborates with the lead –pipe appearance of the tube seen on Hysterosalpingogram. All of these tubal changes cause a spatial anatomical and functional alteration of the tube leading to tubal factor infertility and predispose to ectopic gestation [56].

Microscopically, in advanced cases, the presence of typical tubercles and extensive granulation tissue in the tubal folds with caseation will establish the diagnosis. The tubercles and the chronic inflammatory infiltration involve the mucosa, muscularis as well as the serosa.

However, in most cases, the disease is occult and occasional tubercle may be found in the mucous membrane and the appearance is not different from chronic gonorrhoeal salpingitis. In these early cases, numerous blocks of the tube with study of many sections may be necessary before one finds evidence of tuberculosis. At laparoscopy, one may not find any evidence of infection or inflammation in these cases [38].

When the ovaries are involved, the infection is usually limited to peri-oophoritis due to spread from adjacent peritoneum or fallopian tube. The ovaries are adherent to the ovarian fossa or to the terminal segment of the tube. Occasionally due to haematogenous spread or breaks in the tunica albuginea at ovulation, tubercles can be formed within the ovarian parenchyma. Rarely, the ovaries may harbor a cold abscess. Ovarian involvement can lead to ovulatory failure and infertility [57].

Tuberculous peritonitis is commonly associated with tuberculosis of the pelvis. There are two types of tuberculous peritonitis. In the ‘wet” peritonitis there is out-pouring of straw coloured fluid into the peritoneal cavity, producing ascites. The

peritoneum of the parietal wall and viscera are covered with many small white tubercles. At his stage, the tubes are slightly enlarged and distended and the serosal surface is covered with tubercles and the mucosa of the fallopian tube may not be involved. In the dry or adhesive type of tuberculous peritonitis, there is dense adhesion between the bowel loops; pelvic organs show evidence of advanced disease with salpingitis, pyosalpinx and tubo-ovarian abscess formation [19]. The pelvic adhesions of tuberculous peritonitis distort the anatomical relationship between the ovary and the tube, thereby interfering with ovum pick up.

**Tuberculous Endometritis**

Endometrial tuberculosis is now a relatively infrequent finding in the Western world, but is still a common problem in the developing world, where it occurs in 50-60% of women with genital tuberculosis [58]. Infection occurs by direct transluminal spread from the fallopian tube to the superficial zone of the functionalis. The infected endometrium is shed monthly, allowing only 22-23 days for the establishment of infection. Therefore, it is uncommon to see well established tuberculoid granuloma which typically takes a minimum of 15 days to form [59]. Therefore, caseation is rarely observed except after the menopause or in the unusual event that the severity of the systemic disease impairs ovulation. Nogales – Ortiz et al (1979) have shown that basalis was affected in 40% of cases. Infection may therefore take place from the basalis or by direct haematogenous spread [60].

Advanced endometrial tuberculosis presents with gross infection of the uterine wall, the endometrium is severely ulcerated and the patient presents with caseous and purulent discharge and sometimes with bleeding. The glandular structure of the endometrium is completely destroyed and the curetting reveal typical caseating epitheloid and giant cell granulomas.

However, most commonly early cases of tubercular endometritis are seen where one may find only an occasional tubercle or clusters of tubercles with the

characteristic epitheloid and giant cells. In these cases, the glandular structure may be almost intact and one may find marked chronic inflammatory infiltration [61]. In regularly menstruating women, the lesions are small, may not contain giant cells and rarely show caseation [62]. Therefore, the extent of the inflammatory involvement in TB Endometritis varies from a focal process with very few granulomas to a diffuse process of ulceration of the mucosa and extensive caseous necrosis [63].

Granulomas in different stages of development frequently co-exist suggesting that complete shedding of functionalis does not always occur; and some granulomas being retained until the next cycle. These mature granulomas may be more typical of tuberculosis. Biopsies taken in the immediate premenstrual phase have greatest chance of providing a positive diagnosis since the granulomas have had the largest possible time to develop. Biopsies taken earlier in the cycle may reveal only a non – specific picture of intra stromal plasma cells and lymphocytes or intraglandular polymorphonuclear leucocytes [62].

In some cases, tubercles are not seen and the focal or diffuse infiltration is made up of lymphocytes, plasma cells and a few eosinophils, but the presence of dilated glands with or without intraluminal exudates or glands with micro abscesses should alert the pathologist to the possibility of tuberculous Endometritis [62, 64, 65].

The histological reporting of endometrial tuberculosis is categorized as follows: (Lucas et al 1987) [66]

A) Tuberculous Endometritis

i) Epithelioid and giant cells granuloma with or without caseation.

ii) Non reactive granuloma characterized by foci of necrosis but with poor cellular reaction consisting of scant epitheloid cells and lymphocytes but not giant cells.

B) Doubtful cases of tuberculous endometritis where lesions are composed mainly of lymphocytes and polymorphs with occasional macrophages and minimal epithelial necrosis [66].

Acid – fast / alcohol fast bacilli are seldom found in Ziehl – Neelsen (Z-N) stained section of the infected Endometrium [60]. The exception to this is non-reactive tuberculosis in immuno suppressed patients where abundant bacilli are seen in necrotic tissue that has a poor cellular reaction and no giant cells [66].

The presence of granuloma in the endometrium is usually associated with little or no general disturbance in the maturation and growth of the tissue unless there is disturbance of ovarian function directly attributable to the systemic disease. However, there may be poor secretory pattern [62, 67]. Therefore, during the reproductive years, the endometrial cavity is protected from advanced tuberculous infection by cyclical shedding of the endometrial tissue. Therefore, even in advanced pelvic tuberculous infection, evidence of caseation, fibrosis and calcification are rarely seen in the uterine cavity. Rarely, there is complete destruction of the endometrium including the basal layer, resulting in complete obliteration of the cavity with Asherman's syndrome and secondary amenorrhoea or resulting in the formation of caseous pyometra.

**Prevalence of Genital Tuberculosis**

The actual incidence of genital tuberculosis in the general population cannot be determined accurately, because in a large number of patients, the disease is symptomless and discovered incidentally or may remain undiscovered [58]. At least 11% of patients are asymptomatic and the disease is discovered incidentally [68].

The reported frequency of GTB has been based on postmortem examination, surgical specimens and endometrial biopsies from sterility studies. The incidence also varies greatly according to the socio economic and public health conditions; therefore, there is a wide variation in figures published from various countries. In USA,

Australia and West European countries the incidence of genital tuberculosis is less than 1 %, but the incidence in some African countries and India is 15 – 19 % [69, 70]. Moreover, in less developed areas of the world, there is inadequate availability of diagnostic procedures for diagnosing genital tuberculosis [27]. The incidence is also influenced by the lack of highly sensitive and specific tests to diagnose the condition [71].

Schaefer (1976) estimated that 5-10% of infertile females the world over have genital tuberculosis [18]. Pelvic tuberculosis is an uncommon gynaecological problem in countries like Malaysia and Thailand and is seen in 0.03% to 0.05% of gynaecological cases [55, 72]. Studies from African countries have shown a high incidence of genital tuberculosis in infertile women. In a reproductive biology unit at Western Cape, the prevalence of genital tuberculosis was 7.98% [37].

Over the last decade, in developed countries, there has been a steady decline in the incidence of pulmonary and extra-pulmonary tuberculosis. However, in developing countries, there has been an increased incidence of pulmonary and extra-pulmonary tuberculosis including drug resistant forms due to the emergence of HIV infection [73]. The proportion of extra-pulmonary tuberculosis is increasing in South India and currently stands slightly higher than smear positive pulmonary forms [74].

Genital tuberculosis is responsible for a significant proportion of females presenting with infertility [35]. Genital tuberculosis as a cause of infertility is 10-15 times more common in developing countries [36].

High prevalence of genital tuberculosis has been reported by various authors from African countries. From South Africa, Margolis et al (1992) have reported a 6.15% prevalence of genital tuberculosis in infertile women [75]. A bacteriological study of 114 infertile patients in the Northern Nigeria revealed a prevalence of 16.7% of genital tuberculosis [76]. Oosthuizen et al (1990) have given an incidence of 21% from Africa. [20] In a report from Pakistan, out of 534 infertile women, genital

tuberculosis was diagnosed in 2.43% by histo- pathology and culture in LJ (Lowenstein – Jensen) medium [77].

Various Indian studies have shown that tuberculous endometritis and salpingitis account for 4-16% of all infertility cases [21, 22, 23, 78, 79]. In infertile women undergoing diagnostic laparoscopy, Merchant in 1989, reported an incidence of 14.7% of genital tuberculosis [80]. The study carried out in the females of eastern part of Uttar Pradesh, India , most of which are of low socio economic status and poor hygienic living standards, showed a higher incidence of female genital tract tuberculosis [81].

In Chandigarh, India, by using a simplified tuberculosis algorithm genital tuberculosis was diagnosed in 7.2% of women with infertility [82]. Recently, by using PCR technique along with AFB smear microscopy, HPE and culture, Rozati et al (1996) reported 50% incidence of GTB in 65 infertile women suspected of suffering from female genital tract tuberculosis [71].

Studies have shown that the prevalence of GTB is higher in tubal factor infertility. A study from Cuttack, India (2002) showed that the incidence of GTB was 3% of infertility cases and 41% of tubal factor infertility. [83] Similar findings were shown by Sharman (1952) and Halbrecht (1959).When the tubes were occluded the prevalence of tuberculosis reported was between 25% and 44 % [84, 85].

**Age Incidence of GTB**

Female genital tuberculosis occurs in relatively young females in the reproductive age group. About 80 – 90% of the cases of genital tuberculosis are first diagnosed in patients between the ages of 20 and 40 years. Frequently, the discovery is made during investigation for infertility [58].

After puberty the blood supply to the pelvic organs is increased and as a result, more bacilli could reach the site and infect the reproductive organs or the dormant bacilli can get reactivated. This explains why genital tuberculosis occurs more

frequently in young women [86]. Because of the hormone dependent nature of the disease, many studies have shown that female genital tuberculosis involves women less than 40 years of age. [27, 55, 87] An African study has also shown that 94.7% of the patients were young and were between the ages of 20 and 30 years [76].

In Deshmukh et al (1987) study, the highest incidence of genital tuberculosis was seen in the 19 to 25 age group (57.7%) [22] Similar findings were reported by Trivedi et al (1993) Alwani et al (1995) and Nagpal et al (2001).[42,89,88] A recent study by Rozati et al (2006) also showed that genital tuberculosis affected 52 of 65 patients in the age group of 21 – 39 years [71].

As genital tuberculosis affects young women of reproductive age group it has great implication as an important cause of infertility.

**Clinical Features**

Genital tuberculosis is a disease of varied symptomatology. At one end of the spectrum, the disease may remain asymptomatic with no signs and symptoms and the diagnosis is made during evaluation of infertility or other gynaecological problems. It is estimated that at least 11% of patients are asymptomatic and hence the disease is discovered incidentally [68]. At the other end of the spectrum, the patients may present with typical signs and symptoms of tuberculosis such as fever, loss of weight, loss of appetite, anorexia, malaise and night sweats. There may be a history of poor general health persisting over a period of months or years. Some patients also give a history of recurrent pelvic inflammatory disease that has not responded to the usual antibiotic therapy [58]. The disease can also present with mild, varied clinical symptoms which are also common to other Gynaecological conditions.

Therefore, a high degree of suspicion is necessary to diagnose pelvic tuberculosis.

The symptoms that are usually associated with genital tuberculosis are

- Infertility
- Lower abdomen and pelvic pain

- Menstrual disturbances
- Vaginal discharge

The most common initial symptom for which a woman seeks medical advice is infertility. Approximately 85% of patients with genital tuberculosis have never been pregnant. [58]

In infertile women genital tuberculosis can exist without any apparent signs and symptoms. In these cases, the disease may be latent or may harbor a low grade insidious infection. A study conducted at Institute of Reproductive Medicine, Kolkata, India, indicated that latent tuberculosis should be considered in young Indian patients presenting with unexplained infertility with repeated In Vitro Fertilization (IVF) failures [90]. Many studies have confirmed the presence of a strong association between genital tuberculosis and infertility.

In a study by Chowdhury (1996), the major symptoms of genital tuberculosis were infertility in 45%, pelvic pain in 50%, and poor general health in 25% and menstrual disturbances in 20% of cases [87]. In a Clinicopathological study of 1000 cases of tuberculous endometritis, the commonest presenting symptoms were infertility (47%) and amenorrhoea (26%) [64]. Other studies from Pakistan, Malaysia, Iran and India have also shown that infertility was the most frequent mode of presentation in genital tuberculosis; seen in 42.5%, 50%, 75.6% and 70.8% of cases respectively [91, 72, 25, and 92].

A history of primary infertility in a woman in whom examination reveals no apparent cause and who gives a family history or past history of tuberculosis should arouse suspicion of genital tuberculosis [58].

The next common complaint is lower abdominal pain and pelvic pain reported in 17% to 50% of cases of genital tuberculosis [25, 91, 87]. Pelvic pain is usually present for several months before the patient seeks medical advice. The pain is usually chronic and low grade, although episodes of acute lower abdominal pain due to

secondary infection by pyogenic organisms may also occur. With the progression of genital tuberculosis, pelvic pain becomes more severe and aggravated by coitus, exercise and menses. Sometimes, there may be abdominal swelling associated with pain [58, 93].

The prevalence of menstrual disturbances in female genital tuberculosis varies from 20% to 50% in various studies [42, 81, 87, 88]. The most common change in the menstrual pattern observed is menorrhagia, oligomenorrhoea, amenorrhoea, polymenorrhoea and hypomenorrhoea. In early stages of the disease initial pelvic congestion and increased vascularity of the endometrium cause menorrhagia. Later on, with severe endometritis, oligomenorrhoea or amenorrhoea result [88].

Tyagi et al believe that local endometrial changes with systemic effect account for abnormality in menstruation [94]. Menstrual disturbances such as shortened irregular cycles with heavy bleeding may occur when there is ovarian involvement. In cases of severe endometritis, the periods become scanty or amenorrhoea results due to incomplete or complete obliteration of the uterine cavity due to scarring and dense fibrosis of the uterine cavity [56]. Advanced active pulmonary tuberculosis can produce amenorrhoea, particularly if associated with fever and weight loss. Complete destruction of the ovary by genital tuberculosis seldom occurs and ovarian failure is not the cause of amenorrhoea [58]. Amenorrhoea is the result of end organ failure secondary to endometrial caseation [95, 96].

In women with histologically proven endometrial Koch’s, 40% of patients had scanty period or amenorrhoea not responding to hormone therapy [97].

In Margolis et al study (1992), in 87.7% of cases menstruation was normal. Their findings emphasized that genital tuberculosis is often a disease of absent or few symptoms [75]. A study in 1993 showed that in 475 patients attending a TB hospital with extragenital tuberculosis, in 52.84% of cases, the menstrual pattern was found to be altered. The most common change in the menstrual pattern observed was

oligomenorrhoea in 18.84%, amenorrhoea in 10.52%, hypomenorrhoea in 8.84%, menorrhagia in 5.47% and polymenorrhoea in 4.84%. The highest incidence of altered menstrual pattern was observed in the age group of 21 – 30 years [42].

In Sutherland's study in 1985, the following symptoms were noted in 710 cases of Gynaecological tuberculosis [53].

| | |
|---|---|
| Infertility | 41.16% |
| Pelvic Pain | 25.17% |
| Menstrual disturbances | 17.86% |
| Amenorrhoea | 5.06% |
| Vaginal discharge | 3.79% |

Other symptoms such as abdominal bloating and vaginal discharge are seen less frequently [98]. Dysmenorrhoea is rarely a significant symptom of pelvic tuberculosis. Many women suffering from genital tuberculosis give a history of having had appendicectomy performed for chronic discomfort in the right iliac fossa. Because of the tubal pathology, there is history of ectopic pregnancy in some of the cases [99]. In one study 5.17% of cases of GTB had history of ectopic pregnancy in the past [92]. Tuberculosis should be suspected when the chronic pelvic inflammatory disease is refractory to standard therapy [100].

**Contact History**

Epidemiologic studies of TB transmission in humans proved that person to person airborne transmission occurs. If one patient with pulmonary tuberculosis had 20 close contacts, 5 to 10 of them could be expected to become infected and one of the infected persons would develop active disease at some point in his or her lifetime [101,102,103].

A past history of tuberculosis or history of close contact with a family member suffering from tuberculosis should raise the suspicion of genital tuberculosis in infertile women. However, in most cases, a past history of tuberculosis or a history of

contact with tuberculosis may not be forthcoming as tuberculosis is considered a taboo in India. In a study from Mexico, 39.1% had a history of contact with a relative with tuberculosis [27].

**Past History of Tuberculosis**

Since genital tuberculosis is considered as one of the secondary manifestations of tuberculosis and in majority of cases with its primary site in the lung, one may expect a history of pulmonary tuberculosis or X-ray evidence of tuberculosis. However, in many instances the primary pulmonary lesions have already been arrested. Careful evaluation of the X-ray film is imperative to detect small or healed lesions or signs of pleurisy/pleural effusion. A negative chest X-ray, therefore cannot rule out the possibility of genital tuberculosis.

A definite documented history of tuberculosis of any site in the past is an important consideration in suspecting genital tuberculosis in the presence of infertility or other pelvic symptoms that are not otherwise explained [82].

In a study by Misra et al (1996), a previous history of pulmonary tuberculosis was present in 5% of patients [81]. In a review of sixty cases of genital tuberculosis, in nine cases there was extra – genital tuberculosis in the past [100]. In a study from Mexico, 16% had a history of tuberculosis in the past [27]. A study from Nigeria suggested that, the low incidence of past history of pulmonary tuberculosis in infertile women with genital tuberculosis may relate to a gastro – intestinal source of the infective organisms [76].

However, a number of studies have reported higher incidence of patients with genital tuberculosis, having extra- genital lesion [18, 41]. In Sutherland's large series of 638 patients, 80% had a history of tuberculosis elsewhere in the body [104]. In a recent study from Northern India, past history of pulmonary tuberculosis and / or extra – pulmonary tuberculosis was available in 73.6% of patients which had occurred during childhood or adolescent years [82]. In a report from New Delhi, past history of

tuberculosis was present in 15 of the 40 cases of genital tuberculosis (pulmonary - 9 and abdominal tuberculosis - 6) [105].

Amarnath et al (1987) analyzed 71 cases of genital tuberculosis. In their study there was a history of previous extra – genital tuberculosis in 66.2% of cases, of which 29.58% had pulmonary tuberculosis, 23.35% had abdominal tuberculosis, 5.64% had renal tuberculosis, and 2.82% had erythema nodosum. Concurrent disease was seen in 17.02% of cases [99].

**BCG Vaccination**

Studies have shown varying results with the use of BCG vaccine to prevent tuberculosis. In a large controlled trial from India, it was shown that the risk of sputum positive tuberculosis in persons vaccinated with BCG was not lower than in persons given placebo [106]. However some observational studies have shown a reduction in the prevalence of tuberculous meningitis and miliary tuberculosis in vaccinated children than in unvaccinated controls [107].

**Physical Examination**

Physical examination is important in establishing a diagnosis of genital tuberculosis. However, it should be remembered that in most cases no abnormal findings may be apparent, and if present may be vague.

General examination may reveal an active or healed extra genital lesion especially in the lungs .There may also be evidence of extra-genital lesion in the spine and scars in the cervical node and axillary node areas which may indicate past infection in these sites. However, in most cases the primary site of infection may not always be detected.

There is also little correlation between presenting complaints and physical findings in genital tuberculosis. In most cases, abdominal and pelvic examination may be entirely normal. In various studies, examination was reported to be normal in 56.1% to 90% of cases [41, 75, 81]. In some of the cases, palpation of the abdomen

may reveal a “doughy” sensation, which has been ascribed to tubercle formation on the intestines and peritoneum. Patients can also present with either general or sacculated ascites resulting in abdominal distention. Matting of intestines, omentum and pelvic organs can present with irregular abdominal masses. As the disease is usually silent, on bimanual pelvic examination fallopian tubes may not be enlarged. If adnexal masses are present they vary in size from slight thickening and irregularity of the tube to large tubo-ovarian masses (T-O Mass). Tuberculous T-O masses are less tender than those due to pyogenic disease, although secondary infection and acute exacerbation may produce sharp pain and acute tenderness [58]. In a study from Pondicherry, a doughy abdomen was present in 18% of patients with genital tuberculosis [100]. Nagpal et al (2001) observed 33 % incidence of adnexal masses in genital tuberculosis [88]. Other pelvic lesions such as fibroids, ovarian cysts and adenomyosis may co-exist with genital tuberculosis.

Adnexal masses in younger women, who have a history of pulmonary / extra pulmonary tuberculosis or associated with secondary amenorrhoea, should raise a strong suspicion of tuberculosis [58].

Based on clinical signs and symptoms, the diagnosis of the disease is difficult. Apart from asymptomatic nature to varied clinical presentation, a past history of tuberculosis or a history of contact may not be forthcoming and an evidence of tuberculous lesion elsewhere may be lacking. The abdominal and vaginal examination findings may be normal [73].

**Lab Investigations**

Routine laboratory studies are also of little value in the diagnosis of genital tuberculosis. The laboratory investigations which are said to be characteristic of active pulmonary tuberculosis, are not necessarily so in female genital tuberculosis. Anemia is only a feature of advanced disease. In acute stages, there is mild to moderate leukocytosis with preponderance of monocytes, with a decrease in lymphocytes. As

the acute stage subsides the monocytes fall and the lymphocytes increase. The erythrocyte sedimentation rate is usually elevated, although this is not very specific for diagnosis [56].

The majority of patients have a normal white blood cell count, although there is a tendency to lymphocytosis.In 100 proved cases of genital tuberculosis, ESR was found to be raised in 89% of cases, and the rise was moderate to severe. Lymphocytosis was seen in 31% of cases. [88] In a study by Amarnath et al ESR was elevated in only 59.14% of cases and lymphocytosis was seen in 69.01% of cases [99].

**Tuberculosis and HIV infection**

The incidence of HIV associated tuberculosis is increasing worldwide especially in developing countries [108]. The immuno compromised state due to HIV infection causes reactivation of endogenous tuberculosis infection to development of tuberculosis disease [73]. HIV infected patients rapidly develop clinically significant disease and respond poorly to treatment. It is also known that in HIV positive patients, extrapulmonary tuberculosis occurs more often [109,110].

Although a relative increase in GTB would be expected, this has not been reported. Probably tuberculous systemic disease is diagnosed earlier, before extra – pulmonary manifestation occurs [56]. However, diagnosis of genital tuberculosis should be considered more often and more carefully in all HIV infected women and all patients with tuberculosis should be screened for HIV infection [111]. Giannacopoules et al (1998) reported a case of genital tuberculosis presenting as acute PID in a HIV infected African woman [40].

**The Tuberculin skin test (Mantoux test)**

The tuberculin skin test is based on the hypersensitivity reaction and indicates whether or not sensitivity due to a past or present mycobacterial infection exists in a

person. The test involves intradermal injection of 5 tuberculin units (equivalent to the activity of 0.1 micro gm of the standard purified protein derivative. (PPD – S).

In a sensitive subject, after several hours there is infiltration of mononuclear cells at the injection site. The inflammatory reaction increases progressively over a period of 1 to 4 days producing persistent erythema and induration at the site. The key cells involved are lymphocytes and monocytes. The reaction should be read at 24, 48 and 72 hours, but in most cases this is not possible, and the 48 hour reading is the one most commonly taken. The extent of induration should be measured in two diameters at right angles and the average of these values is recorded as the result.

An induration of $\geq$10mm is taken as a positive Mantoux test. However, in persons with HIV infection, persons in close contact with an infectious patient with tuberculosis, persons whose chest radiograph is consistent with healed tuberculosis, induration of >5mm is taken as positive Mantoux test [112].

A positive tuberculin test is not specific for Mycobacterial tuberculous infection [73]. Various reports have shown that infection with various non – tuberculous mycobacteria may produce skin test responses to PPD – S that fall within the same size range as that found in patients with proven tuberculosis. Therefore, false positive PPD reaction may result from infection with non tuberculous mycobacteria. The test is also positive when there is history of past infection and does not differentiate active disease from past infection [44].

The Mantoux test generally remains positive as long as viable bacilli persist in quiescent foci. When tubercle bacilli establish infection, the host usually contains the infection and a positive tuberculin reaction is the only evidence of infection. These viable but dormant organisms which are enclosed within the granulomatous fibrotic tissue are a potential threat to become metabolically active at a future date producing active disease.

Interpretation of tuberculin test is also complicated by prior BCG vaccination and now by HIV infection [40]. A positive reaction may be produced in BCG vaccinated persons. As the reaction involves cell mediated immunity, in immuno compromised individuals with human immunodeficiency virus (HIV) infection, uremia, sarcoidosis, those on chemotherapy for serious malignant disease, and very ill patients can give a negative response. False negative reaction can also occur with poor technique, such as injecting into the deeper layer of the skin and improper storage of the reagent. In a few patients, no definite cause for the false negative reaction can be found.

Raut et al have analyzed the usefulness of the Mantoux test in the diagnosis of genital tuberculosis in women of child bearing age. This study reported that the Mantoux test had a sensitivity of only 55% and a specificity of 80% in women with laparoscopically diagnosed tuberculosis. They concluded that Mantoux test has limited utility in diagnosing active genital tuberculosis during childbearing age. However the study recommended that, in infertile women with a positive Mantoux test, laparoscopy may be advocated early [113].

In Gupta et al study (2007), out of the 25 infertile women with genital tuberculosis only 2 cases were positive for Mantoux test [105]. However, Figueroa – Damian et al study in 1996, showed a higher positivity of Mantoux test in proved cases of genital tuberculosis [27]. Alwarez and Mc Cabe also reported that 90% of patients with genitourinary involvement had a positive test [114].

**Chest X – Ray**

A careful evaluation of the chest roentgenogram by a trained chest physician is important for locating small or healed lesions. However, majority of pulmonary lesions are arrested by the time genital tract involvement becomes obvious. Therefore, the chest X – ray is normal in most cases [20, 37, 55, 73]. Nagpal et al (2001) reported that chest X – ray was positive only in 1% of the cases with proved genital

tuberculosis.[88] Klein et al (1976) reported that of the 20 patients with pelvic tuberculosis, results of chest x- ray were normal in 15 [41].

The most probable explanation for the negative or occasionally positive finding at chest X – ray is that a clinically undetected lung disease has undergone spontaneous resolution after there has been some type of bacteraemia to which the tubes alone are singularly responsive [40]. A negative chest X – ray does not rule out the possibility of genital tuberculosis. [115]

**IMAGING**

As abdomen and pelvic tuberculosis with peritoneal involvement usually present with general symptoms and obscure abdominal complaints, imaging studies with ultrasonogram (USG), Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) are usually the initial investigations.

Abdominal radiography may show calcified pelvic and abdominal lymph nodes which is a sequelae of healed tuberculosis [116].

Awareness of the sonographic changes associated with tuberculous infection may improve diagnostic accuracy and avoid clinical mismanagement and surgical exploration in wet type of tuberculosis. Sonographic features of wet and dry (adhesive) type of tuberculosis were reported by Yapar et al, (1995). The features of wet tuberculosis were septated ascites, particulate ascites, loculated fluid, thickened peritoneum and adnexal masses. Adhesions, adnexal masses and loculated fluid were found in the dry type [117]. CT and MR imaging studies have shown heterogenous tubo – ovarian masses, dilated fallopian tubes with thickened wall and increased enhancement, ascites, thickened peritoneum, omental masses, enlarged lymph nodes and heterogenous uterine enhancement [118,119].

The CT findings of TB peritonitis are non – specific and mimic disseminated peritoneal malignancy, mesotheliomas and non – tuberculous peritonitis. A high degree of clinical suspicion and familiarity with the abdominal CT manifestations

allow early diagnosis of abdominal tuberculosis. Certain features on CT may help in the diagnosis. The most common manifestation of abdominal tuberculosis is ascites of variable amount and omental infiltration. Due to the high protein and cellular content in the tuberculous exudates, the ascitic fluid ususlly measures higher than water density (25 – 45 HU). The CT appearance of chylous ascites i.e., a fat – fluid level in conjunction with caseated lymph nodes has also been described. The presence of a slight and smooth peritoneal thickening is more suggestive of TB, while nodular irregular thickening is more compatible with peritoneal carcinomatosis. TB lymphadenitis is a common manifestation of abdominal tuberculosis and reported in 55 – 60% of cases. Visceral lymph nodes are usually affected. They present with enlarged nodes with central hypodensity [120].

**Hysterosalpingogram**

As a part of infertility workup, in order to ascertain the tubal patency, HSG is a frequently performed investigation in cases of infertility. HSG findings in genital tuberculosis were described by Chavhan et al [121].

**Tubal Tuberculosis:**

- Tubal occlusion is the most common HSG finding reported in 81% of cases of genital tuberculosis. Occlusion occurs most commonly at the junction between the isthmus and ampulla. Cornual occlusions are not very common in tuberculosis and are produced more commonly by other organisms. Other causes of tubal occlusion include endometriosis, prior pelvic surgery and PID due to Chlamydia and gonococci.
- As the caseous ulceration of tuberculosis heals, the entire tube becomes encased in heavy connective tissue scar. Scarring in the mucosa of the entire tube gives an appearance of “rigid pipe” to the tube.
- Scarring and multiple contractions can give rise to “beaded appearance” to the tube.

- Mild to moderate hydrosalpinx with thickened mucosal folds within the dilated tube is a common feature in tuberculosis. Other causes of tubal dilatation include other inflammatory diseases, adhesions and obstruction of any cause. Tuberculous hydrosalpinx is a common finding in India [80].
- Calcification of the fallopian tubes and ovaries may also be seen in genital tuberculosis. Tubal calcification takes the form of linear streaks along the course of the tube or may appear as dense tiny nodules.
- Peritubal adhesions can be caused by genital tuberculosis which may appear as convoluted tubes, peritubal halo, tubal fixation and loculated spillage of contrast material.

Chavhan et al (2004) reported tuberculosis in 7.5% of HSG performed for infertility. In this study calcification was seen in 5.4% of cases, irregularity of tube in 16%, tubal occlusion in 81% of cases, tubal dilatation in 46% of cases and adhesions in 11% of patients [121].

- Intrauterine scarring may lead to obliterated uterine cavity, giving an appearance of “pseudounicornuate” uterus, ‘T’ shaped uteri or asymmetrical small uterus.[80]

Although the various tubal and uterine features described are not specific for genital tuberculosis, they are highly suggestive of it [121]. The diagnostic criteria established by Klein et al (1976) are very useful for this purpose.

**Diagnostic criteria by Klein et al, 1976 [41].**

- Calcified lymph nodes or small, irregular calcification in the adnexal area.
- Obstruction of fallopian tube in the zone of transition between the isthmus and ampulla.
- Multiple contractions along the course of fallopian tube.
- Endometrial adhesions or deformity or obliteration of the endometrial cavity in the absence of curettage or surgical termination of pregnancy.

The above features are suggestive of GTB; although a conclusive diagnosis can be made only from a positive culture or histologic specimen.

**Endometrial tuberculosis**

HSG appearances of endometrial tuberculosis are synechiae, distorted uterine contour and venous and lymphatic intravasation.

- The synechiae in tuberculosis are characterized by irregular, angulated and stellate shaped well demarcated borders – filling defects.
- Venous and lymphatic intravasations are good indicators suggesting endometrial tuberculosis; reported in 27% of cases [121].
- It is not specific for tuberculosis and can be seen if HSG was done in the early menstrual cycles, shortly after endometrial instrumentation and any condition causing obstruction to flow such as intra – uterine adhesions and tubal obstruction of any aetiology [122].

30% incidence of intra uterine adhesions was reported in one study with genital tuberculosis [97]. HSG is a very useful tool in establishing the diagnosis [41]. Nagpal et al (2001) showed that 72.10% of cases associated with proved tuberculosis had HSG findings suggestive of tuberculosis with cornual block, fimbrial block, beaded tubes, hydrosalpinx, localized spill, intra and extravasation and filling defect in the uterine cavity [88].

**Laparoscopy**

In today's Gynaecological practice, laparoscopy has become an important tool in the evaluation of infertility and in the diagnosis of various pelvic conditions. Endoscopy has the dual advantage of pelvic organ visualization and sample collection from inaccessible sites for laboratory diagnosis [123].

Laparoscopy has been used as an additional tool to evaluate women with high suspicion of genital tuberculosis. Endoscopy helps to obtain microbiological samples, evaluate the condition of the organ and the extent of the damage and provides an

opportunity for therapeutic intervention [82]. Laparoscopy has been found to be a superior method of bacteriological sampling, since the laparoscopic collection is done under direct vision [124,125,126].

Three clinical forms of tuberculosis of the uterine appendages are distinguished: latent or minor inflammation, marked inflammation with tubo – ovarian lesion and tuberculomas. Early / latent tuberculosis do not produce tubal or peritoneal changes. Evidence of acute infection by laparoscopy is small miliary tubercles, peritoneal congestion, swollen and reddened serosa of uterus and tubes. Chronic infection manifests as thickened tubes, terminal hydrosalpinx with retort shaped tubes, T-O mass, flimsy adhesions in the POD and intravasation or extravasation on chromopertubation [88].

Visual diagnosis alone at laparoscopy is insufficient [127]. Certain conditions like Tubo-Ovarian masses of gonococcal / pyogenic origin, pelvic endometriosis, small ovarian cyst and old pelvic haematocele may closely mimic a T-O mass of tuberculous origin. Rarely, the whole appearance may be difficult to distinguish from that of ovarian malignancy [56].

Therefore, definite diagnosis can only be made by positive histology of tissue or by positive culture of tissue or POD aspirate

Semenovski et al (1999) showed that laparoscopy may detect pathognomonic signs of rashes on the visceral peritoneum and enlarged mesenteric lymph nodes. Their study showed that laparoscopy increases the diagnostic potentiality by 19.7% in diagnosing abdominal and genital tuberculosis [128].

Avan et al 2001 compared the clinical and laparoscopic features which may help to differentiate between infertility in females due to genital tuberculosis from pelvic inflammatory disease (PID) and endometriosis. This study reported that tortuous, bilaterally blocked and thickly adherent tubes are common in genital tuberculosis when compared to other groups. The primary infertility patients with

chronic malnutrition and masses and adhesive fallopian tubes on laparoscopic examination should be evaluated for genital tuberculosis [129].

The following laparoscopic findings were reported in patients with proved genital tuberculosis by Amarnath et al: pelvic adhesions in 46 – 48 %, tubercles in 33.8%, and adnexal masses in 32.3% of cases and encysted effusion in 8.45% of cases [99]. In Agrawal et al study, authors have compared the histology, bacteriology, HSG and diagnostic laparoscopy in clinically suspected cases of genital tuberculosis. Their observation showed that bacteriology could detect only 2.8% of cases, histology 21.71%, HSG 51.11% of cases and laparoscopy was suggestive of tuberculosis in 74% of cases. Their study concluded that laparoscopic visualization of genital tract is more effective as compared to bacteriology and histological methods and laparoscopy helps in diagnosing genital tuberculosis at an early stage [130].

In early and latent cases, there may not be evidence of pelvic tuberculosis at laparoscopy. In a study by Vynck et al (1990) from South Africa, in cases that were positive by microbiological studies in menstrual fluid, there was laparoscopic evidence of tuberculosis only 55.5% of cases and in the remaining 44.5% of cases, the pelvis was considered normal [37]. Similar finding was also noted in Deshmukh et al study, where out of 45 cases with histologically proven genital tuberculosis, 3 cases did not show evidence of tuberculosis at laparoscopy [22].

Moreover laparoscopy is an invasive procedure and should be done carefully to avoid injury to an adherent bowel. Matted tuberculous adhesions do not always give rise to a “doughy” abdomen and in fact they can be remarkably silent and prone for dangerous injury to the bowel [131,132].

Genital tuberculosis presents unique diagnostic challenges including subtle clinical manifestations that may be overlooked at laparoscopy during early stages of infection [133].

**Hysteroscopy**

Hysteroscopic visualization of the uterine cavity is not very specific for diagnosing tuberculous infection [56,108]. But some of the common findings are:

- A normal cavity with obliterated fibrosed tubal ostiae.
- Intracavity adhesions, more commonly dense fibrous or fibromuscular adhesions.
- Fibrosed tubular cavity with complete absence of endometrial proliferation.
- Complete obliteration of the cavity
- Tubercles and microcaseation may also be seen.

The best way to detect intra – uterine adhesions is by hysteroscopy, as many times, intra uterine adhesions are mistakenly reported as small uterus or malformation of uterus by HSG [97].

**Urinary System**

10% of females with genital tuberculosis also show evidence of renal involvement [134]. While investigating a woman with infertility, investigations for genitourinary tuberculosis should be initiated in the presence of unexplained urinary symptoms, haematuria, persistent pyuria or abacteriuric pyuria [40]. Three first morning voided midstream urine specimens are sent for AFB stain and culture [16]. In a study by Namavar et al, urine culture was negative in 41 cases of proved genital tuberculosis [25]. In a study by Marna et al, (1992) in infertile patient genital tuberculosis was diagnosed by isolating *MTB* only from the urine [135]. In 31 patients with proved tuberculosis of urogenital system diagnosed by culture and HPE, *MTB* was detected by PCR in urine in 81.25% of patients [136].

Sputum examination for AFB, though not advisable as a routine investigation in cases of infertility, should be done if there is clinical or radiological evidence of tuberculosis [21]. In cases of proved genital tuberculosis, prior to commencement of drug treatment, baseline investigations must include chest X – ray, urine examination,

three early morning sputum or gastric aspirate samples for the presence of *MTB*. Full urological examination is indicated when urine is positive for tuberculosis [93, 16].

**Detection of AFB in Direct Smears**

Tubercle bacilli may be demonstrated by direct microscopy, by culture and by animal inoculation.

Direct microscopy is important because the results are available immediately. It may be of value in the primary care setting because it is simple and it is relatively easy to perform and interpret. It often gives preliminary confirmation of the diagnosis while waiting for culture report. Direct microscopy will reveal whether acid fast bacilli are present or absent.

The acid fast bacilli (AFB) could be any pathogen or saprophytic mycobacteria [137].

Though smears are an important adjunct to the detection of *MTB*, they are not adequate criteria alone and must be followed by culture to determine the species of mycobacterium or whether the organism belongs to members of another genus [138]. Though less sensitive than culture, the acid fast smear examination is specific, rapid and simple to perform. The sensitivity of a single smear test of a respiratory specimen varies between 22% and 42%; when multiple specimens are examined the detection rate improves to 96% in patients with pulmonary tuberculosis [139]. The sensitivity of smears of other specimen sources however is not as great [140].

For a smear test to be positive there should be 10,000 organisms per milliliter of specimen [141,142]. Therefore respiratory specimens are more likely than other types of specimen to be positive.

There are three methods of preparing acid fast smears [138].

- Ziehl – Neelsen stain
- Kinyoun stain

- Use of Fluorochrome dye such as auramine O.

All Mycobacteria have a lipid rich cell wall that retains carbol Fuchsin, rhodamine or auramine dye even in the presence of acid, alcohol or formalin. The molecular target of acid fast staining dyes (Fuchsin, auramine or rhodamine) is the mycolate on the bacterial surface.

Smears examined by Fluorochrome method have been found to have a greater sensitivity than those stained with Fuchsin (Ziehl – Neelsen, Kinyoun stains). Fluorochrome stained smears are examined more effectively as they are viewed under high dry magnification rather than oil immersion required for fuchsin stained smears. Moreover, with a fluorochrome dye, the mycobacteria appear bright against dark background, therefore easier to detect. As Fluorochrome stains are more sensitive and easier to read than Fuchsin stains they are recommended by the CDC. [143,144].

The acid fast bacilli appear as purple to red, slightly curved, short or long rods (2 to 8 μm), occasionally beaded or banded. In general, the appearance of an acid fast bacillus does not provide a species identification.

The frequency and morphology of acid – fast bacilli found in specimens can be variable; therefore, the laboratory staff should be vigilant while interpreting the smears. It is also important to develop quantitative criteria for reporting smear results which can provide useful clinical information.

The presence of small number of bacilli in extra – pulmonary sites contribute to difficulty in visualization of acid fast bacilli.

The AFB bacilli are very rarely found in endometrium and cervical granulomas even with the use of fluorescent techniques [60,145]. The incidence of AFB positivity in GTB varies from 0 to 63.5% as reported by Reddy et al (1975), Tyagi et al (1977) and Khan et al. (1982) [146,147,148]. Bobhate et al and Misra et al observed AFB positivity in 1.6% and 1.23% of cases respectively in genital tuberculosis [81,149]. In Abebe et al (2004) study, AFB smear was positive in only

one case of 25 clinically suspected female genital tract tuberculosis [150]. Rozati et al gave the detection rate of 5.2%. In yet another study, the AFB was seen in direct smears in 12.19% of cases [71, 25]. Absence of AFB does not exclude the diagnosis of female genital tract tuberculosis [25].

Eisenach study showed that PCR could distinguish between tuberculosis and non tuberculous mycobacteria in cases of smear positive disease [151]. In specimens positive for AFB, American Thoracic Society recommended to use PCR as additional evidence [152].

Studies on tissue section to demonstrate AFB has shown that immune histochemical staining for mycobacteria is a better method than acid fast staining to demonstrate AFB at extra pulmonary site of tuberculosis [153].

**Microbiological Techniques for the diagnosis of Tuberculosis**

Accurate identification of Mycobacterium tuberculosis through culture is presently the yardstick for diagnosis and remains the gold standard technique. However, the time required for the report and frequent negative results in paucibacillary specimens are important limitations.

Variety of laboratory media for the cultivation of Mycobacteria has been described.

**1. Traditional Method**

- Lowenstein Jensen (LJ) medium containing albumin in an agar base.
- Middlebrook 7 H 10 or 7 H 11 medium containing egg and potato base.

These media support the growth, from which colonial morphology, growth rate and pigment production are studied. Colonies are seen if the bacillary count is more than 1000 bacilli. In these methods several weeks of incubation is necessary and may take upto 8 weeks to grow the organisms.

**2. BACTEC System**

Improvements in media have allowed colonies to grow even when the count is 100 bacilli. This is possible with the use of liquid based media radiometric growth detection such as BACTEC 460 or non radiometric $CO_2$ growth detection with BACTEC ALERT 3. It is a Broth - based growth system developed by Becton Dickinson and is based on generation of radioactive carbondioxide from substrate palmitic acid. The Bactec system has the advantage of reduced culture time of only 2 weeks, high sensitivity and also provides rapid assessment for drug sensitivity patterns. [154,137]The radiometric culture BACTEC has a sensitivity of 80 – 90%, whereas the LJ medium has a sensitivity of only 30 – 35%. This high sensitivity is particularly useful in cases of genital TB as traditional methods show poor recovery of AFB [123,137].

The other advances in the detection of *MTB* are the Isolator Tube (Wampole) and MGIT – (Mycobacteria Growth Indicator Tube) techniques [155].

**Identification of Mycobacterium Tuberculosis**

An isolate that is slow growing, non- pigmented, non – photochromogen, with a rough colony and a tendency towards cord formation should raise a high index of suspicion as *MTB.*

For conventional assignment to the species of *MTB* standard biochemical tests are carried out [156]. However biochemical testing requires an additional 2 to 4 weeks for completion after mycobacteria have appeared in culture. *MTB* would typically display positive reaction on the niacin production and nitrate reduction tests. Bio Chemical tests for the identification of *MTB* are time consuming and sometimes ambiguous results are obtained. To overcome these limitations chemical methods based on lipid profiles and Nucleic acid amplification (NAA) tests have been described.

**Analysis of lipid profiles:**

Mycobacteria have characteristic lipid profiles. The lipid profiles can be analyzed by high – performance liquid chromatography (HPLC) and thin – layer Chromatography (HP–TLC). This allows quick identification of mycobacterial colonies on a solid medium in 2 to 4 hours. This strategy has been successfully used in several laboratories [157].

Culture methods are widely used in the detection of genital tuberculosis. However, negative cultures can occur,

1. When the specimen of endometrium used for culture is unrepresentative
2. When the sample is from a scanty paucibacillary extra – pulmonary site. A substantial number of TB lesions of the genital tract are bacteriologically mute [133].
3. When the patient has had previous anti – tuberculous chemotherapy
4. When there is a prolonged time interval between biopsy and bacteriological examination [158].
5. In the presence of bacteriostatic substance in the tissue that inhibits the growth of bacilli [159].

In order to increase the microbiological yield, the optimal time for sampling is at the end of the menstrual cycle or within 12 hours following the onset of menses to allow maximum time for the endometrial granulomas to develop [16].

Various studies have evaluated histological and bacteriological methods in the diagnosis of endometrial tuberculosis.

Francis in 1964 reported 96 cases of positive endometrial culture in which only 48 showed histological evidence. The diagnosis would have been missed in 50% of cases had cultures for bacteriology been omitted [160].

In a study from South Africa, 1985, the incidence of endometrial tuberculosis in infertile women was 4.85%. In these cases culture was positive in 57% of cases,

Guinea pig inoculation (GPI) was positive in 71% of cases and histological findings were positive in 42% of cases. Cost analysis of culture & GPI showed that culture of endometrial specimen is most cost – effective than GPI. [161].

In Chhabra et al study, (1986) endometrium was subjected for tuberculosis study in 150 cases and 21 cases were found to be positive either by histopathology or by bacteriology. Only in two of them both histology and bacteriology were positive. In 9, only bacteriology was positive and in 10 only histology was positive. The study concluded that both methods of diagnosis are complementary and neither is completely dependable [162].

In Manjari et al study (1995), combined histological and bacteriological study was carried out on 1124 endometrial curetting from cases of sterility. On histological examination 1.87% had tuberculous endometritis whereas on bacteriological examination 2.05% were positive for *MTB.* The study concluded that the use of the two methods simultaneously yielded a better result [163]. Deep Jyoti et al (1990) reported 6% incidence of GTB by culture [21]. In recent years, Abebe et al (2004), Bhanu et al (2005) and Rozati et al
(2006) have reported 12%, 3.2% and 7.8% of positive endometrial culture respectively (150, 133, 71).

In early lesions, the epithelium of endometrial glands exhibit necrosis and the lumen contains exudates, often eosinophilic and inflammatory cells including polymorphs and macrophages. Histologically these lesions may appear normal; however such lesions contain plenty of AFB when grown in culture [164].

Tuberculous endometritis can masquerade as a non – specific non – granulomatous lesion [62, 64]. The diagnosis of tuberculosis should be based on the demonstration of acid – fast bacilli, because the epithelial granulomas represent a non – specific form of inflammation.

Numbers of studies have evaluated the value of microbiological culture in cases where normal endometrial histology or chronic endometritis was reported. In Deep Jyoti et al (1990) study, the incidence of genital tuberculosis was found to be 6% by histopathological and culture studies. In this study among the 200 infertile women, eight cases were diagnosed as tubercular endometritis by histopathology as well as culture of AFB. In yet another 4 cases, where histology revealed chronic endometritis, the AFB culture was positive [21]. Kothadia et al (1989) made similar observations [165].

By Chakraborty et al (1993), combined histological and bacteriologicl study was conducted on 1320 endometrial curettings. By initial histologic findings only 5.3% of cases could be detected, while combined evaluation by bacteriological methods increased the prevalence to 6.2%., an increase in the diagnostic acumen by >14.5% [166].

Bacteriological study was of greater value in doubtful cases where there was absence of granuloma or epithelioid cells but presence of inflammatory exudates consisting of polymorphs, lymphocytes, macrophages along with minimal necrosis of epithelial cells. In this study, histologically 8 doubtful cases and 4 normal cases were picked by culture. The study concluded that simultaneous use of both histologic and bacteriological methods increase the detection rates [166]. Similarly, in Roy et al study (1993), a combination of histopathology and microbiological evaluation increased the diagnostic rate by approximately 10% [79].

A non- invasive way of getting samples for culture / HPE / and PCR could be menstrual blood. Menstrual blood culture has higher pick up rate in early and latent cases. In treated latent cases, fertility could be restored. Microbiological studies of cervical discharge, menstrual blood or endometrium yield greater number of positive results [167,168,169]. In a study from South Africa, of the 23 cases of genital

tuberculosis, menstrual blood was positive in 69.6% of cases, 17% of endometrial tissue and 26% of peritoneal fluid were positive [20].

In Margolis et al study (1992), menstrual fluid collection and culture proved to be the most reliable diagnostic procedure, since it was positive in 11 patients in whom pre menstrual endometrial sample cultures were negative and also in 17 patients in whom histological examination of premenstrual endometrial samples for tuberculosis were negative [75].

The value of culture lies not in the diagnosis of tuberculosis, but in the assessment of drug resistance and in distinguishing NTM from *MTB* [170]. NTM organisms have been cultured in extra-pulmonary sites such as lymph nodes [171]. Few studies have also reported atypical mycobacterium in culture while investigating cases of infertility for GTB.

In 1978, Khiancy et al reported 7 cases of atypical mycobacteria grown in culture [172]. In Chhabra et al study (1986) out of 150 endometrial samples 9 *MTB* and 2 atypical mycobacterium had grown in culture [92]. In Chakraborty et al study, (1993) out of 1320 endometrial samples *MTB* was grown in 80 cases and atypical mycobacteria in 2 cases [166].

Although the most important human pathogenic species is Mycobacterium tuberculosis, the significance of NTM organisms increased after the onset of the AIDS epidemic [173]. Singh et al (2007) have isolated NTM in 12.5% of cases with lymphadenitis, the highest rate so far reported from India [174].

**Histopathological Examination (HPE)**

A definite diagnosis of genital tuberculosis is possible by the recovery of *MTB* from the genital tract or by the histological demonstration of tuberculous granulomas in tissue samples. Histo pathological examination is easy, quick and cheap and provides characteristic features of *MTB.*

In female genital tuberculosis, the fallopian tube is the initial site of involvement, affected in almost all cases, followed by secondary extension to the endometrium in 50 to 90% of cases (18, 23,145,149,177). In as many as 50% of cases, infection may be limited to the fallopian tube [20].

In a recent report, endometrium was affected by tuberculosis in 50% of cases of tubal infertility due to genital tuberculosis [97]. It seems probable that tuberculous endometritis is almost 100% proof of tubal tuberculosis even without clinical and palpable evidence of adnexal disease [61].

Without resorting to laparoscopy or laparotomy, the source of material generally available for culture or biopsy of the female genital tract is the endometrium and menstrual discharge. As the uterus is more accessible for taking biopsies, most studies use endometrial curettage to collect the material for the diagnosis of genital tuberculosis [25].

Histology of endometrium provides lesser diagnostic information and is often not satisfactory for the following reasons:

- Due to the secondary nature of the genital tuberculosis, the infecting organisms are sparse in number.
- When there is extension of tuberculosis from the fallopian tube to the endometrium, the small inoculums of mycobacteria and the slow growth of the organism reduce the chance of obtaining a positive result from a single endometrial biopsy or menstrual culture.
- Moreover due to the cyclical sloughing and shedding of the endometrium every month granulomas do not have enough time to form, so the endometrium may not show evidence of tuberculosis in all cycles [41]; the specimen obtained by biopsy may be small.

- Or the endometrium is severely damaged resulting in amenorrhoea and no tissue is available for HPE.

Therefore, the diagnosis based on histology from endometrial curettage alone and a report based on a single sample could result in false negativity.

The reasons for false negative results with HPE are:

- Inadequate specimen.
- Non-representative tissue samples.
- Infected area can be easily missed.
- Technical failure in processing biopsy.
- Period of specimen collection in relation to disease stage.
- Effect of HIV co-infection - due to poor cell mediated immune response [71].

Due to the above reasons, to ensure maximum yield multiple specimens from several sources should be collected. The best time for examining the endometrium is in the premenstrual phase, at which time the tubercles reach their maximum growth. The portion of the endometrium most likely to show tubercles is in the region of the uterine cornua where the spread from the tube first occurs.

A diagnosis of endometrial TB made on histological grounds alone is reasonably dependable. The typical and almost pathognomonic lesion of the tuberculous endometritis in regularly menstruating woman is the non-caseating granulomas composed of epithelioid cells, giant cells and peripheral lymphocytes. The granulomas are usually situated in the superficial part of the endometrium often in close opposition to a gland.

In those cases presenting with amenorrhoea, endometrial replacement with caseous granuloma is a predominant finding because there is ample time for the lesions to progress to full blown caseous granuloma [145,175]. Granulomatous lesions

are highly indicative of, but not exclusive to tuberculosis unless TB Bacilli are seen [123].

Fluorescent auramine phenol and rhodamine staining has also been used to identify tubercle bacilli. Granulomatous endometritis, even with a negative acid fast stain and culture is generally presumed to be of tuberculous origin. Endometrium is one of the few tissues in which granulomas that simulate TB such as sarcoidosis, foreign body reaction are extremely rare. When a granulomatous endometritis is present, it is probably tuberculosis [61]. In the absence of typical granuloma, dilatation of glands, active destruction of epithelium and inflammatory exudates in the lumen suggest tubercular aetiology [64].

The reported incidence of endometrial tuberculosis varies. In a recent study in 2005, none of the biopsy specimens had histopathological indication of tuberculosis [133].

Manjari et.al. (1995) reported 1.87% positivity in histology [163]. In Nagpal's series (2001), positive histology of endometrial tuberculosis was observed in 3.8% of cases [88]. A histopathological diagnosis of tuberculous endometritis has been reported in 2.3% of 42770 Gynaecological specimens [64]. In Misra et al study, tubercular endometritis was recognized in 4.92% of cases and the incidence was found to be more in secondary infertility than primary infertility [81]. The nature of tuberculous lesion was classified into Proliferative type, exudative type and mixed type. The commonest histopathology in endometrial TB was Proliferative type, reported in 50.1% of cases [149]. The endometrium can almost be completely replaced by tuberculous granulation tissue. In one study the incidence of lost endometrium was reported to be 50.95% [176].

Though endometrial curettage is the commonest procedure to obtain endometrial tissues, studies have looked at endometrial aspiration cytology for the diagnosis of genital tuberculosis. Endometrial aspiration cytology has been claimed to

be simple, inexpensive and less traumatic, can be repeated more than once and can be done in the out-patient departments (OPD) [178,108].

Baijal L et al study showed that in one procedure, the procedure of uterine aspiration cytology provided adequate material both for cytological and histological evaluation by cell block [179]. Endometrial aspiration cytology is characterized by the presence of epitheloid cells, giant cells and plenty of histiocytes in the smear [108]. In cases with intense caseation, eosinophilic granular material was noted [179]. The accuracy of endometrial aspiration cytology has been reported to be 100% when compared with histology and aspiration cytology has been recommended as a screening procedure in the diagnosis of genital tuberculosis [180]. In Baijal et al study, eight smears showed evidence of endometrial tuberculosis and the diagnosis was confirmed by histology either by cell block or the endometrial biopsy [179]. Examination of menstrual blood by direct smear for mycobacterium tuberculosis gives very poor results [108].

**The Polymerase chain reaction (PCR) in the diagnosis of genital tuberculosis**

Accurate identification and early diagnosis of tuberculosis is vital in the management of pulmonary and extra-pulmonary tuberculosis. In extra-pulmonary forms of tuberculosis such as genital tuberculosis, the diagnosis of the condition is more challenging. In recent years, there has been an exciting development in the field of molecular biology and the last decade has seen major advances in the understanding of the genetic structure of mycobacteria. This has lead to the development of specific gene sequences, probes and gene amplification system for the diagnosis of tuberculosis.

Nucleic acid amplification tests (NAA) enable the clinician to make a rapid and accurate diagnosis. All NAA tests amplify target nucleic acid region (DNA or RNA) that uniquely identify the mycobacterial tuberculosis. Because NAA tests can

be used directly on clinical specimens, they are also called "Direct amplification tests" (DAT) [181].

PCR technique is the best known and most widely used NAA test and has evolved as a rapid, sensitive and specific molecular biological method for detecting mycobacterial DNA in both pulmonary and extra-pulmonary samples from patients suspected of suffering from tuberculosis [182].

PCR was first described in 1988 and was used to detect *MTB* a year later [183]. A variety of PCR methods have been developed for detection of specific sequences of MTB and other mycobacteria [137].

The PCR technique is capable of amplifying minute amounts of DNA, even a single copy of DNA into millions of identical copies of DNA sequence. The specific DNA sequence may be a gene, part of a gene or a stretch of nucleotides.

**PCR Technique**

The specimen that contains the DNA sequence of interest is heated to denature double stranded DNA. The specific oligonucleotide primers (short single stranded pieces of DNA) bind to the DNA sequence of interest. Now a heat stable DNA polymerase extends the primers to create a complete and complimentary strand of DNA. The process in repeated sequentially 20-40 times, thereby creating millions of copies of DNA sequence. The amplified sequences are now easily detected by gel electrophoresis. If the target DNA sequence is not present in the sample examined, primers have nothing to bind to, and no amplification occurs. So PCR offers unmatched sensitivity and specificity [184].

In developing countries, clinical samples are often stored for subsequent analysis since molecular tests are conducted at only a limited number of laboratories. A study was conducted to assess the speed at which mycobacteria undergo autolysis and free DNA is detected in the supernatant during low-temperature storage. Study by Pathak et al (2007) propose that supernatant fluid is a valuable sample for PCR for

both fresh and stored specimens, particularly those with a low bacterial load in addition to conventional sediment [185].

PCR tests are rapid and the results are usually obtained within 6-12 hours. Many studies have shown PCR to be the most sensitive and rapid method to detect extra-pulmonary mycobacterial tuberculosis [186,187,188]. A report from Uganda showed that PCR resulted in a 75% reduction in cost for a single test compared with BACTEC and concluded that PCR is a reliable and cheaper alternative for the identification of Mycobacterium tuberculosis complex [189].

Before PCR could be applied to the study of tuberculosis, a specific DNA amplification target has to be identified. The sequence should not be shared by other pathogens and other mycobacterial species.

**Several target sequences have been described to detect *MTB*:**

Ribosomal RNA (r RNA) sequence [190,191], single copy genes encoding structural proteins of 65 and 38 Kilo Dalton. (KD) [183], Gene sequence that encodes for ESAT-6 protein [71], MPB 64[192], Insertion element IS 6110[193] and TRC 4 Primers [194].

**IS 6110 Primer:**

The insertion sequence of IS 6110 belongs to a class of molecules known as transposons, which are self-replicating stretches of DNA that can become permanently integrated into the host genome. IS 6110 sequence has been found in the *MTB* complex of organisms. (Mycobacterium Tuberculosis, Mycobacterium Africanum, Mycobacterium Bovis), but in no other mycobacterial species. Except for MTB the other members of the *MTB* complex are not usual human pathogens or colonizers and could not be likely to cause false positive results. Thus IS 6110 serves as a useful amplification target [195].

Generally the IS 6110 element is present in high copy numbers in most strains of *MTB* and low copy numbers is M.Bovis strains [196,197]. The

IS 6110 primers amplify a fragment with a length of 123 bp. [198]. Report by Negi et al (2007) suggests that the presence of IS6110 correlates more closely with the diagnosis of clinical tuberculosis than that of 65kDa, 38kDa and 85B proteins [199].

The most widely used primers to detect *MTB* in clinical specimens in PCR are from the insertion element IS 6110. However, Narayanan et al. (1997) had reported that 40% of the strains of MTB isolated from patients in Chennai had only a single copy of IS 6110 and 4% did not carry even a single copy of IS 6110 [194]. Since IS 6110 based PCR for diagnosis may in some cases lead to false negative results, a new target for PCR using repetitive element called pTRC $_4$ was developed at the Tuberculosis Research Centre, Chennai [194].

**TRC $_4$ primer**

This is a conserved sequence and repeats at least four times in the genome of *MTB.* From the deduced sequence of 2.126 kb of $TRC_4$ several primer pairs have been designed and one set of primers amplifies a target sequence of 173 bp consistently. Its nucleotide sequence has been assigned GenBank Accession No. µ 84405. $pTRC_4$ was found to be specific for M.tuberculosis complex, and did not cross react with any of the non-mycobacterial species. This $pTRC_4$ clone in addition to being specific for M.tuberculosis complex has also been found to be a repetitive element. Therefore, it is an ideal target for diagnosis.

The PCR with both probes IS 6110 and $TRC_4$ can be used as a fast and sensitive adjunct to other conventional techniques in the diagnosis of extra-pulmonary tuberculosis [200].

Some of these above assays have been repeatedly found to be reproducible, highly sensitive and specific in double blind evaluation [183]. In general, gene amplification methods have been found to be highly sensitive and specific for diagnosis of tuberculosis directly from the clinical specimen [137].

**Sensitivity and specificity of Diagnostic methods**

Various studies have reported sensitivity between 70-100% and specificity between 80-100% by gene amplification methods.

Comparative data for sensitivity and specificity of various PCR methods.

| S.No. | PCR | Specimen | Sensitivity % | Specificity % |
|---|---|---|---|---|
| 1. | PCR – 36 KD [201] | Sputum | 88.9 | 93.9 |
| 2. | PCR – IS 6110[202] | CSF | 81 | 80 |
| 3. | PCR-TRC-4 [202] | CSF | 91 | 76 |
| 4. | PCR-MPB64 [192] | CSF | 75 | 100 |

A number of variables can affect the sensitivity and specificity of a PCR reaction [203].

1. Temperature at which the primers are allowed to anneal to the template DNA in the samples.
2. The composition of the buffer in which the reaction is carried out (particularly the magnesium concentration).
3. The number of cycles over which the DNA amplification is continued.
4. The concentration of primers and the DNA template.
5. And the method used to examine the DNA amplification products. (Agarose gel electrophoresis, dot blot hybridization etc.

In Bhanu et al. study (2005), in order to exclude the possibility of false negativity, 37 PCR products negative by gel electrophoresis were hybridized to 32 p-MPT 3. The PCR by gel electrophoresis and hybridization were concordant in 97.3% of the negative results and in 2.7% it was positive by hybridization method [133].

A Meta analysis [181] on the accuracy (test performance characteristics such as sensitivity and specificity) and reliability (repeatability) of NAA tests for tuberculosis showed that:

1. Nucleic acid amplification tests (NAA), in general have high specificity and positive predictive value. A positive test in a patient with a reasonably high pre test probability is fairly confirmatory of tuberculosis.
2. NAA tests, however, have a lower sensitivity and negative predictive value. Therefore, a negative test does not exclude the diagnosis of tuberculosis.
3. NAA tests have the highest sensitivity in patients with smear-positive pulmonary tuberculosis. Their sensitivity tends to be poor in patients with smear-negative pulmonary tuberculosis and extra pulmonary form of tuberculosis. In these patients negative NAA does not rule out tuberculosis.
4. Therefore, a negative NAA test in a patient with a high index of clinical suspicion should prompt continued investigation.
5. NAA tests amplify dead bacilli and cannot distinguish viable from non-viable bacilli, therefore, they should not be used to monitor response to anti-tuberculosis therapy, (ATT).

**Clinical application of the polymerase chain reaction to tuberculosis**.

**Advantages:**

1. It is a rapid test with results available within a day of the DNA being extracted from the sample.
2. Using IS 6110 sequence as an amplification target; there is virtually no misidentification of *MTB* with any other mycobacterial species. PCR could distinguish between tuberculosis and non tuberculous mycobacteria in cases of smear-positive disease [204].
3. PCR technique requires only < 10 bacteria /ml of specimen to achieve a positive test.

4. It can also be applied to sterile fluids like peritoneal fluid where the culture is difficult due to low bacterial load [182].
5. When a histopathological diagnosis of chronic granulomatous inflammation is reported which may be due to Mycobacterium tuberculosis, foreign body reaction, sarcoidosis and brucellosis, TB-PCR should be performed to enable a definitive diagnosis of tuberculosis [205,206].
6. Similarly, in situations where tuberculosis is suspected clinically and in patients who are immunocompromised, where the HPE reveals non-specific inflammation, the TB-PCR would be a rapid and sensitive method for a definitive diagnosis

**Limitations of PCR**

1. NAA tests should be performed only in established laboratories that have adequate quality assurance and monitoring system in place. In the absence of such system, these tests can produce false-positive results and lead to unnecessary intervention.
2. Complex technique, quality control, reproducibility of results and variation from laboratory to laboratory are other limitations.
3. In developing countries like India the high cost of the test should be taken into account.
4. In specimens from extra-pulmonary sites there are difficulties in diagnosis even by PCR, due to small volume of samples and an irregular dispersion of the organisms in the traditionally paucibacillary sites [207,208].
5. PCR may detect dead organisms in patients with clinically inactive disease. In a patient with a known history of tuberculosis, a positive PCR must be interpreted with caution and may not indicate active disease. In patients receiving chemotherapy, PCR should not be used as an indicator of infectivity at present as the assay remains positive for a greater time than mycobacterial culture.
7. False positive and false negative results of PCR.

False positivity can occur due to contamination occurring in clinics and laboratories. The problem of false positivity can be substantially reduced by proper laboratory design, strict discipline about collection and processing of the specimen, handling of reagents and use of certain blocking agents [137].

In cases of false negative results, very small number of organisms and inhibitors in paucibacillary specimens are especially important. False negative result can occur when there is contamination of the sample with heparin which is a known PCR inhibitor [123].

The analyzed specimen may contain inhibitors of PCR. A thorough DNA purification is of the greatest importance in testing blood containing samples. The inhibitory activity of clinical material can be decreased by additional purification of DNA by gel filtration on micro columns.

Several strategies have been used to improve the sensitivity; such as use of immunomagnetic beads, [209] and capture resins [210].

Cultivation of *MTB* is considered the Gold standard for the diagnosis of tuberculosis. However, this gold standard lacks sensitivity and is negative in specimens from majority of paucibacillary cases. This poses great dilemma for comparing gene amplification methods which are vastly more sensitive but have danger of false positivity due to contamination.

Due to various limitations of false negativity and danger of false positivity, no clear guidelines for application of gene amplification methods are available [137].

When an absolute gold standard diagnostic test is not available against which a new test should be compared, one should develop and justify a combination of criteria against which a new test is to be assessed [211].

American Thoracic Society recommended using PCR as additional evidence in specimens smear positive for AFB. For smear negative cases cautious approach of

using gene amplification as one of the evidences would be preferable which should be repeated in case of doubt [212,213].

**PCR in Extra pulmonary tuberculosis:**

The diagnosis of extra pulmonary tuberculosis is complicated because:

- There is difficulty in obtaining adequate material for examination.
- An invasive procedure is frequently required to obtain adequate material for examination.
- Often there is low yield of material.
- Due to paucibacillary nature there is diagnostic difficulties by direct examination and culture.

Vishnevskaia (214,214A) has shown that PCR would be a valuable adjunct in the diagnosis of extra-pulmonary tuberculosis. Study by Negi et al (2006) has shown that PCR test is more sensitive than ZN smear examination, LJ medium culture and BACTEC culture for diagnosing TB in pulmonary and extra-pulmonary clinical samples [215].

Involvement of genital tract by *MTB* has been demonstrated in historic population. PCR analysis conducted on mummies of the Andes mountain region, South America, dating from A.D.140-1200 showed the presence of *MTB* in the genital area and the study confirmed the existence of *MTB* in historic population [11].

In recent years, PCR technique has been found to be useful in confirming the diagnosis in a substantial number of cases of tuberculosis of female genital tract [216,217,218,219]. PCR was used to examine endometrial curettage specimens in 44 patients with different nosological entities, which showed a high sensitivity (80%) and a high specificity in the diagnosis of genital tuberculosis [220].

In a report by Baum et al. 2001, PCR of *MTB* was used to support a clinical and histological diagnosis of a typical case of culture negative female genital tract

tuberculosis [216]. Gupta et al. study (2007) showed PCR positivity in 9 cases among the 40 infertile women affected with genital tuberculosis [105].

A study from Ethiopia (2004) showed that among the 25 clinically suspected female genital tuberculosis, one was AFB smear positive, three were culture positive , seven were histology positive and 12 ( 48% ) were positive by PCR. Samples taken from the fallopian tube were more frequently positive than those from the endometrium. The study concluded that, to achieve sufficient sensitivity and specificity for the diagnosis of female genital tract tuberculosis, PCR should be combined with the other available methods of diagnosis [150].

Himanshu et al. (2003), conducted PCR studies using IS 6110 repeat sequence primers on the endometrial tissue on 150 sub-fertile women. In this study 57.33% were found to be PCR positive and 42.67% were PCR negative. The findings at HSG and laparoscopy and Mantoux positivity between the PCR positive and PCR negative group were not statistically significant. On histo pathological examination none of the PCR positive or PCR negative group showed evidence of endometrial tuberculosis. The study concluded that tuberculosis remains undiagnosed in many women by traditional methods and it can be diagnosed by PCR on endometrial tissue [57].

Few studies have looked at the sensitivity and specificity of PCR in the diagnosis of female genital tract tuberculosis.

Rozati et al. (2006), investigated the value of different diagnostic techniques for the diagnosis of genital tuberculosis in 65 infertile women, suspected of having genital tuberculosis on clinical grounds. In this study *MTB* was diagnosed by AFB smear in 5.2%, by positive culture in 7.8%, by histopathology in 11.05% and by PCR in 43.1% of suspected cases. Their study showed that without PCR; 50% of the clinically suspected cases would have been missed. Interestingly, four of the seven histology positives were not supported by PCR [71].

The sensitivity and specificity of various diagnostic methods used in this study are as follows:

| Procedure | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|
| Smear microscopy | 87.5% | 86.36% | 70% | 95% |
| Histopathology | 82.3% | 84.6% | 87.5% | 78.5% |
| Culture of Mycobacterium | 91.6% | 88.88% | 84.6% | 94.1% |
| PCR | 96.4% | 100% | 100% | 66.66% |
| Combination of culture and PCR | 100% | 100% | 100% | 100% |

The study concluded that when the clinical suspicion of tuberculosis is high, PCR is the best method of diagnosing genital tuberculosis and the technique of choice for its high sensitivity and specificity [71].

In a double blind study, by Bhanu et al. (2005), the presence of the mpt 64 gene of *MTB* by PCR was investigated on 61 samples obtained from 25 infertile women and correlated with laparoscopic findings. PCR demonstrated *MTB* DNA in 56% of cases, AFB smear was positive in 1.6% of cases and culture was positive in 3.2% of cases. The sensitivity of PCR in both endometrial biopsy and endometrial aspiration samples were 76.9%. Comparing PCR with laparoscopy findings, all patients with laparoscopy suggestive of tuberculosis with caseation, granulomas, tubercles and beaded tubes, 60% of those with a probable diagnosis of TB with hydrosalpinx without frank tubercles or caseation, T-O masses, adhesion and 33% with incidental findings were positive by PCR.

PCR was also positive in one infertile patient with normal laparoscopy. The possibility of patient harbouring a latent infection was considered in this case. To rule out false positivity in this case, PCR assay was repeated in a second series of samples and the reproducibility was confirmed. The study showed that PCR shows a seven

fold increase in the sensitivity of detection compared to the culture and 14 fold more sensitive compared to the smear. The study concluded that proper sampling, multiple sampling and repeat sampling from the patient will enhance the sensitivity of PCR as a diagnostic tool of genital tuberculosis [133]. In this study, 53.3% of endometrial biopsy samples, 47.6% of endometrial aspiration samples and 16% of POD aspirate were positive for PCR.

**Immuno diagnosis of tuberculosis:**

There has been interest shown in immunodiagnosis in situations where infective agent is sparse or may not be available for investigations.

Immunoassay is useful in tuberculosis:

1. In situations where there is relative difficulty in obtaining specimens as in extra pulmonary tuberculosis.
2. Whenever there is relative difficulty in culturing the mycobacteria
3. In situations where longer time is required for culture.

The Sero diagnostic techniques use

1 Antibody detection against mycobacterial antigens

2 Identifying specific antigens of mycobacteria in clinical specimens.

Antibody detection against *MTB.*

Elisa methodology is used to measure antibodies against various mycobacterial antigens: 38 – Kilo Dalton, 30 Kilo Dalton, 16 Kilo Dalton, A 60 and Lipoarabinomannan.

Antibodies that are tested are IgG, IgM, IgA

IgM:

IgM is the initial antibody response, but the levels are very low and the measurement is difficult.

IgG:

Greater and more frequent exposure to antigen generally leads to a more vigorous IgG antibody response. Small antigenic exposures are seen in primary tuberculous infection, BCG vaccination and contact with environmental bacteria. These situations do not induce a substantial IgG antibody response.

**Serodiagnosis of Extrapulmonary tuberculosis**:

Miliary tuberculosis, TB meningitis, pleurisy with effusion is often acute and may occur immediately after primary infection. In these conditions sero diagnosis may not be reliable. Whereas TB osteomyelitis, renal tuberculosis and genito urinary tuberculosis are late manifestations of disease and these patients often run a chronic course, and sero diagnosis may be of value in these conditions.

Antibody response is markedly decreased or absent in all HIV infected tuberculous patients. So, Elisa antibody detection has limited potential in AID prevalent population [221].

**TREATMENT**

Early diagnosis of the disease with culture for AFB, but before the development of histological evidence of disease may be associated with a more favourable outcome if the disease is treated promptly and adequately [222].

Based on the extent of involvement of genital organs, genital tuberculosis manifests as minimal and advanced disease.

Minimal genital tuberculosis is usually asymptomatic except for sterility. T-O masses are not palpable although induration in the tubes may be present. Diagnosis is based on bacteriological or microscopic evidence of tuberculosis. Successful pregnancy can be achieved if treated adequately at this stage. In advanced tuberculosis, palpable T-O masses are present with extensive tubal damage [223].

In cases of proven genital tuberculosis, prior to commencement of drug treatment, baseline investigations must include chest X– ray, urine examination, three

early morning sputum or gastric aspirate samples for the presence of *MTB.* Full urological examination is indicated when urine is positive for tuberculosis [93, 16].

Treatment strategies of genital tuberculosis are similar to those of extra – pulmonary tuberculosis. Treatment of genital tuberculosis with 9 month short course chemotherapy has been shown to be effective [82, 54,108]. The 6 month regimen that is now the standard for pulmonary tuberculosis has shown to be successful in treating active genital tuberculosis also.

The recommended treatment regimen by the Revised National Tuberculosis Control Programme (RNTCP) for extrapulmonary tuberculosis (category III) is as follows: These patients receive a 6-month regimen consisting of isoniazid (600 mg.), rifampicin (450 mg.)and pyrazinamide (1500mg) thrice weekly for 2 months followed by isoniazid and rifampicin thrice weekly for 4 months ( 2 HRZ thrice-weekly/ 4 HR thrice- weekly) [223A].

After treatment of GTB follow up is important to check for endometrial conversion. Following medical treatment, the patient with GTB should be followed closely for an indefinite period of time. Chest X-ray and the uterine curettage should be repeated at the end of the treatment course. Endometrial biopsy should be repeated every 6-12 months for a few years [104].

Surgical treatment of pelvic tuberculosis is usually contemplated for persistent/ recurrent pelvic mass or abnormal bleeding [134]. Kardos believes that tubercular foci in the adnexae should be removed if there is no clinical improvement after 6 months of chemotherapy [223].When surgery is contemplated, chemotherapy should be started at least 1-2 weeks pre-operatively and medical treatment to be continued for 6-9 months after surgery. Under the cover of chemotherapy, morbidity and mortality are significantly reduced [224].

If the patient does not conceive after one year of completion of therapy, HSG or laparoscopy is carried out to check tubal patency. Though tubal patency may have

been restored following ATT, the tubes may remain rigid and beaded in most cases. Despite the advances in chemotherapeutic treatment, pregnancy after treating genital tuberculosis is rare and when it does occur is more likely to be an ectopic or result in spontaneous abortion [225,226,227].

The outcome of pregnancy in treated cases of GTB is not very optimistic. Among 97 cases of infertile women with GTB, Tripathy et al (2002) reported a conception rate of 19.2% and a live birth rate of 7.2% [228]. Investigators have supported the use of In vitro fertilization and embryo transfer (IVF – ET) as a successful method for treating infertility of tuberculous origin [43,229].

Total corporeal synechiae due to tuberculosis carries a very poor prognosis. Surrogacy is the only option in this group of women. Among patients treated with IVF-ET, patients with GTB are a less favourable subset among other tubal infertility cases. [43] Sadhana et al reported 11 pregnancies, one ectopic pregnancy and one second trimester abortion of a twin gestation among the 39 cases of IVF-ET who were treated for genital tuberculosis earlier [97].

# CHAPTER III

## AIMS AND OBJECTIVES OF THE STUDY

The aims of this study are:

1) To analyze the symptomatology and clinical parameters to identify risk factors to arrive at a presumptive diagnosis of genital tuberculosis in infertile women.

2) To evaluate the efficacy of Histo-pathological examination, culture studies and Polymerase Chain Reaction Technique in the diagnosis of genital tuberculosis in female infertility.

3) To identify a suitable diagnostic test which can be used in clinical practice.

4) To establish an early diagnostic strategy.

5) To evaluate the efficiency of two sets of primers used in PCR in diagnosing genital TB.

# CHAPTER IV

# METHODS & MATERIALS

Methodology of the study included research design, population, sample size, data collection procedures, and description of diagnostic tests and statistical analysis of data.

**RESEARCH APPROACH &DESIGN:**

The research approach used in this study was evaluative approach. In this prospective study, the clinical methods and various diagnostic methods were analyzed and evaluated for their effectiveness in diagnosing Genital Tuberculosis.

**SETTING OF THE STUDY:**

This study was conducted at the Fertility Research Clinic of the Institute of Obstetrics and Gynaecology (IOG), Chennai, in collaboration with the Tuberculosis Research Centre (TRC), Indian Council of Medical Research (ICMR), Chennai.

This hospital is a tertiary care centre and a referral hospital located in the city of Chennai. In this hospital there are 752 beds with General Obstetrics and Gynaecology units, and the specialty departments of Endocrinology, Genetics, Medical Oncology, Radio-oncology, Neonatology and Fertility Research Centre. (FRC)

**POPULATION:**

Women who attended the FRC clinic of the Institute of Obstetrics and Gynaecology and Govt. Hospital for Women and Children, Egmore, Chennai, with a complaint of inability to conceive were selected as the population for this study. Approximately 1200 new sub- fertility cases are registered in the Fertility clinic every year, and nearly 3000 cases are being followed up and treated.

**SAMPLING TECHNIQUE:**

Convenience Sampling Technique was employed for this study.

**SAMPLE SIZE AND PERIOD OF STUDY:**

The study was conducted from January 2005 to December 2007.

The material for the study included 173 subjects who met the inclusion and exclusion criteria of this study.

**CRITERIA FOR SAMPLE SELECTION:**

**Inclusion Criteria:**

Infertile women who presented with one or more of the following findings were included in the study.

1. Women with tubal factor infertility proved by either hysterosalpingogram and / or laparoscopy.
2. Those presenting with unexplained infertility
3. Cases presenting with recurrent pelvic inflammatory disease (PID), refractory to conventional therapy.
4. Those cases with adnexal masses and ascites diagnosed by ultrasound scan.
5. Those cases with past history of tuberculosis and those with history of close contact with a person suffering from tuberculosis were also included in the study.
6. Clinical features suggestive of active tuberculosis in extra genital sites.

**Exclusion Criteria:**

Those women in whom infertility was due to the following factors were excluded from the study.

1. Abnormalities of ovulation
2. Male Factors
3. Endocrine problems
4. Sexual disorders
5. Endometriosis

6. Peritoneal adhesions due to previous abdominal surgery.

**DESCRIPTION OF INSTRUMENT /TOOL:**

The investigator developed the instrument for this study based on review of books, journals and research report. The specific diagnostic tests were carried out as per the protocol followed in the Department of Microbiology and Immunology of TRC, and the Pathology Department of the Institute of Obstetrics and Gynaecology. The content validity was obtained from the Guide and Co- Guides as well as from the Advisory Committee for PhD, The Tamilnadu Dr.M.G.R. Medical University, Chennai.

**FORMAT OF THE TOOL:**

The tool consisted of four sections

| | | |
|---|---|---|
| Section I | : | Deals with the demographic data, Gynaecological symptoms and symptoms related to tuberculosis. |
| Section II | : | Gives the examination findings |
| Section III | : | Includes investigations and Gynaecological procedures |
| Section IV | : | Includes specific diagnostic tests for tuberculosis |

**Section 1: CLINICAL PROFILE.**

A detailed history and a thorough clinical examination were carried out and the following clinical profiles of the patients were noted in the proforma. (Annexure I)

- Age, duration of marital life
- Type of infertility – primary / secondary
- Menstrual abnormalities – secondary amenorrhoea / oligomenorrhoea / menorrhagia / metrorrhagia
- Dysmenorrhoea, Dyspareunia**,** Discharge per vaginum, chronic pelvic pain, abdominal pain / bloating, treatment for PID.
- Bowel / Urinary Symptoms

- History of previous surgery done for appendicitis, ovarian cysts were looked for.
- Constitutional symptoms such as loss of weight, loss of appetite and evening rise of temperature suggesting active disease was also looked for.
- Subjects were screened for evidence of past history of tuberculosis in various sites such as lungs, abdomen and lymph nodes. In those women who have had TB in the past, the time since diagnosis and the duration of treatment were also noted.
- In order to obtain additional information, a history of close contact with family members and neighbors who suffered from tuberculosis was also obtained.
- In cases of secondary infertility, delivery details and previous abortion details were also noted.

**Section II: EXAMINATION**

All subjects were examined for anemia, Body Mass Index (BMI) noted and evidence of previous tuberculosis and respiratory system involvement were looked for.

A thorough abdominal examination and bimanual pelvic examination were carried out for detection of any evidence of tuberculosis. On abdominal examination findings such as abdominal mass, abdominal distension ascites and doughy feel of abdomen were looked for. By vaginal examination findings such as tubo-ovarian mass (T-O Mass) and irregularity of pouch of Douglas (POD) were noted.

**Section III: INVESTIGATIONS**

All patients were subjected to the following preliminary investigations:

1. Complete blood count
2. Erythrocyte sedimentation rate (ESR)
3. Tuberculin Test: MANTOUX TEST – (Mx)

   Tuberculin test was performed for all patients by intra cutaneous injection of 0.1ml purified protein derivative – tuberculin (5 tuberculin units) and assessed

for the presence of induration within 48-72hours. A positive tuberculin test is defined as an induration size equal to or more than 10mm. [230].

4. All cases were evaluated by chest X-ray for the presence of active or old foci of pulmonary tuberculosis.
5. In cases where pelvic ultrasound showed evidence of calcification, abdomen and pelvic X-rays were taken for confirmation.
6. In 131 subjects hysterosalpingogram (HSG) was done and characteristic findings such as cornual blocks, distal blocks and beaded tubes; retort shaped hydrosalpinges, localized spill, intravasation and filling defect in the uterine cavity were looked for.
7. Imaging with abdominal ultrasound (USG) was done as a routine in all infertile patients. The finding of a large amount of loculated fluid containing septations with bilateral hydropic fallopian tubes and T-O masses was suggestive of tuberculosis.
8. Laparoscopy was carried out in all cases to ascertain the tubal patency / tubal block and to diagnose other pelvic pathology. During the procedure, features suggesting acute TB infection such as frank miliary tubercles with peritoneal congestion, caseation, granulomas and straw coloured ascitic fluid were looked for. Evidence of past chronic infection in the form of thickened tubes, intraluminal caseation, and terminal hydrosalpinx with retort shaped tubes, tubo-ovarian masses, flimsy adhesions in the pouch of Douglas were looked for. Also, at the time of laparoscopy fluid from the pouch of Douglas was aspirated for culture and PCR studies.
9. Wherever possible, tissue biopsy was also taken from the tube, ovary and the peritoneal surface.
10. In all cases the endometrial tissue was curetted in the pre-menstrual phase of the cycle for specific investigations.

11. In 52 cases, three consecutive urine samples were collected for culture and PCR studies to look for associated urinary tract involvement.
12. HIV I and II were tested in all cases for both wife and husband.

**Section IV: Specific Investigations:**

The material for the study was collected from three sources.

1. Pre-menstrual Endometrium
2. Fluid from the Pouch of Douglas
3. Urine.

- The Endometrial specimens were divided into three portions and subjected to smear for AFB, Culture for *MTB* infection, PCR studies and Histo – Pathology examination (HPE)
- Pouch of Douglas aspirate and urine samples were divided into two portions and subjected to smear for AFB, culture and PCR studies.
- Culture and PCR studies were carried out at the Tuberculosis Research Centre, ICMR, Chennai.
- Histo-pathology examination of the specimens was carried out at the Pathology Department of the Institute of Obstetrics and Gynaecology, Chennai.

## DESCRIPTION OF SPECIFIC DIAGNOSTIC TESTS

## MICROBIOLOGICAL TESTS FOR MYCOBACTERIUM TUBERCULOSIS (SMEAR AND CULTURE)

Microbiological tests for *MTB* were carried out as per the guidelines of TRC, ICMR, Chennai [231,232,233].

**COLLECTION OF SPECIMEN:**

In 173 infertile women laparoscopy was carried out and during the procedure fluid from the POD was aspirated and sent for culture for *MTB* and smear study. A premenstrual curettage was done and the curettings were also sent for microbiological tests. In 52 patients urine samples were collected on 3 consecutive days.

**TRANSPORTATION:**

The POD aspirate, the biopsy material and urine samples were transported in sterile containers within one hour of collection and were processed within a few hour of collection to retain the viability of tubercle bacilli.

**PROCESSING OF SAMPLES AND INOCULATION:**

Since extra pulmonary specimens, in general are paucibacillary in nature, their processing methods require milder decontamination. Further, these specimens are inoculated onto multiple media, namely

- Lowenstein Jensen Medium (L.J.)( It is an egg based Medium)
- L-J enriched with sodium pyruvate (L.J.P.)
- Kirchner’s liquid medium (K)
- Selective Kirchner’s medium (SK)

In order to selectively inhibit the growth of other micro organisms, Kirchner's medium is made selective by incorporating polymyxin, amphotericin B, carbenicillin and trimethoprim. (PACT)

**PREPARATION OF POUCH OF DOUGLAS (POD) ASPIRATE**

**SMEAR**:

Using a 5mm loop, one loopful of POD aspirate is placed in the middle of the slide, when dry; one more drop is placed in the same spot and allowed to dry. Another drop from the deposit is smeared on the same spot and fixed.

**CULTURE**:

- Before processing, one loopful of peritoneal fluid is inoculated onto LJ, LJ-P solid medium, while 0.2ml is transferred to SK medium, using a sterile pipette. (A Set)
- The remaining specimen is processed and decontaminated, then inoculated into culture media.
- Decontamination:

  Centrifuge the peritoneal fluid at 3000rpm for 15 minutes. Decant the supernatant fluid. To the deposit add 1ml of sterile distilled water and 1ml of 5% $H_2SO_4$. Shake well and keep aside for 15 minutes. Neutralize with distilled water up to the neck of the bottle and centrifuge again as above. Decant the supernatant. After inoculating one loopful of deposit into solid media, the remaining deposit is transferred as a whole to the SK medium. (B Set)

**PREPARATION OF TISSUES / BIOPSY MATERIAL (ANNEXURE IA)**

- On receipt in the laboratory, the tissues are aseptically taken out with a sterile forceps, cut into small pieces with sterile scissors and transferred into a sterile tissue grinder tube.
- To this 5ml of sterile distilled water is added and homogenized with a sterile Teflon grinder.

- From this homogenate, with one loop full of ground biopsy material a direct smear is made.
- The homogenate is then centrifuged at 3000g for 15 minutes.
- The supernatant is discarded and the deposit is decontaminated with 5% $H_2SO_4$ and inoculated on LJ, LJ-P and SK media.

**PROCESSING OF URINE SAMPLES**

No smear is made for urine specimens.

Urine samples are processed as per the protocol of the TRC laboratory (ANNEXURE I B).

**SMEAR STUDY FOR ACID FAST BACILLI**

Microscopic examination for acid fast bacilli is done by two techniques.

- Conventional Ziehl-Neelsen (Z-N) staining
- Fluorescence Staining.

In this study, a fluorescent dye (auramine – O, rhodamine, auramine – rhodamine, acridine orange etc.,) is used for staining and the slides are examined using fluorescence microscope.

**Principle:**

Mycobacteria retain the primary stain even after exposure to decolorizing with acid-alcohol, hence the term “acid-fast”. A counter – stain is employed to highlight the stained organisms for easier recognition. With auramine staining, the bacilli appear as slender bright yellow fluorescent rods, standing out clearly against a dark background. [Fig.1]

**Reporting Of Results**

- ❖ A smear is called positive if it contains a minimum of 4 AFB of typical morphology in the entire smear.
- ❖ If less than 4 bacilli are present, the smear is reported negative.

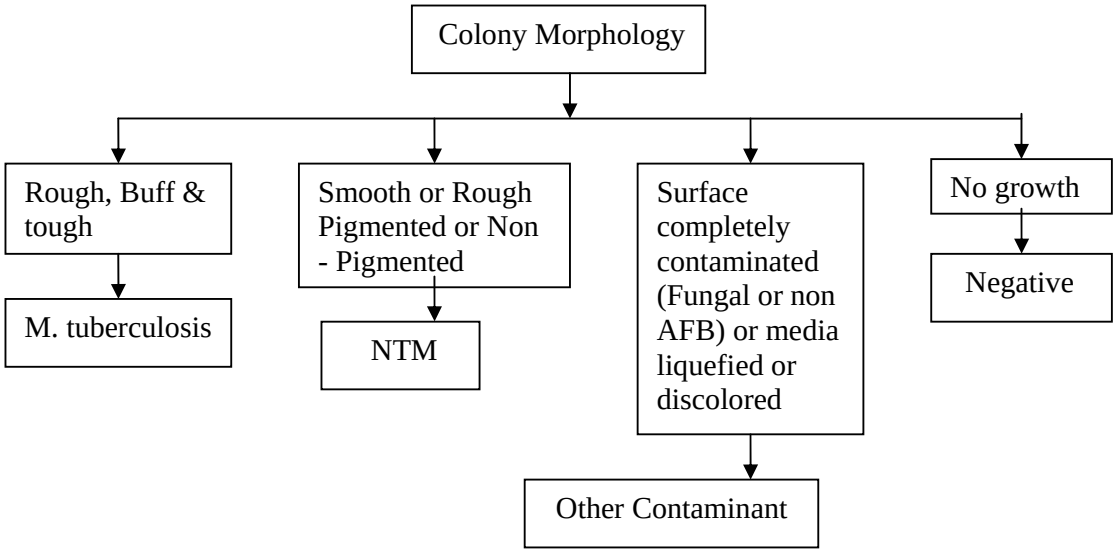
❖ For positive smears at least 50 fields have to be screened.

Grade positive smears into three degrees of positivity using the high power objective as below.

| **No. of bacilli per High Power Field** | **GRADE** |
|---|---|
| Less than 6 per field | 1+ |
| 6-100 bacilli per field | 2+ |
| More than 100 per field or large clumps | 3 + |

**APPEARANCE AND REPORTING OF POSITIVE CULTURE IN SOLID AND LIQUID MEDIA**

Typical colonies of Mycobacterium Tuberculosis are rough, crumbly, waxy, non- pigmented (buff coloured) and slow growers; having the appearance of breadcrumbs or cauliflower. [Fig. 2, 3]



**READING OF THE EXTRA-PULMONARY SPECIMEN CULTURES:**

**Reading of the solid medium slopes:**

All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Mycobacterium tuberculosis proliferates extremely slowly and tubercle bacilli do not grow in primary culture in less than one week and usually requires two to four weeks to give visible growth from specimens. Therefore, cultures are examined weekly, up to eight weeks on a specified day of the week. During examination, slopes in which the surface has been completely contaminated or where the medium has been liquefied or discoloured were discarded.

**The reading of the SK medium bottles:**

The SK medium bottles are observed up to six weeks. When growth is observed in the SK bottles or when a gross turbidity is seen, the bottles are removed, decontaminated with NaOH and inoculated onto two slopes of L-J medium. These are read weekly for a further period of eight weeks.

**IDENTIFICATION OF MYCOBACTERIA [234,235,236]**

Although a presumptive diagnosis of *MTB* may be made on the basis of the morphological characteristics of tubercle bacilli, it is necessary to do confirmatory tests. Identification of mycobacterial species requires a battery of biochemical tests to decide whether the isolate is a pathogenic species of M.tuberculosis complex (M.tuberculosis, M.bovis, M.africanum) or not. The biochemical tests along with the morphological characteristics will enable a precise identification of more than 95% of the M.tuberculosis strains. Niacin production test, Nitrate reduction test, Susceptibility to p-nitro benzoic acid

( PNB ) and Catalase activity at $68^0$C /Ph 7 are commonly used to identify the strain of M.tuberculosis (Fig 4).

The following are the characteristic features of M.tuberculosis [234,235,236].

1. Growth rate is slow
2. Grow at temperature of $35^0 – 37^0$ C only.
3. No pigmentation is produced
4. Niacin positive ( figure 5)
5. Catalase negative at $68^0$ C
6. No growth on LJ medium containing p – nitro benzoic acid.
7. Positive reaction for nitrate production

In this study Niacin production test was used for confirmation of *MTB.*

**Drug Susceptibility Tests (Figure 6)**

In all cases, which were positive for mycobacterial culture, drug susceptibility tests were carried out to confirm the choice of the initial course of chemotherapy. By direct and indirect tests, the susceptibility of mycobacterial bacilli to Isoniazid, Rifampicin, Ethambutol, Ofloxacin, Kanamycin and Ethionamide was studied.

## TISSUE PROCESSING FOR HISTOPATHOLOGICAL EXAMINATION

Tissue biopsies taken from the Endometrium, ovary, tube and the peritoneum were processed as described below:

**Pre-processing:**

Pre-processing involves 1) identification and labeling of the specimen 2) proper fixation of tissue with 10% formalin. The selected tissue bits are put in Tissue Tek cassettes and fixed in formaldehyde overnight.

**Processing:**

The processing of tissue consists of the following steps.

**Dehydration**

This is to remove the fixative and water from the tissues and replacing them with dehydration fluid. The tissue is dehydrated with graded alcoholic solution; 50%,

70%, 95% and 100%, each for one hour. Four changes of 100% alcohol are done, the first three for one hour each, and the last change for 12 hours.

**Clearing**

This is to replace the dehydration fluid. Here two changes of xylene are used for half an hour each. This gives a translucent appearance to the tissue.

**Impregnation and Embedding:**

In order to get thin sections, the tissue is embedded in molten paraffin wax and made into blocks.

**Cutting sections into blocks:**

This is sectioning of paraffin wax embedded tissues in the blocks into thin sections of 6 micron thickness using a Microtome. These sections are then mounted on glass slides (76x25mm) coated with egg albumin and dried in oven for 30 minutes.

**Dewaxing:**

The slides are then dewaxed by treating the slide with two changes of xylene and two changes of absolute alcohol, each for one hour; a total of four hours.

**Staining:**

These dewaxed slides are then stained with routine Haematoxylin and Eosin (H&E) stains. The stained slides are then dried, dehydrated with xylene and alcohol and mounted with DPX mounting medium and are ready for histopathological reporting.

## POYLMERASE CHAIN REACTION TECHNIQUE [198,200]

DNA extraction chemical and PCR chemicals were obtained from USB, Amersham Bioscience.

**I. PROCESSING OF SAMPLES**

The endometrial tissue was finely chopped using a sterile scalpel and homogenized manually in TE buffer (TRIS – EDTA – 10mM Tris.cl. pH 8.0; 1mM

EDTA pH 8.0) until the solution becomes turbid. This was centrifuged at 10,000 rpm for 20 minutes. The supernatant was discarded and the pellet was processed for further studies.

**Processing of Pouch of Douglas (POD) aspirate and urine samples:**

POD aspirate and urine samples were centrifuged at 2500 rpm for 15 minutes. Supernatant was discarded and the pellet was used to extract DNA.

**II. ISOLATION OF DNA**

Pellets were re suspended in 500µl of TE buffer by repeated pipetting. Then 50µl of 10mg/ml of lysozyme was added, mixed well and incubated for one hour at $37^{o}$C. To this sample 70µl of 10%. SDS (Sodium Dodecyl Sulfate) and 6µl of 10mg/ml of proteinase K are mixed and incubated for 10 minutes at $65^{o}$C. After incubation 100µl of 5 M NaCl is added and mixed thoroughly. The samples were further incubated with 80µl of CTAB / NaCl (Cetyl trimethyl ammonium bromide in sodium chloride) solution for 10 minutes at $65^{0}$C. For DNA extraction, to this prepared sample approximately equal volume (700 - 800µl) of chloroform / Isoamyl alcohol are added, mixed thoroughly and spinned in a micro centrifuge for 10 minutes. The aqueous supernatant is removed to a fresh micro centrifuge tube. To this 0.6 volume Isopropanol is added to precipitate the nucleic acids and placed at -$20^{0}$C for 60 minutes. The resultant sample is spinned at 12,000 rpm for 20 minutes at $6^{0}$C. The resulting DNA pellet is washed with 70% ethanol to remove residual CTAB and re-spin at room temperature for 5 minutes to repellet it. The supernatant is carefully removed and the pellet is dried. The prepared pellet is re dissolved in 25µl of TE buffer (910mM TRIS and 1mM EDTA) and stored at $4^{0}$C for future use.

**AMPLIFICATION OF MYCOBACTERIAL DNA**

Polymerase chain reaction (PCR) was performed using Gene amplification 9700 Thermal cycler with standard 25µl working volume. (Gene Amplification PCR System 9700- Applied Biosystems, USA)

Precautions were taken to avoid false positivity. Preparation of PCR reagents, addition of template DNA and analysis of amplified products was done in three different rooms to avoid carryover contamination. Reagents were aliquoted and each aliquot was used only once. Wax beads were added to minimize non-specific amplification.

PCR was carried out in 25 micro liter volumes consisting of the following:

- Template DNA (2-10ng)
- 10 x reaction buffer
- d $NTP_3$ (deoxy nitro tri phosphate (dATP, dTTP, dCTP, dGTP ) - 2.5mM
- MgCl
- Tag DNA polymerase (1µ / µlit)

  Primer 1 (10 pmols / µl) - 1µlit

  IS 6110a (5’ CCT GCG AGC GTA GGC GTC GG – 3’)

  IS 6110b (5’ CTC GTC CAG GCG CGC TTC GG – 3’)

  (PRIMER DESIGNER – Version 2.0 – Copy right 90, 91 Scientific & Educational Software)
- Primer 2 (10pmols/ µl ) - 1µl

  $TRC_4$ primer 1 (5’ GAC AAC GAC GTG CGC CTA CT – 3’)

  $TRC_4$ primer 2 (5’ GAC CGA ATT AGC GTA GCT CC – 3’)

  The IS 6110 primers amplify a fragment with a length of 123 bp, while the 18me $TRC_4$ primers amplify the fragment with a length of 173 bp.
- Milli Q Water.

DNA extracted from H37 Rv served as template for positive control. A negative control was always included along with the clinical samples and positive control. A negative control had PCR buffer, dNTPs and primers except template DNA.

**Cycling Parameters**

The reaction was performed on ice to minimize non-specificity. The cycling parameter used was initial denaturation at $95^0$C for 5 minutes, followed by denaturation at $94^0$C for 30 seconds, annealing at $58^0$C for 30 seconds, extension at $72^0$C for 30 seconds with 25 cycles and a final extension at $72^0$C for 5 minutes.

Detection of amplified products was done by agarose gel electrophoresis (2%) at 80 volts for 45 minutes. Gel was stained with ethidium bromide and viewed under UV transilluminator. (VILBER-LOURMAT, France, TCP- 20.M)

The technician performing the PCR technique was blinded to the clinical impression of tuberculosis and the results of other investigations.

Figure 7 shows PCR results using IS 6110 and TRC4 probes for the detection of Mycobacterium Tuberculosis.

## DIAGNOSIS OF CHLAMYDIA TRACHOMATIS & NEISSERIA GONORRHOEA BY PCR TEST

In order to rule out other organisms such as Chlamydia trachomatis & Neisseria gonorrhoea which can also cause tubal block leading to infertility, the AMPLICOR CT/NG PCR Test (Roche Diagnostic Systems, Inc.,) [237] was performed on the endocervical swabs taken from 173 women. The test was performed at the Venereology Department of Madras Medical College, Chennai.

## STATISTICAL ANALYSIS

Data entry was done using Microsoft Excel in Windows$^{xp}$. Analysis was performed in OPEN EPI VERSION 2.2.1 software.

**Descriptive Statistics**

Descriptive statistics such as frequencies, percentages, ranges and means were used to describe demographic variables and specific tests.

For the diagnosis of genital tuberculosis there is no absolute gold standard test available to say whether for certain , the disease is present or not. In these situations, one may need to develop and justify a combination of criteria against which the new test is to be assessed [211].

Therefore, a combination of criteria has been developed for the diagnosis of genital TB, against which the various diagnostic tests used in this study were validated.

Based on the clinical profile and laparoscopic evaluation of patients a diagnostic criterion was derived to suspect tuberculosis.

A woman was said to be suspected of having genital tuberculosis if she has had findings suggestive of tuberculosis at laparoscopy with one or more of the following findings: A definite past history of tuberculosis, in the presence of active extra-genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux test , evidence of calcification / complex adnexal mass by scan.

**Inferential Statistics**

The specific diagnostic tests: AFB smear, culture, HPE and PCR were evaluated against the newly derived criteria by using bivariate two by two tables. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated. Also, chance corrected agreement was evaluated using Kappa statistics for PCR results of endometrium sample and POD aspirate and for evaluation of two primers for PCR used in the study.

**CONSENT AND ETHICAL CONSIDERATION**

Before undertaking this study Institutional Ethics Committee approval was obtained (Annexure II).

Each subject was explained about the need for this study and the research method in detail in her own language (Annexure III, IV) and the informed consent was obtained. (Annexure V, VI)

The investigator ensured that the privacy and dignity of the subjects were maintained. Their religious and cultural beliefs and ethical values were respected during the process of data collection and investigations.

Refusal to participate in the study was respected and necessary treatment was given as for other cases .In patients who participated in the study, at each stage of investigation findings were explained to them and appropriate treatment was given as and when necessary.

**Figure 1**

AFB Bacilli under Fluorescent Microscopy

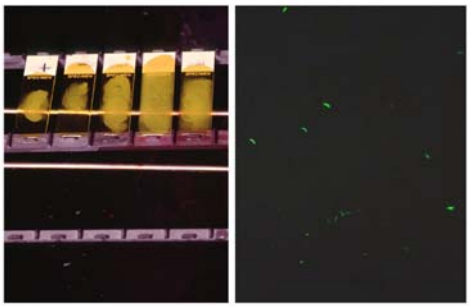


**Figure 2**

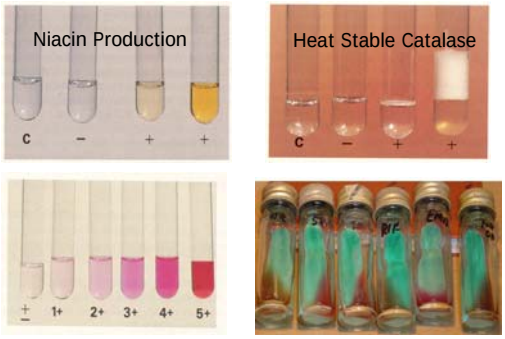
Typical Rough, Buff and Tough Colony of *MTB*

**Figure 3**

**Typical MTB Colony- breadcrumbs or cauliflower appearance**

**Figure 4**

Biochemical Reactions for *MTB*




Aryl Sulphate Nitrate Reduction

**Figure 5**

Positive Niacin Test – confirmatory biochemical test for *MTB*

**Figure 6**

Microscopic observation drug susceptibility (MODS) Assay

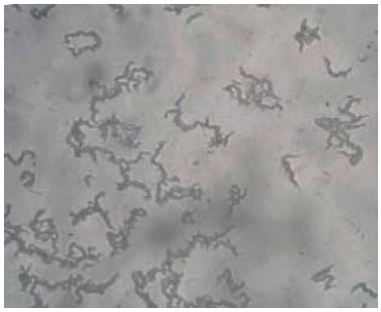
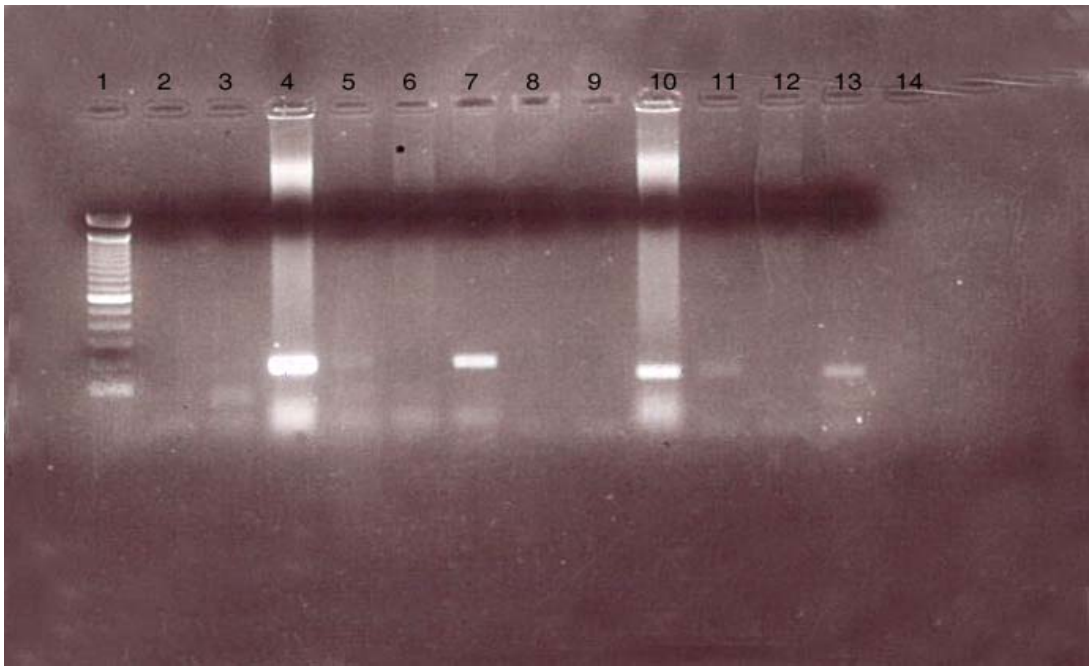


**Figure 7**

PCR Results Using IS6110 and TRC4 Probes for the Detection of Mycobacterium DNA




Well NO. 1- 100 base pair DNA ladder
Well 2 – Negative control TRC4 (no template added)
Well 3 – Processing Blank – TRC4
Well 4 – Positive control TRC4
Well 5 – Patient sample TRC4 Positive (faint)
Well 6 – Patient sample – negative
Well 7 – Patient sample positive (dark)
Well 8-Negative control IS6110
Well 9 – Processing Blank IS6110
Well 10 – Positive control IS6110
Well 11 – Patient sample positive IS6110
Well 12 – Patient sample negative IS6110
Well 13 – Patient sample positive IS6110

# CHAPTER V

# RESULTS

This prospective study was conducted at the Fertility Research clinic of the Institute of Obstetrics and Gynaecology, Chennai in collaboration with Tuberculosis Research Centre, Indian Council of Medical Research (ICMR), Chennai. The study was conducted from January 2005 to December 2007.

The study consisted of 173 subjects who attended the Fertility Research Clinic of this hospital for investigation of infertility of more than 2 years duration. The subjects fulfilled the inclusion and exclusion criteria of the study. All 173 women belonged to lower socio economic group. All the women had infertility as their presenting complaint.

A detailed history of all participants was taken regarding demographic details, type of infertility and duration of marital life. Details were also taken with special reference to gynaecological symptoms, menstrual disturbance, history of contact or past history of TB and history suggestive of active disease. Details of previous investigations and treatment were also noted to rule out other causes of infertility.

Apart from physical examination a detailed gynaecological examination was carried out in all cases.

All the patients underwent a Complete Blood Count, Chest X-ray and a Mantoux test.

All 173 patients underwent Transabdominal USG and laparoscopy and HSG was carried out in 131 cases.

For specific investigations the material for the study was collected from three sources.

1. Pre-menstrual Endometrium

2. Pouch of Douglas aspirate
3. Urine. Three first morning voided midstream urine specimens were sent for AFB stain and culture in 52 cases. Specimens were also tested for the presence of PCR DNA.

The collected samples were subjected to AFB smear examination, culture, HPE and PCR studies.

Based on the clinical profile, laboratory investigation results and findings at Laparoscopy a clinical criterion was derived to suspect GTB.

The specific diagnostic test results were evaluated against the newly derived diagnostic criteria.

The results of all the clinical findings, laboratory results, Laparoscopy findings and the results of specific diagnostic tests are submitted in this chapter.

**ORGANISATION OF DATA**

The data obtained were mainly classified into following six sections.

| | |
|---|---|
| Section A | Distribution of demographic variables and evaluation of clinical symptoms |
| Section B | Examination findings |
| Section C | Analysis of Laboratory Investigations |
| Section D | Results of gynaecological procedures: USG, HSG, Laparoscopy and Hysteroscopy |
| Section E | Results of specific diagnostic tests for genital tuberculosis |
| Section F | Evaluation of specific diagnostic tests in the diagnosis of genital tuberculosis |

All the above details were entered in the Master sheet and Coding were given for Data entry into the Computer.

**SECTION A: CLINICAL SYMPTOMS ANALYSIS**

**AGE DISTRIBUTION:**

The women in this study were aged between 20 and 37 years. The mean age of presentation was 27.35 years with a standard deviation of 3.93years.Table I shows the age distribution among 173 infertile women.

**Table 1: Age Distribution among 173 Infertile Women**

| Age in years | NO. of cases | Percentage |
|---|---|---|
| 20-24 | 45 | 26% |
| 25-29 | 79 | 45.7% |
| 30-34 | 37 | 21.4% |
| 35& above | 12 | 6.9% |
| Total | 173 | 100% |

In this study, 124 (71.7%) patients were aged less than 30 years; showing that female genital tuberculosis is seen in relatively young females.

**DURATION OF MARITAL LIFE: (Diagram 1)**

The duration of marital life ranged between 2 and 15 years. The mean duration of marital life was 6.73 years with a standard deviation of 3.62years. 79 (45.7%) women were married between 2 and 5years, 76 (43.9%) were married between 6 and 10 years and another 18 (10.4%) were married for more than 10 years.

**TYPE OF INFERTILITY (Diagram 2)**

As shown in diagram 2, one hundred and sixty one (93.1%) patients sought medical advice for primary infertility and the remaining 12 (6.9%) were investigated for secondary infertility. Those women who presented with secondary infertility have had spontaneous abortions in the first trimester 2 to 5 years earlier.

**GYNAECOLOGICAL SYMPTOMS**

In this study, 56.6% of women did not have any Gynaecological symptoms other than infertility. Forty eight (27.7%) women presented with menstrual disturbances; oligomenorrhoea was the predominant menstrual disturbance seen in 34 (70.8%) of them. Secondary amenorrhoea was seen in three cases, metrorrhagia and polymenorrhoea were seen in one case each. In this study, menorrhagia was seen in five cases and hypomenorrhoea in three cases. Decrease in menstrual flow is more common with long standing infertility cases. In 20 of the 34 cases who presented with oligomenorrhoea, the duration of infertility was more than 6 years.

Symptoms such as dysmenorrhoea and dyspareunia were seen in 15.6% and 1.7% of cases, respectively. Vaginal discharge not responding to treatment for more than two years was seen in five cases. Other symptoms such as abdominal bloating, bowel disturbance were seen in two cases each. Urinary symptom with haematuria was seen in one case. Chronic pelvic pain not responding to treatment was seen in 9 (5.2%) cases.

One patient presented with active abdominal tuberculosis with ascites and adnexal mass. In this woman, ESR was elevated, Mantoux was positive with an induration of 14 mm. Laparoscopy was highly suggestive of tuberculosis and the tissue taken from the tubercles on the surface of the tube and peritoneum were positive for Mycobacterium Tuberculosis by HPE and PCR. However, culture was negative.

Table 2 shows the prevalence of various gynaecological symptoms seen in 173 infertile women.

**Table 2: Symptoms observed in 173 infertile women**

| Symptom | Number | Percentage |
|---|---|---|
| Menstrual Disturbance | 48 | 27.7% |
| Dysmenorrhoea | 27 | 15.6% |
| Dyspareunia | 3 | 1.7% |
| Chronic vaginal discharge | 5 | 2.9% |
| Chronic pelvic pain | 9 | 5.2% |
| Abdominal bloating | 2 | 1.2% |
| Urinary symptoms | 1 | 0.6% |
| Bowel symptoms | 2 | 1.2% |
| No symptoms other than infertility | 98 | 56.6% |

**PAST HISTORY OF TUBERCULOSIS**

A definite past history of tuberculosis was available in 11 (6.4%) cases. These women had tuberculosis in sites such as lungs, axillary nodes, cervical nodes and gastro – intestinal tract. In these women, tuberculosis was diagnosed 2-15 years earlier and was treated with ATT for 9 months to 2 years.

Analysis of these 11 cases with past history of tuberculosis showed that in 10 of them there was definite evidence of tuberculosis on laparoscopy with tubercles, caseation and calcification. Mantoux test was positive in 9 cases, ESR was elevated in 7 cases, and PCR was positive in 5 of 8 cases in whom the test was done. Culture and HPE were positive in 2 cases (TABLE 3).

**Table 3: Results of additional diagnostic parameters done on 11 cases with Past History of Tuberculosis**

| Site of TB | Laparoscopy | Mantoux | Culture | HPE | PCR | ESR |
|---|---|---|---|---|---|---|
| Axillary adenitis | + | + | + | + | + | Elevated |
| Abd.TB | + | Negative | - | - | + | Normal |
| Abd. TB | + | + | - | - | - | Normal |
| Cervical adenitis | + | + | - | - | + | Elevated |
| Abd.TB | + | + | - | - | + | Normal |
| Pulmonary | + | + | + | + | + | Elevated |
| Pulmonary | + | + | - | - | - | Elevated |
| Pulmonary | + | + | - | - | ND | Elevated |
| Pulmonary | + | + | - | - | ND | Elevated |
| Pulmonary | + | + | - | - | ND | Elevated |
| Pulmonary | Normal | Negative | - | - | - | Normal |

As in 10 of the 11 cases, either one or more of the other diagnostic parameters were positive; a definite past history of tuberculosis was taken as one of the parameters to arrive at a diagnostic criterion to suspect genital tuberculosis.

- ***A definite documented history of extra genital tuberculosis in the past is an important consideration in suspecting genital tuberculosis in the presence of infertility.***

Diagram 3 shows the pictorial depiction of past history of tuberculosis and corroborative evidence from other test results:

**CONTACT HISTORY**

In seven cases (4%) there was a history of close contact with family members who were treated for tuberculosis in the past. In one case more than one family member were affected.

In one case there was a history of contact with a neighbour who was treated for pulmonary TB for many years. This index patient developed tuberculous cervical adenitis at the age of 12 years, and subsequently found to be suffering from genital tuberculosis while investigating for infertility.

**SECTION B: EXAMINATION FINDINGS**

Anaemia was not a common feature and was seen only in 8 (4.6%) cases. Old healed scar following cervical adenitis was present in one case. Abdominal and pelvic examination did not reveal any abnormality, except the one case who presented with ascites and adnexal mass.

**BODY MASS INDEX**

Distribution of Body Mass Index (BMI) among the 173 infertile women showed that 30 (17.4%) women were underweight with a BMI of less than 19 and 143 (82.7%) women had normal or above normal BMI. (Diagram 4) During the same period nearly 21% of women attending the general Gynaecological Out Patient Department were also undernourished. In general, women attending the Government Institutions are from lower socio-economic group and tend to be undernourished.

**SECTION C: INVESTIGATIONS**

The haemoglobin values ranged between 6 and 13 Gms /dl, and in eight (4.6%) patients the levels were less than 11 grams %.

The total leucocyte count (TC) ranged from 7-11 x $10^9$ / l, and the differential count (DC) was within normal limits in all cases including the case which presented with active disease.

**ERYTHROCYTE SEDIMENTATION RATE (ESR)**

In our study, in 27 (15.6%) cases ESR was elevated more than 15 mm in one hour. Among the 27 cases in which ESR was elevated, in 21 cases there was corroborative evidence of TB from other test results. Laparoscopy was suggestive of TB in 21 cases, PCR was positive in 14 cases and Mantoux test was positive in 20 cases. Conventional methods of diagnosis, culture and HPE were positive in six cases, and one case was positive by HPE alone.

Diagram 5 shows ESR positivity and positive results of other diagnostic parameters

The efficiency of ESR in diagnosing GTB was also evaluated against the conventional methods of diagnosis. (TABLE 4)

**Table 4: Evaluating ESR with HPE &Culture**

| **ESR** | **HPE & Culture** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 5 | 22 | 27 |
| Negative | 2 | 144 | 146 |
| Total | 7 | 166 | 173 |

When the results of ESR was evaluated against the conventional methods of diagnosis, the sensitivity of ESR was found to be 71.4% (95% CI 35.89, 91.78) and the specificity was 86.7 %( 95% CI 80.75, 91.08) The PPV was low at 18.52% and the NPV was 98.63%.

As shown in table 4, in 22 cases, where ESR was elevated, the conventional methods of diagnosis were negative. This could suggest false positive results of ESR; however in three of them HPE showed evidence of non- specific endometritis which could suggest tuberculosis.

Among the 27 cases with elevated ESR, in 21 of them the other diagnostic parameters were also positive as shown in diagram 5. Moreover, compared to the conventional methods, the sensitivity and specificity of ESR in diagnosing genital tuberculosis is also high.

***Therefore, an elevated ESR in an infertile woman should be further evaluated.***

**Chest X-ray**

In the present study, chest X – ray was done in all 173 cases and was evaluated carefully by a trained chest physician for locating small or healed lesions. Only four patients (2.3%) showed evidence of healed lesions with fibrous scars.

As none of the patients had symptoms pertaining to the respiratory system, suggesting active pulmonary tuberculosis, sputum for AFB was not done in these cases.

**HIV Testing**

As tuberculosis and HIV infection can co-exist, all subjects and their partners were checked for HIV Infection and all were found to be negative.

**TUBERCULIN TEST (MANTOUX TEST)**

Tuberculin test using 0.1 ml of purified protein derivative was carried out in all 173 women and was assessed for induration in 48-72 hours. A positive tuberculin test was defined as induration size equal to or more than 10 mm. A positive tuberculin test was seen in 37 (21.4%) cases. The mean induration among the positive cases was 13.8 mm with a standard deviation of 2.7mm (range: 10-20 mm).

Diagram 6 shows the result of Mantoux test on 173 infertile women.

In our study, out of the 37 cases who showed positive Mantoux reaction, there was also corroborative evidence of TB from other test results (diagram 7).

In 29 of them; laparoscopy showed evidence of tuberculosis. (Caseation and granulomas were seen in 21 cases, adhesions and hydrosalpinx in 8 cases.) Other

diagnostic tests such as PCR were positive in 18 cases, culture was positive in 6 cases, and HPE was positive in 6 cases.

In order to evaluate the usefulness of Mantoux test in diagnosing GTB, the test was evaluated against the known conventional methods, namely, culture and HPE. (TABLE 5)

**Table 5: Evaluating Mantoux test with HPE and Culture**

| **Mantoux** | **HPE & Culture** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 6 | 31 | 37 |
| Negative | 1 | 135 | 136 |
| Total | 7 | 166 | 173 |

The sensitivity of Mantoux test in diagnosing GTB was found to be 85.7% (95% CI 48.69, 97.43) and the specificity was 81.3 % (95%CI 74.71, 86.52). The PPV was 16.22% and the NPV was 99.26%.

- ***Based on the corroborative results of other diagnostic tests and the results of evaluation against the conventional methods, a positive Mantoux test in an infertile woman should be taken as an important criterion for further evaluation for tuberculosis.***

**BCG Vaccination**

In this study 105 (60.7%) women showed evidence of vaccination with the presence of deltoid scar.

**SECTION D SPECIFIC GYNAECOLOGICAL PROCEDURES**

**ULTRASOUND EXAMINATION (USG)**

Awareness of the sonographic changes associated with tuberculous infection may improve diagnostic accuracy and avoid clinical mismanagement and surgical exploration in wet type of tuberculosis.

Abdominal ultrasound examination was carried out in all the 173 cases. USG findings were normal in 150 (86.7 %) cases. Findings suggestive of tuberculosis were seen in 23 (13.3%) cases. Adnexal masses and cysts were seen in 17 cases, ascites was present in three cases, calcification in two cases and endometrial fluid was seen in one case. The results of specific diagnostic tests in these cases showed that HPE, culture and PCR were positive in 13 of the 23 cases.

**HYSTEROSALPINGOGRAM: (HSG)**

As a part of infertility work up, HSG is a frequently performed investigation to ascertain the patency of fallopian tubes.

Tubal evaluation was carried out by Hysterosalpingogram (HSG) in 131 cases. Among these, HSG was found to be normal in 74(56.5%) cases and 57 (43.5%) cases showed abnormal findings (Diagram 8).

In those who had abnormal findings, characteristic features of tuberculosis such as calcification (Figure 8), beaded appearance of tube, distorted uterine cavity(Figure 9), intravasation of dye (Figure 10,10A), mid tubal block ( Figure 11) and fimbrial block with large hydrosalpinx (Figure 12,13), were seen in 22 cases. Of these, 18 were positive by PCR and one case was positive by culture and HPE. In the remaining 35 cases, cornual blocks (Figure 14,15), were found, in whom 13 were positive by PCR and in two cases culture and HPE were positive.

**Abnormal HSG findings:**

| | | | |
|---|---|---|---|
| Cornual Block: 35 | | PCR – Positive | 13 |
| | | HPE & culture Positive | 2 |
| Characteristic Features of TB on HSG: 22 | | | |
| Beaded tubes | 4 | | |
| Calcification | 2 | | |
| Fimbrial Block with hydrosalpinx | 12 | PCR – Positive | 18 |
| Intravasation with tubal block | 3 | HPE & culture positive | 1 |
| Distorted cavity | 2 | | |

In 31 of the 57 cases with abnormal HSG findings, other diagnostic parameters such as ESR, Mantoux test, HPE, Culture and PCR were also positive (Diagram 9).

- ***Characteristic features on HSG should alert the clinician to the possibility of MTB and definitive diagnostic tests should be carried out in these cases.***

**Laparoscopy in the diagnosis of Genital Tuberculosis**

In this study, after obtaining necessary consent, laparoscopy was carried out under general anaesthesia in all the 173 cases. A systematic and thorough evaluation of pelvis and abdominal cavity was carried out for evidence of TB and findings such as granulomas, caseation, calcification, tubercles and loculated ascites were looked for. The fallopian tubes were also evaluated for the presence of proximal and distal blocks and hydrosalpinx. Presence of adhesions was also noted. Pelvis and peritoneal cavity were also evaluated for the presence of other pathology. In 81 cases there was fluid in the POD. Therefore, in these cases, the fluid was also aspirated and sent for culture and PCR studies.

Among the 173 cases, the findings were suggestive of tuberculosis in 80 (46.2%) of them. In 20 of these 80 cases, there was a definite evidence of tuberculosis with findings such as granulomas (Fig.16), caseation, calcification (Fig.17) and tubercles (Fig.18-21). In another 18 cases, there was probable evidence of tuberculosis with hydrosalpinx (Fig.22, 23), dilated retort shaped tubes (Fig.24), tubes covered with white plaques and exudates, dense adhesions (Fig.25, 26) and loculated ascites and intravasation of dye into the parametrium. In the remaining 42 cases, there was suspicion of tuberculosis because of minimal adhesions (Fig.27), free fluid in Pouch of Douglas and cornual block. (TABLE 6)

**Table 6: Findings at Laparoscopy In 173 Infertile Women**

| Findings | No. of cases | Percentage |
|---|---|---|
| Normal | 93 | 53.8% |
| Definite evidence of TB | 20 | 11.6% |
| Probable evidence of TB | 18 | 10.4% |
| Suspicion of TB | 42 | 24.2% |
| Total | 173 | 100% |

**Hysteroscopy**

Hysteroscopy is also a useful tool in assessing the endometrial cavity in suspected GTB. At the time of the study hysteroscopy instrument was not available in the hospital. Therefore, hysteroscopic evaluation of the uterus was not included in the study protocol. However, during a workshop we were able to do hysteroscopy in seven cases. Two of these cases presented with secondary amenorrhoea. In one case there was evidence of calcification (Fig.28) and the other case showed intra uterine adhesions. There was no menstrual disturbance in the other five women. However, in

two of these cases findings were suggestive of tuberculosis; in one case there was a white patchy lesion visualized near the left cornua of the tube (Fig.29). The other case showed small tubercles in the endometrium near the left cornua and a small lesion within the cornua itself. (Fig.30)These two cases were positive by PCR. However, HPE and culture were negative .In these two cases laparoscopy showed congestion of tubes with minimal adhesions.

**SECTION E: SPECIFIC DIAGNOSTIC TESTS FOR TUBERCULOSIS**

Specific diagnostic tests included AFB smear, Culture, HPE and PCR studies. From 173 infertile women the following samples were available for various specific diagnostic tests.

- Endometrial samples - 173
- Pouch of Douglas Aspirate - 81
- Urine samples - 52

**Detection of AFB in Direct Smears.**

Smears were prepared from 173 endometrial samples and 81 POD aspirate and were examined by Fluorochrome method. AFB smears are not normally done on urine samples.

Out of the 173 cases, 8 (4.6%) were positive by direct microscopy in the endometrial samples. Among the 8 cases that were positive for AFB smear, none were positive for MTB in culture. However, in three cases NTM organisms were grown. In another two cases, PCR was positive.

Also, in 81 POD aspirate samples AFB smear was positive in 5 (6.2%) cases. On culture MTB was negative in all the 5 samples and NTM organisms had grown in two cases.

In two of the above samples NTM organisms were positive both in the endometrium and POD aspirate.

In two of the five positive smears in POD fluid, PCR was also positive in 2 cases; in one of them positive in both endometrium and POD fluid.

Table 7 shows the results of AFB smear on endometrium and POD fluid

**Table 7: Results of AFB Smear of Endometrium and POD Fluid**

| Specimen | Sample size | No. Positive | Percentage |
|---|---|---|---|
| Endometrium | **173** | **8** | **4.6%** |
| POD Fluid | **81** | **5** | **6.2%** |

**Microbiological diagnosis of Genital Tuberculosis**

In this study, culture was carried out in 173 premenstrual endometrial samples, 81 POD aspirate and 52 urine samples. Since these extra-pulmonary lesions are paucity bacillary in nature, their processing included milder decontamination and inoculation into multiple media. At the end of 8 weeks species of MTB was identified in positive cultures.

Three first morning voided midstream urine specimens were sent for AFB stain and culture.

In spite of inoculation into multiple media, our study revealed that only six samples (3.5%) yielded microbiological proof of *MTB.*

Interestingly, in our study, NTM was isolated in 41 cases. TABLE 8 shows the result of endometrial culture from 173 infertile women

**Table 8: Result of Endometrial Culture (N=173)**

| Result | Number | Percentage |
|---|---|---|
| Positive for MTB | 6 | 3.5% |
| Negative for MTB | 124 | 71.7% |
| Non Tuberculous Mycobacteria | 41 | 23.6% |
| Contamination | 2 | 1.2% |
| Total | 173 | 100% |

When 81 samples from the POD were cultured, none of the samples were positive for *MTB* and two samples were contaminated. However, 16 samples had grown Non Tuberculous Mycobacterial Organisms (NTM).

As genital tract is closely associated with urinary tract, urine samples were collected in 52 cases for culture and PCR study. In this study, none of the urine samples were positive for culture and six samples were positive for NTM organisms. Table 9 shows the culture results from various sources

**Table 9: Comparative Study of Culture Results from Various Sources**

| Result | Endometrium | Pod aspirate | Urine |
|---|---|---|---|
| Positive for MTB | 6 (3.5%) | 0 | 0 |
| Negative for MTB | 124 (71.7%) | 63 (77.8%) | 46 (88.5%) |
| NTM Positive | 41 (23.7%) | 16 (19.8%) | 6 (11.5%) |
| Contamination | 2 (1.2%) | 2 (2.5%) | 0 |
| Total | 173 | 81 | 52 |

In our study in 41 (23.7%) cases NTM (previously known as atypical mycobacteria) had grown in culture. Figure 31 shows the NTM growth with smooth morphology, mucous colonies with coloured appearance. Among these, in 25 cases, the samples were analyzed by High Performance Liquid Chromatography (HPLC) to identify the Mycolic acid characteristics of various NTM organisms (Figure 32).

HPLC analysis showed that Mycobacterium chelonae / Mycobacterium abscessus was the predominant organism grown in 16 samples. Mycobacterium Fortuitum was grown in 4 samples, Mycobacterium Kansasii in 1 sample, and Mycobacterium simiae was positive in 2 samples, Mycobacterium Intracellulare and Mycobacterium marinum were grown in one sample each. (TABLE 10)

**Table 10 showing result of NTM organisms grown in culture**

| Organism | Number positive |
|---|---|
| Mycobacterium chelonae / Mycobacterium abscessus | 16 |
| Mycobacterium Fortuitum | 4 |
| Mycobacterium Kansasii | 1 |
| Mycobacterium simiae | 2 |
| Mycobacterium Intracellulare | 1 |
| Mycobacterium marinum | 1 |
| Total | 25 |

**Histopathological Examination:**

For histopathological studies, a portion of the endometrial tissue/tissue from the lesion over the tube or peritoneum was fixed in 10% formalin; routine processing was done and stained with haemotoxylin and Eosin. Presence of caseating

granulomas surrounded by epitheloid cells, lymphocytes, plasma cells and giant cells was diagnostic of tuberculosis.

In this study, in 173 infertile women endometrial samples were taken by pre menstrual curettage for HPE. And 7 of the 173 samples (4%) were reported positive for TB. In these cases the histology showed granulomas, surrounded by epithelioid cells, lymphocytes and giant cells. (Figure 33, 34)

Another 6 specimens were reported as non specific endometritis. Histology of these specimens showed destruction of glandular epithelium with exudates and inflammatory cells with polymorphs and macrophages. (Figure 35)

In two cases only fibro collagenous tissue was obtained.

TABLE 11 shows endometrial biopsy result of 173 infertile women.

**Table 11: Endometrial Biopsy Result (N=173)**

| Result | Number | Percentage |
|---|---|---|
| Tuberculous Endometritis | 7 | 4% |
| Non Specific Endometritis | 6 | 3.5% |
| Secretory Phase | 102 | 59% |
| Proliferative Phase | 53 | 30.6% |
| Hyperplastic Endometrium | 3 | 1.7% |
| Fibro collagenous tissue | 2 | 1.2% |
| Total | 173 | 100% |

In one case who presented with active abdominal tuberculosis, at laparoscopy, there were tubercles on the surface of the fallopian tube and peritoneum and biopsies were taken from these lesions. The histology in this case was reported as tuberculous

salpingitis. (Figure 37) The peritoneum also showed evidence of tuberculosis (Figure 38).

In one case besides the endometrium, the endo cervix also showed histopathological evidence of tuberculosis (Figure 39). In yet another case, who presented with T-O mass, there was evidence of tuberculosis both in the endometrium and the ovary. (Figure 40)

**RESULTS OF POLYMERASE CHAIN REACTION**

The Polymerase Chain Reaction was carried out on Endometrium, POD aspirate and Urine samples for identification of M.tuberculosis using both IS 6110 and $pTRC_4$ primers.

PCR testing was done on 160 endometrial samples.

Out of the 160 endometrial samples 45 (28.1%) samples were PCR positive.16 (19.8%) of the 81 POD aspirate and 4 (7.7%) of the 52 urine samples were also PCR positive. Table 12 shows the results of PCR study.

**Table 12 Comparative Study of PCR Results From Various Sources**

| Specimen | No. | Positive | Percentage |
|---|---|---|---|
| Endometrium | 160 | 45 | 28.1% |
| POD aspirate | 81 | 16 | 19.8% |
| Urine | 52 | 4 | 7.7 % |

The endometrial samples showed higher PCR positivity compared to the POD aspirate and urine samples.

The results of specific diagnostic tests on endometrial samples were compared. (TABLE 13)

**Table 13: Results of specific diagnostic tests on endometrial samples.**

| Test | No. | Positive result | Percentage |
|---|---|---|---|
| AFB Smear | 173 | 8 | 4.6% |
| Culture | 173 | 6 | 3.5% |
| HPE | 173 | 7 | 4.0 % |
| PCR | 160 | 45 | 28.1 % |

On analyzing the results of specific diagnostic tests performed on the Endometrium, it was seen that the AFB smear was positive in 4.6% of cases, Culture was positive in 3.5%, HPE was positive in 4% and PCR was positive in 28.1% of cases.

In 81 cases POD fluid was available for various studies. As shown in Table 14, AFB smear was positive in 6.2% of cases, culture was negative in all the samples and PCR was positive in 19.8% of cases.

**Table 14: Results of specific diagnostic tests on POD fluid (No.81)**

| Test | No. | Positive result | Percentage |
|---|---|---|---|
| AFB Smear | 81 | 5 | 6.2% |
| Culture | 81 | 0 | 0% |
| PCR | 81 | 16 | 19.8 % |

In 52 urine samples culture was negative in all the cases, and PCR was positive in 7.7% of cases.

**SECTION F**

**EVALUATION OF SPECIFIC DIAGNOSTIC TEST**

Cultivation of *MTB* is considered the Gold standard for the diagnosis of tuberculosis. However, this gold standard lacks sensitivity and is negative in specimens from majority of paucibacillary cases.

In genital tuberculosis one cannot use a gold standard technique as the detection rate of mycobacterial culture and HPE are very low.

***In conditions , where there is no gold standard technique available to evaluate a diagnostic test, one may have to develop and justify a combination of clinical profile and criteria against which the new test has to be assessed.*** (211)

Therefore, based on the results of clinical profile and laparoscopic evaluation which were discussed in earlier chapters, a group of clinical parameters were identified and using these parameters a diagnostic criterion was derived to suspect GTB.

***A possible diagnosis of GTB should be considered in infertile women in the presence of following criteria:***

1. A definite past history of tuberculosis
2. Presence of active extra-genital tuberculosis
3. A positive Mantoux test
4. Elevated ESR
5. Characteristic findings on HSG
    - Distorted endometrial cavity
    - Beaded appearance of tubes
    - Retort shaped hydrosalpinx
    - Intravasation of dye

- Presence of calcification

6. Sonographic features of tuberculosis
    - Particulate ascites and loculated fluid
    - Endometrial fluid with or without adnexal masses.
    - Complex adnexal mass
7. Positive findings at laparoscopy in the absence of gonococcal or chlamydial infection.

A woman was said to be suspected of having genital tuberculosis if she has had findings suggestive of tuberculosis by laparoscopy with one or more of the following findings: A definite past history of tuberculosis, presence of active extra genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux and evidence of calcification / complex adnexal mass by scan.

As there is no gold standard technique available to diagnose GTB, the newly derived diagnostic criteria was used to evaluate the various specific diagnostic tests: **AFB smear, culture, HPE and PCR, so as to identify a suitable test which can be used in clinical practice.**

Of the 173 samples that were tested for Chlamydia trachomatis & Neisseria gonorrhoea infection, 13 were found to be positive for Chlamydia and 3 were found to be positive for gonococcal infection. Therefore, these 16 cases were excluded from the study.

In the remaining 157 cases, in 153 endometrial samples the results of all the four specific tests were available and therefore taken for further evaluation. Based on the newly derived clinical criterion cases were divided into two groups.

| | | |
|---|---|---|
| **Group A:** | **Those with suspicion of tuberculosis** | **61/153** |
| **Group B:** | **Those in whom tuberculosis was not Suspected** | **92/153** |

By bivariate analysis, the sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated with 95% confidence interval (CI) for the specific diagnostic tests (AFB smear, culture, HPE and PCR).The results are as follows:

**I. Evaluation of AFB smear against the clinical criteria**

The results of AFB smear were evaluated against the clinical criteria and the findings are as follows:

Of the 61 clinically positive samples, 4 (6.55%) were positive by AFB smear. Of the 92 clinically negative samples, 91 (98.91%) were also negative by AFB smear, and the crude agreement between the results was 62.09%.

The sensitivity of AFB smear in diagnosing GTB was 6.56% and the specificity was 98.9 %.The PPV was 80% and the NPV was 61.49%. (TABLE 15)

**Table 15: Evaluation of AFB Smear and Clinical Criteria**

| **AFB smear** | **Clinical Criteria** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 4 | 1 | 5 |
| Negative | 57 | 91 | 148 |
| Total | 61 | 92 | 153 |

| | | |
|---|---|---|
| Sensitivity | 6.56% | (95%CI 2.579, 15.68) |
| Specificity | 98.9% | (95%CI 94.1, 99.81) |
| PPV | 80% | (95%CI 37.55, 96.38) |
| NPV | 61.5 % | (95%CI 53.45, 68.94) |

**II. Evaluation of culture results against the clinical criteria**

The results of culture were evaluated against the clinical criteria and the findings are as follows:

Of the 61 clinically positive samples, 4 (6.6%) were positive by culture. All the 92 clinically negative samples were negative by culture also. Therefore, the specificity of culture in the diagnosis of GTB was 100% and the sensitivity of culture in diagnosing GTB was 6.6 %.( TABLE 16)

**Table 16: Evaluation of Culture Results and Clinical Criteria**

| **Culture** | **Clinical Criteria** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 4 | 0 | 4 |
| Negative | 57 | 92 | 149 |
| Total | 61 | 92 | 153 |

Sensitivity 6.6% (95%CI2.579, 15.68)

Specificity 100% (95%CI95.99, 100)

PPV 100% (95%C51.01, 100)

NPV 61.7% (95%CI53.74, 70.01)

**III. Evaluation of HPE and clinical criteria**

On evaluating the HPE results against the Clinical Criteria, the sensitivity of HPE was 8.2% and the specificity was 100% in the diagnosis of GTB.

There was 100% concordance between the negative HPE results and the negative clinical findings. However, among the 61 clinically positive cases only 5(8.2%) were positive by HPE. The crude agreement between the results was 63.4 %.( TABLE 17)

**Table 17: Evaluation of HPE and Clinical Criteria**

| HPE | Clinical criteria | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 5 | 0 | 5 |
| Negative | 56 | 92 | 148 |
| Total | 61 | 92 | 153 |

| | | |
|---|---|---|
| Sensitivity | 8.2% | (95%CI3.552, 17.79) |
| Specificity | 100% | (95%CI95.99, 100) |
| PPV | 100% | (95%CI56.55, 100) |
| NPV | 62.1% | 95%CI54.13, 69.57) |

**IV. Evaluation of PCR and Clinical Criteria**

In 153 infertile women, PCR results by either or both probes (IS6110 & $TRC_4$) were evaluated against the clinical diagnosis of GTB. A positive clinical diagnosis of GTB was made in 61 of the 153 infertile women.

Of the 61 clinically positive samples, 27 (44.3%) were positive by PCR. Of the 92 clinically negative samples 74 (80.4%) were negative by PCR also. 18 patients whose samples were found to be positive by PCR were clinically assessed to be negative for GTB. And 34 patients who were clinically confirmed to have GTB were determined to be negative by PCR.

The crude agreement between the results was 66%, and the chance corrected agreement was 26% (Kappa). There was marginal agreement between the results of PCR and clinical results confirming the disease.

Table 18 represents the concordance between PCR results using either or both probes and clinical results confirming GTB.

**Table 18: Evaluation of PCR and Clinical Criteria**

| PCR | Clinical criteria | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 27 | 18 | 45 |
| Negative | 34 | 74 | 108 |
| Total | 61 | 92 | 153 |

The sensitivity and specificity of PCR in diagnosing GTB are given below.

Sensitivity 44.3 % (95% CI 32.51, 56.7)

Specificity 80.4% (95% CI 71.18, 87.25)

PPV 60% (95% CI 45.45, 72.98)

NPV 68.5% (95% CI 59.25, 76.51)

TABLE 19 shows the test performance of various diagnostic tests on 153 endometrial samples.

**Table 19: Comparative evaluation of various diagnostic tests**

| Test | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| AFB smear | 6.7% | 98.9% | 80% | 61.5% |
| Culture | 6.6% | 100% | 100% | 61.7% |
| HPE | 8.2% | 100% | 100% | 62.1% |
| PCR | 44.3% | 80.4% | 60% | 68.5% |

In this study, PCR in endometrial samples showed a sensitivity of 44.3% which was highest compared to the other diagnostic tests.

However, the specificity was high with AFB smear, culture and HPE testing.

## EVALUATION OF PCR TEST IN THE DIAGNOSIS OF GTB

This study has shown that the sensitivity of PCR is higher as compared to the other diagnostic methods. Therefore, it was evaluated further for false negative and false positive results.

False negative PCR

Out of the 153 cases, in 34 patients there were positive clinical and laparoscopic features of genital TB but the PCR results were negative. This could indicate the possibility of false negative PCR results.

When comparing PCR results with the conventional methods of diagnosis in the five cases that were positive by either culture or HPE, PCR was positive only in three cases. (IS 6110 was positive in one case and $TRC_4$ was positive in two cases). (TABLE 20)

**Table 20: Comparing PCR results with conventional methods of diagnosis**

| PCR | HPE & Culture | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 3 | 42 | 45 |
| Negative | 2 | 106 | 108 |
| Total | 5 | 148 | 153 |

Among the 34 cases with possible false negative results, in 11 samples NTM organisms were grown in culture. Whether NTM organisms could result in

inflammatory changes in the pelvis giving rise to positive findings at laparoscopy and negative PCR results should be ascertained.

False positive PCR

Of the 153 women, in 18 of them PCR was positive but the clinical profile was negative. This raises the possibility of false positive PCR results by way of contamination, dead bacilli or previous infection. The problem of false positivity can be minimized by way of multiple areas of sampling and repetitive sampling. In our study, in 13 of the 18 cases with possible false positive results, PCR was positive both in the endometrium and POD aspirate and in 2 cases urine samples were also positive for PCR.

Also, in six of the 18 cases, the HPE findings on the endometrium were reported as chronic endometritis which could be the earliest finding in endometrial tuberculosis.

Analysis of Endometrial and POD Aspirate Samples

The PCR results were analyzed from samples taken from different sources on the same patient. (TABLE 21)

In 81 women PCR results were available from samples taken from both endometrium and POD aspirate. Out of 16 cases that showed positivity in POD aspirate, 13 of them were positive in the endometrial samples also. In 66 (81.5%) of the 81 samples there was (crude) agreement in the results and the Kappa statistics of chance corrected agreement was 52% which indicates good agreement.

**Table 21 PCR results of 81 Endometrium and POD aspirate samples.**

| **POD aspirate** | **Endometrium sample** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 13 | 3 | 16 |
| Negative | 12 | 53 | 65 |
| Total | 25 | 56 | 81 |

Kappa = 52% (good agreement)

Evaluation of two sets of primers in PCR technique:

IS 6110 primers are widely used in the diagnosis of *MTB.* Studies have shown that use of more set of primers along with IS6110 will increase the sensitivity of PCR. Therefore, along with IS6110 primers we have used another primer called $TRC_4$ primer in this study.

Evaluating IS 6110 and clinical criteria

Of the 61 clinically positive samples 9 (14.8%) were positive by PCR using IS6110 probes .Of the 92 clinically negative samples, 85 (92.4%) were negative by IS6110 probes. The crude agreement between the results was 61.4%. The false negative rate of IS 6110 was 85.2%.

Table 22 presents the concordance between the PCR results using IS6110 probes and clinical criteria confirming GTB.

**Table 22: Evaluation of IS 6110 and Clinical Criteria**

| **IS 6110** | **Clinical criteria** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 9 | 7 | 16 |
| Negative | 52 | 85 | 137 |
| Total | 61 | 92 | 153 |

Sensitivity 14.8% (CI 7.961, 25.72)

Specificity 92.4% (CI 85.12, 96.27)

PPV 56.2% (CI 33.18, 76.9)

NPV 62.0% (CI 53.69, 69.74)

Evaluating $TRC_4$ and clinical criteria

On evaluating the PCR using $TRC_4$ probes against the clinical criteria, of the 153 samples 22 were positive and 79 were negative by both clinical criteria and $TRC_4$ results. Using TRC4 probe alone, the crude agreement between the results was 66.0 % (Table 23). The false negative rate of $TRC_4$ was 63.9%.

**Table 23: Evaluation of $TRC_4$ and Clinical Criteria**

| **$TRC_4$** | **Clinical criteria** | | **Total** |
|---|---|---|---|
| | **Positive** | **Negative** | |
| Positive | 22 | 13 | 35 |
| Negative | 39 | 79 | 118 |
| Total | 61 | 92 | 153 |

| | | |
|---|---|---|
| Sensitivity | 36.1% | (CI 25.17, 48.61) |
| Specificity | 85.9% | (CI 77.31, 91.55) |
| PPV | 62.9% | (CI 46.34, 76.83) |
| NPV | 66.9% | (CI 58.05, 74.78) |

Comparing results of PCR using IS 6110 and $TRC_4$ probes

On evaluating the PCR results using two sets of primers against the clinical criteria, it was seen that PCR with TRC4 primers had a higher sensitivity (36.1%) than PCR with IS6110 primers (14.8%) in detecting clinically positive GTB. (Diagram 10)

Concordance in diagnosis by PCR with two sets of primers.

Six samples were positive while 108 samples were negative by both probes. Thus, there was agreement between the results of the two primers among 114 of 153

samples. The crude agreement was 74.5%, and chance corrected agreement (Kappa) was 15.8%. There was marginal agreement between the two methods. (Table 24)

**Table 24 Concordance in the results of PCR using IS 6110 & $TRC_4$ probes.**

| TRC4 | IS 6110 | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 6 | 29 | 35 |
| Negative | 10 | 108 | 118 |
| Total | 16 | 137 | 153 |

Reliability of PCR in diagnosing GTB.

Reliability of PCR in diagnosing GTB was checked by repeat sampling on patients and re-testing of saved samples.

76 women who lived closer to the hospital were chosen and letters were sent out to them asking them to come for repeat sampling. However, only 10 women responded to the letter. Therefore, repeat endometrial sampling was possible only in 10 cases. On repeat PCR testing on these 10 samples, in 9 of the 10 cases there was agreement in the results of PCR between the first visit and second visit. (Table 25)

**Table 25: Results of PCR on Repeat Sampling**

| Sl. No. | Case No. | First visit | | Repeat visit | | Clinical criteria |
|---|---|---|---|---|---|---|
| | | IS 6110 | $TRC_4$ | IS 6110 | $TRC_4$ | |
| 1 | 16 | Neg. | Pos. | Neg. | Pos. | Pos. |
| 2 | 36 | Neg. | Neg. | Neg. | Neg. | Pos. |
| 3 | 81 | End – Pos<br>POD – Pos<br>Uri. - Pos | Neg. | Pos. | Neg. | Neg. |
| 4 | 96 | Pos. | Neg. | Pos. | Neg. | Neg. |
| 5 | 100 | Neg. | Neg. | Neg. | Neg. | Neg. |
| **6** | **108** | **Neg.** | **Pos.** | **Neg.** | **Neg.** | **Neg.** |
| 7 | 111 | Neg. | Neg. | Neg. | Neg. | Pos. |
| 8 | 114 | Neg. | Neg. | Neg. | Neg. | Pos. |
| 9 | 120 | Neg. | Neg. | Neg. | Neg. | Pos. |
| 10 | 128 | Neg. | Neg. | Neg. | Neg. | Neg. |

- In 9 of the 10 repeat samples, there was concordance in the results of first sample and repeat samples.
- In four cases, where the clinical criterion was positive for GTB, the results of PCR in the initial sample and repeat samples were consistently negative.
- In two cases, where the clinical criteria were negative, the PCR results were consistently positive. In one of these cases, the initial PCR was positive in the endometrium, POD fluid and the urine sample and the repeat endometrial sample was also positive.
- Only in one case, the $TRC_4$ was positive in the initial visit, but in the subsequent visit was negative. The clinical criterion was also negative in this case.

Re-testing was also carried out in 10 of the 34 reported false negative samples and 9 of the 18 reported false positive samples. On repeat testing, all the 10 negative samples were consistently negative and 8 of the 9 positive samples were consistently positive.

**TREATMENT:**

Among the 173 infertile women studied, 7 were positive by conventional methods, either by culture or HPE. These women were treated with standard Anti tuberculosis treatment (ATT) with short course of chemotherapy for 9 months; initial two months with isoniazid (600 mg.), rifampicin (450 mg.) and ethambutol (1200mg.) thrice weekly followed by isoniazid and rifampicin thrice weekly for the next 7 months. No pregnancies have been reported in two years of follow up.

# CHAPTER VI

# DISCUSSION

Infertility affects approximately 10-15% of couples. Tubal and peritoneal pathology are among the most common causes and the primary diagnosis in 30-40% of infertile couples. The genital tract tuberculosis is one of the most common causes of tubal factor infertility. When tuberculosis affects the genital organs of young females, the disease often remains silent, or may present with non specific symptomatology. As a result, the disease is either not diagnosed at all or diagnosed at an advanced stage when tubal damage has already occurred. At this stage in spite treatment with medical and surgical methods the prognosis for fertility is poor.

The need for this study was to identify strategies to diagnose GTB early, so that treatment with medical methods may improve the prospects of cure, thereby preserving fertility. The justification for undertaking this study was based on the fact that, GTB is one of the major causes of infertility in women, but the diagnosis of the condition is difficult and more challenging. Though, tuberculosis of the other parts of the body, especially of lungs is diagnosed without much difficulty, GTB still eludes diagnosis and remains undiagnosed. A high degree of suspicion aided by intensive investigations is important in the diagnosis of the disease.

One of the aims of this study was to arrive at a diagnostic criterion to suspect GTB which would indicate the need for further investigations. Routine laboratory investigations are of little value. Though clinical parameters such as Mantoux, ESR, chest X-ray, USG and HSG would indicate the need for further evaluation, they are not sufficient criteria to diagnose the disease.

Therefore, specific diagnostic tests are needed to diagnose GTB. A definite diagnosis can be made by positive mycobacterial culture and by demonstrating lesions on histopathology. But these methods have low pick up rate. In recent years, PCR

technique has evolved as a useful and rapid technique for the diagnosis of pulmonary and extra- pulmonary tuberculosis.

Therefore, in this study an attempt was made to analyze the effectiveness of specific diagnostic tests –AFB smear, culture, HPE, PCR in diagnosing GTB, and also to identify a suitable diagnostic test which can be used in clinical practice to identify early lesions.

The study consisted of 173 infertile women, who fulfilled the inclusion and the exclusion criteria. Convenience sampling technique was used in this study. A detailed clinical assessment was done; routine laboratory investigations and specific gynaecological procedures were carried out in these women. Emphasis was laid on diagnosing / excluding GTB by utilizing 4 specific diagnostic parameters (AFB smear, culture, HPE, PCR).

After excluding cases who were positive for Gonococcal and Chlamydia infections, 153 cases were available for further study. Among these 153 cases, based on the clinical assessment and results of gynaecological procedures, a presumptive diagnosis of GTB was made in 61 women in whom laparoscopic findings were suggestive of TB along with one or more of the historical/clinical/lab parameters/specific gynaecological procedures suggestive of tuberculosis were positive.

Specific diagnostic tests were evaluated against these presumed cases of GTB.

**Prevalence of Genital Tuberculosis**

The actual incidence of genital tuberculosis in the general population cannot be determined accurately, because in a large number of patients, the disease is symptomless and discovered incidentally or may remain undiscovered [18].

The incidence also varies greatly according to the socio – economic and public health conditions. Therefore, there is a wide variation in figures published from various countries. Moreover, in less developed areas of the world, there is inadequate

availability of diagnostic procedures to diagnose genital tuberculosis [27]. The incidence is also influenced by the lack of highly sensitive and specific tests to diagnose the conditions [71].

In USA, Australia and West European countries, the incidence of genital tuberculosis is < 1 %. [69] Pelvic tuberculosis is also an uncommon gynaecological problem in some of the Asian countries like Malaysia and Thailand, the reported prevalence being .03% to .05% of gynaecological cases [72, 55].

However, studies from Africa have shown a higher incidence of genital tuberculosis in infertile women. Various Indian studies have also shown higher incidence among infertile women. (Table 26)

**Table 26: Prevalence of genital Tuberculosis among infertile women from various studies.**

| SI no. | Author | Year | Country | Sample Size | Method of Diagnosis | Prevalence |
|---|---|---|---|---|---|---|
| 1. | Dc Vynck [37 ] | 1990 | South Africa | 451 | Menstrual blood culture | 7.9% |
| 2. | Oosthuizen et al. [ 120 ] | 1990 | South Africa | 109 | Menstrual blood and Endometrial culture | 21% |
| 3. | Margolis et al [ 75 ] | 1992 | South Africa | 650 | Menstrual blood culture | 6.1% |
| 4. | Emembolu et al [76] | 1993 | Nigeria | 114 | Culture | 16.7% |
| 5. | Shaheen et al [77] | 2006 | Pakistan | 534 | Endometrium HPE & Culture | 2.43% |
| 6. | Sivanesaratnam et al [72] | 1986 | Malaysia | Not Specified | Culture | .03% |
| 7. | Deshmukh et al [22] | 1987 | India | 500 | HPE | 9% |
| 8. | DeepJyoti et al [21] | 1990 | India | 200 | HPE & Culture | 6% |
| 9. | Chakraborthy et al [166] | 1993 | India | 556 | HPE & Culture | 7.6% |
| 10. | Manjari et al [163] | 1995 | India | 1124 | Culture<br>HPE | 2.05%<br>1.87% |
| 11. | Rozati et al [71] | 2006 | India | 65 | AFB Smear, HPE, Culture, PCR | 50% |
| 12. | Present Study | 2008 | India | 173 | End – Culture<br>End – HPE<br>End – PCR | 3.5%<br>4%<br>28.1% |

End.- Endometrium

Genital tuberculosis is the major causative factor for severe tubal disease requiring assisted reproduction in developing countries like India. In a study by Singh et al (2008), among the 140 infertile women seeking IVF, 70 patients had tubal factor infertility, and the prevalence of GTB in tubal factor infertility was 48.5% [24].

**Age Incidence:**

The 173 study patients were aged between 20 and 37 years, and the median age of presentation was 27. The study has shown that genital tuberculosis is seen in relatively young females as 124 (71.7%) patients were less than 30 years of age. A similar observation has been made by several authors [22, 42, 76, 87, 88].

This preponderance of genital tuberculosis in young women of reproductive age group is explained by the fact that, after puberty the blood supply to the pelvic organ is increased, and as a result, more bacilli could reach the site and infect the reproductive organs or the dormant bacilli can get reactivated. [86] This increased blood supply to the pelvic organ is hormone dependent; therefore, many studies have shown that genital tuberculosis involves women aged less than 40 years of age [55, 87].

As genital tuberculosis affects young women, it has great implication as an important cause of infertility. In this study, 54.3% of women were married for more than 6 years.

One hundred and sixty one (93.1%) patients sought medical advice for primary infertility and the remaining 12 (6.9%) were investigated for secondary infertility. Those women who presented with secondary infertility have had spontaneous abortions in the first trimester 2 to 5 years earlier.

**Analysis of Symptoms**

Genital tuberculosis is a disease of varied symptomatology. At one end of the spectrum, the disease may remain asymptomatic with no signs and symptoms and the diagnosis is made during evaluation of infertility or other gynaecological problems.

At the other end of the spectrum, the patients may present with typical signs and symptoms of tuberculosis such as fever, loss of weight, loss of appetite, anorexia, malaise and night sweats. The disease can also present with mild, varied clinical symptoms which are also common to other Gynaecological conditions.

***Therefore, a high degree of suspicion is necessary to diagnose pelvic tuberculosis***.

The most common initial symptom for which a woman seeks medical advice is infertility and is seen anywhere between 42% to 85% of cases [25, 72, 91, 92,238]. In infertile women genital tuberculosis can exist without any apparent signs and symptoms. In our study, 56.6% of women did not have any gynaecological symptoms other than infertility. Margolis et al study (1992) emphasized that genital tuberculosis is often a disease of absent or few symptoms [75].

In our study, 48 (27.7%) women presented with menstrual disturbances. oligomenorrhoea was the predominant menstrual disturbance seen in 34 (70.8%) of them. Menorrhagia was seen in five cases, secondary amenorrhoea and hypomenorrhoea were seen in three cases each, metrorrhagia and polymenorrhoea were seen in one case each. The prevalence of menstrual disturbances in female genital tuberculosis varies from 20% to 50% in various studies.

Table 27 shows the prevalence of menstrual disturbance in GTB from various studies.

**Table 27: Studies Showing Prevalence of Menstrual Disturbance in GTB**

| Sl. No | Author | Year | Sample size | Menstrual disturbance |
|---|---|---|---|---|
| 1 | Nagpal et al [ 88 ] | 2001 | 100 | 34% |
| 2 | Chowdhury [ 87 ] | 1996 | Not specified | 20% |
| 3 | Trivedi et al[ 42 ] | 1993 | 475 | 52.84% |
| 4 | Misra et al[ 81 ] | 1992 | 162 | 26.62% |
| 5 | Sutherland et al[ 53 ] | 1985 | 710 | 17.86% |
| 6 | Present study | 2008 | 173 | 27.7% |

Trivedi et al study (1993) showed that, menstrual pattern was found to be altered in 52.8% of 475 patients, and the most common change in the menstrual pattern observed was oligomenorrhoea seen in 18.7% of cases [42]. Tyagi et al believe that local endometrial changes with systemic effect account for abnormality in menstruation [94].

In early stages of the disease, initial pelvic congestion and increased vascularity of the endometrium cause menorrhagia and is often associated with active disease. When the ovaries are involved irregular shortened cycles with heavy bleeding may occur.

In cases of severe chronic tuberculous endometritis, there is end organ failure secondary to endometrial caseation and fibrosis of endometrial cavity. As a result, the periods become scanty and eventually amenorrhoea ensues. Genital tuberculosis appears to be an important and common cause of Asherman's syndrome in India, causing oligomenorrhoea and amenorrhoea with infertility [239].

In Sharma et al study (2008), among 28 women with suspected Asherman's syndrome diagnosis of GTB was made by histopathology on endometrial biopsy in 28.6%, positive culture in 3.6% and positive PCR in 46.4% of cases [239].

Decrease in menstrual flow is more common with long standing infertility cases. In our study, in 20 of the 34 cases who presented with oligomenorrhoea, the duration of infertility was more than 6 years. Symptoms such as dysmenorrhoea and dyspareunia were seen in 15.6% and 1.7% of cases, respectively. Symptoms such as chronic vaginal discharge, abdominal bloating and urinary symptoms were rarely seen as in other studies [98].

Tuberculosis should be suspected when the chronic pelvic inflammatory disease is refractory to standard therapy [100]. In our study, 9 cases (5.2%) presented with chronic pelvic pain. In Sutherland's series of 710 cases, pelvic pain was seen in 25.17% of cases [98].

**Past History of Tuberculosis**

A past history of tuberculosis or history of close contact with a family member suffering from tuberculosis should raise the suspicion of GTB in infertile women. However, in most cases a past history of tuberculosis may not be forthcoming as tuberculosis is considered a taboo in India.

In this study, a definite past history of tuberculosis was available in only 11 (6.4%) cases. These women had tuberculosis in sites such as lungs, axillary nodes, cervical nodes and gastro-intestinal tract. They were treated with ATT 2 -15 years earlier. A similar observation was made by Misra et al in 1996 where a past history of tuberculosis was available in only 5% of cases. [81]In a study from Mexico, 16% had a history of tuberculosis in the past [27].

However, number of studies has shown a higher incidence of extra genital tuberculosis in the past in infertile women (TABLE 28). In Sutherland's large series of 638 patients (1979) 80% had a history of tuberculosis elsewhere in the body [104]. Similar observations were made by other authors as well. In Amarnath et al study (1987) from India, there was a past history of extra – genital tuberculosis in 66.2% of cases [99].

Since genital tuberculosis is considered as one of the secondary manifestations of tuberculosis and in majority of cases with its primary site in the lung, one may expect a history of pulmonary tuberculosis in most cases. However in our study, only 6 of the 11 cases gave a past history of pulmonary tuberculosis. Among them only in four cases there was evidence of old healed lesions on X-Ray.

**Table 28 showing Past History of Tuberculosis in Genital Tuberculosis**

| Sl.No. | Author | Year | Country | Cases studied | Past history % |
|---|---|---|---|---|---|
| 1 | Gupta et al[105] | 2007 | India | 40 | 37.5% |
| 2 | Jindal[82] | 2006 | India | 150 | 73.6% |
| 3 | Misra et al[81] | 1996 | India | 162 | 5% |
| 4 | Figueroa et al[27] | 1996 | Mexico | 25 | 16% |
| 5 | Bhides et al[99] | 1987 | India | 71 | 66.2% |
| 6 | Sutherland[104] | 1979 | | 638 | 80% |
| 7 | Present study | 2008 | India | 173 | 6.4% |

Analysis of these 11 cases with past history of tuberculosis showed that in 10 of them there was definite evidence of tuberculosis on laparoscopy with tubercles, caseation and calcification. Mantoux test was positive in 9 cases, ESR was elevated in 7 cases, and PCR was positive in 5 of 8 cases in whom the test was done. Culture and HPE were positive in 2 cases.

As in 10 of the 11 cases, either one or more of the other diagnostic parameters were positive; a definite past history of tuberculosis was taken as one of the parameters to arrive at a diagnostic criterion to suspect genital tuberculosis.

- ***A definite documented history of extra genital tuberculosis in the past is an important consideration in suspecting genital tuberculosis in the presence of infertility.[82]***

**Contact History**

In seven cases (4%) there was a history of close contact with family members who were treated for tuberculosis in the past. In one case more than one family member were affected. It is possible that these women acquired the infection from family members in childhood or adolescence which manifested as disease later in the

reproductive years. In a study from Mexico, 39.1% had a history of contact with a relative with tuberculosis [27].

**Physical Examination**

On general examination, in most cases no abnormal findings may be apparent, if present, may be vague. In this study, anaemia was not a common feature and was seen only in 8 (4.6%) cases. Old healed scar following cervical adenitis was seen in one case. Abdominal and pelvic examination did not reveal any abnormality, except the one case who presented with ascites and adnexal mass.

Distribution of Body Mass Index (BMI) among the 173 infertile women showed that 30 (17.3%) women were underweight with a BMI of less than 19; 75.7% had normal BMI and 6.9% had above normal BMI. During the same period nearly 21% of women attending the general Gynaecological Out Patient Department were also undernourished. In general, women attending the Government Institutions are from lower socio-economic group and tend to be undernourished.

In various studies, abdominal and vaginal examinations were reported to be normal in 56.1% to 90% of cases. [41, 75, 81] A “doughy” abdomen which has been ascribed to tubercle formation on the intestine and peritoneum was reported in 18% of patients in a study from Pondicherry [100].

Adnexal masses in younger women, who have a history of pulmonary and extra-pulmonary tuberculosis or associated with secondary amenorrhoea, should raise a strong suspicion of tuberculosis [58]. In younger women, in the differential diagnosis of an ovarian tumour and ascites, tuberculosis should always be considered [240].

***Based on the clinical signs and symptoms, the diagnosis of the disease is difficult*** **[73].**

**Laboratory Investigations:**

The laboratory investigations which are said to be characteristic of active pulmonary tuberculosis are not necessarily so in female genital tuberculosis.

Total count (TC) and Differential count (DC):

In this study TC and DC were within normal limits in all cases; including the one who presented with active disease. However, various authors have reported Lymphocytosis in GTB [88, 99].

**Erythrocyte Sedimentation Rate:**

In our study, ESR was elevated in 27 (15.6%) cases. Elevated ESR was reported in 89% of cases in Nagpal study and 59.1% of cases in Bhides et al study [88]. Among the 27 cases in whom ESR was elevated, laparoscopy was suggestive of TB in 21 cases, PCR was positive in 14 cases and Mantoux test was positive in 20 cases. Conventional methods of diagnosis, the culture and HPE were positive in six cases, and one case was positive by HPE alone.

When the efficiency of ESR in diagnosing GTB was evaluated against the conventional methods, the sensitivity of ESR was found to be 71.4% and the specificity was 86.7%. As shown in table IV, in 22 cases, where ESR was elevated, the conventional methods of diagnosis were negative. This could suggest false positive results of ESR; however in three of them HPE showed evidence of non-specific endometritis which could suggest tuberculosis.

Among the 27 cases with elevated ESR, in 21 of them the other diagnostic parameters were also positive. Moreover, compared to the conventional methods the sensitivity and specificity of ESR in diagnosing genital tuberculosis is also high.

***Therefore, an elevated ESR in an infertile woman should be further evaluated.***

Elevated ESR has been included as one of the components of diagnostic criteria to suspect tuberculosis.

**HIV TESTING**

The incidence of HIV associated tuberculosis is increasing worldwide especially in developing countries. [108] HIV infected patients rapidly develop clinically significant disease and respond poorly to treatment. It is also known that in HIV positive patients, extrapulmonary tuberculosis occurs more often [109,110].

Although a relative increase in GTB would be expected, this has not been reported. Probably tuberculous systemic disease is diagnosed earlier, before extra – pulmonary manifestation occurs [56]. However, diagnosis of genital tuberculosis should be considered more often and more carefully in all HIV infected women and all patients with tuberculosis should be screened for HIV infection [111].

In this study all subjects and their partners were checked for HIV infection and all were found to be negative. Giannacopoules et al (1998) reported a case of genital tuberculosis presenting as acute PID in a HIV infected African woman [40].

**Chest X Ray:**

A careful evaluation of the chest roentgenogram by a trained chest physician is important for locating small or healed lesions. However, majority of pulmonary lesions are arrested by the time genital tract involvement becomes obvious. Therefore, the chest X – ray is normal in most cases [20, 37, 55, 73]. In the present study, chest, X – ray was done in all 173 cases and only four patients showed evidence of healed lesions with fibrous scars. A similar observation was made by Nagpal et al (2001) where chest X– ray was abnormal only in 1% of the cases with proved GTB [88].

A negative chest X – ray does not rule out the possibility of genital tuberculosis [16]. As none of the patients had symptoms pertaining to the respiratory system, suggesting active pulmonary tuberculosis, sputum for AFB was not done in these cases. Sputum examination for AFB, though not advisable as a routine

investigation in cases of infertility, should be done if there is clinical or radiological evidence of pulmonary tuberculosis [21].

**Tuberculin test: Mantoux test**

Tuberculin test using 0.1 ml of purified protein derivative was carried out in all 173 women and a positive tuberculin test with an induration of ≥ 10mm was seen in 37 (21.4%) cases. The mean induration among the positive cases was 13.8 mm (range: 10-20 mm).

The Mantoux positivity in various studies varies from 8% - 90% and is shown in Table 29

**Table 29: Studies Showing Positive Mantoux test In Genital Tuberculosis**

| Author | Year | Sample size | % Positivity |
|---|---|---|---|
| Gupta et al [105 ] | 2007 | 25 | 8% |
| Figueroa et al [27 ] | 1993 | 16 | 87.5% |
| Alwarez et al [ 114 ] | 1982 | Not specified | 90% |
| Present study | 2008 | 173 | 21.4% |

The Mantoux test generally remains positive as long as viable bacilli persist in quiescent foci. When tubercle bacilli establish infection, the host usually contains the infection and a positive tuberculin reaction is the only evidence of infection. These viable but dormant organisms which are enclosed within the granulomatous fibrotic tissue are a potential threat to become metabolically active at a future date producing active disease.

A positive tuberculin test is not specific for Mycobacterial tuberculous infection [73]. The test can produce a positive reaction, when there is infection with non – tuberculous mycobacterial (NTM) organisms. Similarly the test can also be positive when the patient has had a past infection [44]. Interpretation of tuberculin test is also complicated by prior BCG injection; as it can give a positive reaction [40].

However, studies on children have shown that, even in BCG vaccinated children, a positive tuberculin test of 10 mm or more is a definitive indicator of natural infection with mycobacteria [241,242]. A reactive test in a BCG vaccinated individual indicates that the individual is infected with MTB and is at risk of disease [243]. Therefore, positive Mantoux test should be interpreted in the same way both in vaccinated and unvaccinated individuals [244]. In our study 105 out of 173 (60.7%) cases had been vaccinated.

Mantoux test can be negative in immuno compromised individuals such as HIV infected women [40]. In our study, in one case conventional methods of diagnosing GTB was positive, but, Mantoux was negative. This could be attributed to anergy to tuberculin [245].

In the present study, out of the 37 cases who showed a positive reaction to Mantoux test, 6 of them showed definite evidence of tuberculosis with positive HPE and culture. In another 5 cases, culture was positive for NTM organisms. Other findings that were suggestive of tuberculosis in these 37 cases were: 9 cases were treated in the past for extra genital tuberculosis (includes 6 cases with confirmed GTB). In 18 cases PCR was also positive. In 29 cases there was evidence of tuberculosis at laparoscopy.

In order to evaluate the usefulness of Mantoux test in diagnosing GTB, the test was evaluated against the known conventional methods, namely, culture and HPE.

The sensitivity of Mantoux test in diagnosing GTB was found to be 85.7% and the specificity was 81.3%. Raut et al have shown that, the Mantoux test had a sensitivity of 55% and a specificity of 80% in women with laparoscopically diagnosed tuberculosis [113].

Based on our results showing high sensitivity and specificity of Mantoux in diagnosing GTB, as well as from reports of other authors quoting high positivity of Mantoux test in GTB, tuberculin test has been taken as one of the components of

diagnostic criteria against which various specific diagnostic tests will be evaluated later.

- ***A positive Mantoux test in an infertile woman should be taken as an important criterion for further evaluation of tuberculosis.***

**Hysterosalpingogram**

As a part of infertility work up, HSG is a frequently performed investigation to ascertain the patency of fallopian tubes. Various characteristic features in HSG have been described to diagnose GTB by Klein et al. [41].

In our study tubal evaluation was carried out by HSG in 131 cases and the findings were abnormal in 57 (43.5%) cases.

In Nagpal et al. study, the HSG findings were suggestive of TB in 72.1% of proved cases of GTB [88]. Sharma et al (2008) analyzed the findings at HSG from 70 infertile women diagnosed as GTB. Their findings showed a normal uterine cavity in 57.1% of women, an irregular uterine cavity in 18.5%, a shrunken cavity in 2.8% and an irregular filling defect in 18.5%.Synechiae were observed in 17.1% of women [246].

Using Klein et al diagnostic criteria, our findings were as follows:

**Calcification:**

Calcification of ovary and tube were seen in one case. 29 years old Mrs. S. married for 4 years was investigated for primary infertility. She had a history of contact with a neighbour who was treated for pulmonary tuberculosis. She was treated for confirmed axillary tuberculous lymphadenitis 9 years earlier. Except oligomenorrhoea she did not have any other symptoms. Her BMI was 18, ESR was elevated to 60 mm at one hour and the Mx was positive with 11 mm induration. USG and HSG showed calcified right ovary measuring about 7-8 cm in diameter. Laparoscopy showed evidence of calcified adnexal mass with dense adhesions. The endometrial specimen was positive for *MTB* by HPE, culture and PCR.

Another case showed evidence of calcification in the centre of the pelvis, therefore calcification of the endometrial cavity was suspected.

**Obstruction at the junction of isthmus and ampulla:**

Studies have reported that in TB, tubal occlusion more commonly occurs at the junction of isthmus and ampulla resulting in mild to moderate hydrosalpinx. In our study, 12 cases presented with distal blocks with hydrosalpinx.

**Multiple contractions along the course of fallopian tube**:

Beaded tubes were identified in 4 cases. Distorted uterine cavity was diagnosed only in 2 cases

In the above cases PCR was positive in 18, conventional methods HPE and cultures were positive in one case.

Studies have shown that cornual occlusion is not very common in TB [41]. However, in our study, in 35 cases there was evidence of cornual block. In these cases PCR was positive in 13, HPE and culture results were positive in two cases. In these 35 cases it is unlikely that cornual blocks were caused by other organisms, because gonococcal and Chlamydia infections have already been ruled out in this study. Other interventions like uterine curettage is also unlikely to be the cause for cornual block as only two patients have had previous abortions in this group of women.

- ***Characteristic features on HSG should alert the clinician as to the possibility of MTB and definitive diagnostic tests should be carried out in these cases.***

**Ultrasound examination (USG)**

Awareness of the sonographic changes associated with tuberculous infection may improve diagnostic accuracy and avoid clinical mismanagement and surgical exploration in wet type of tuberculosis.

Abdominal ultrasound examination was carried out in all the 173 cases. USG findings were normal in 150 (86.7 %) cases. Findings suggestive of tuberculosis were

seen in 23 (13.3%) cases. Adnexal masses and cysts were seen in 17 cases, ascites was present in three cases, calcification in two cases and endometrial fluid was seen in one case. The results of specific diagnostic tests in these cases showed that HPE, culture and PCR were positive in 13 of the 23 cases.

Sonographic features of wet and dry (adhesive) type of tuberculosis were reported by Yapar et al, (1995) [117]. The features of wet tuberculosis were septated ascites, particulate ascites, loculated fluid, thickened peritoneum and adnexal masses. Adhesions, adnexal masses and loculated fluid were found in the dry type [117].

**Laparoscopy in the diagnosis of Genital Tuberculosis**

In today's Gynecological practice, laparoscopy has become an important tool in the evaluation of infertility and in the diagnosis of various pelvic conditions. In women with high suspicion of genital tuberculosis endoscopy helps to obtain microbiological samples under direct vision, evaluate the condition of the organ and the extent of the damage and provides an opportunity for therapeutic intervention [82,123,124,125].

The symptoms of abdominal tuberculosis vary greatly, and laparoscopy can be essential for its diagnosis and management [247]. Significant pelvic morbidity and tubal damage can be diagnosed by laparoscopy [248].

In our study laparoscopy was carried out in all the 173 cases and the findings were suggestive of tuberculosis in 80 (46.2%) of them. In 20 of these 80 cases, there was definite evidence of tuberculosis with findings such as granulomas, caseation, calcification and tubercles. In another 18 cases, there was probable evidence of tuberculosis with hydrosalpinx, dilated retort shaped tubes, tubes covered with white plaques and exudates and loculated ascites and intravasation of dye into the parametrium. In the remaining 42 cases, there was suspicion of tuberculosis because of minimal adhesions, free fluid in Pouch of Douglas and cornual block.

In Bhides et al (1987) study the following laparoscopic findings were reported in 70 patients with proved genital tuberculosis: pelvic adhesions in 48 %, tubercles in 33.8%, adnexal mass in 32.3% and encysted effusion in 8.45% of cases [99].

Three clinical forms of tuberculosis of the appendages are described [88].

1. Early or latent TB where there is no tubal or peritoneal changes.
2. Acute infection showing miliary tubercles, swollen and reddened tubes
3. Chronic infection with terminal hydrosalpinx with retort shaped tubes, T-O masses and intravasation of dye.

Studies have looked at the efficacy of laparoscopy in diagnosing GTB.

Semenovski et al (1999) showed that laparoscopy increases the diagnostic potentiality by 19.7% in diagnosing abdominal and genital tuberculosis [128].

Observation by Agarwal et al showed that bacteriology could detect only 2.8% of cases, histology 21.71%, HSG 51.11% of cases and laparoscopy was suggestive of tuberculosis in 74% of cases. Their study concluded that laparoscopic visualization of genital tract is more effective as compared to bacteriology and histological methods and laparoscopy helps in diagnosing genital tuberculosis at an early stage [130].

In Yang et al study (1996) pelvic tuberculosis was diagnosed in 63.6% of tubal infertility cases by laparoscopy. They have described four types of tuberculosis lesions: miliary ascites, adherent mass, calcification and nodular sclerosis [249].

In our study, laparoscopy was suggestive of TB in 80 (46.2%) cases. But a definite diagnosis could be made only in 20 cases by the presence of granulomas, caseation,calcification and tubercles. In the remaining 60 cases, though a definite diagnosis was not possible, it was assumed that the positive findings were probably due to TB because gonococcal and chlamydial infections were already ruled out.

Visual diagnosis alone at laparoscopy is insufficient [127]. Certain conditions like Tubo – Ovarian mass of gonococcal / pyogenic origin, pelvic endometriosis, and ovarian malignancy may closely mimic a T. O mass of tuberculous origin [56].

Moreover in early and latent cases, laparoscopy may not show evidence on tuberculosis.

Vynck et al from South Africa (1990) reported that, in proved cases of GTB by microbiological studies on menstrual fluid, in 44.5%, the pelvis was clear at laparoscopy [37]. Similar findings were shown by Deshmukh et al from India, where 3 of the 45 histologically proved GTB did not show evidence of TB at laparoscopy [22]. Therefore, during early stages of infection subtle clinical manifestation may not be evident at laparoscopy [133].

***Therefore, a definite diagnosis can only be made by positive histology of tissue or by positive culture of tissue or POD aspirate.***

**Hysteroscopy [56,108]**

Hysteroscopy is also a useful tool in assessing the endometrial cavity in suspected GTB. As the instrument was available only for a few cases, hysteroscopy was done only in seven cases. Two of these cases presented with secondary amenorrhoea. In one case there was evidence of calcification and the other case showed intra uterine adhesions. There was no menstrual disturbance in the other five women. However, in two of these cases findings were suggestive of tuberculosis; in one case there was a white patchy lesion visualized near the left cornua of the tube. The other case showed small tubercles in the endometrium near the left cornua and a small lesion within the cornua itself. These two cases were positive by PCR. However, HPE and culture were negative .In these two cases laparoscopy showed congestion of tubes with minimal adhesions.

Microbiological Techniques for the diagnosis of Tuberculosis

Tubercle bacilli may be demonstrated by direct microscopy, by culture and by animal inoculation.

**Detection of AFB in Direct Smears**

Direct microscopy is important because the results are available immediately. It may be of value in the primary care setting because it is simple and it is relatively easy to perform and interpret. It often gives preliminary confirmation of the diagnosis while waiting for culture report. Direct microscopy will reveal whether acid fast bacilli are present or absent. For a smear test to be positive there should be 10,000 organisms per milliliter of specimen [141,141].The presence of small number of bacilli in extra – pulmonary sites contribute to difficulty in visualization of acid fast bacilli.

In our study, 8 (4.6%) of the 173 endometrial samples and 5 (6.2%) of the 81 POD aspirates were positive for AFB smear.

The AFB bacilli are very rarely found in endometrium and cervical granulomas even with the use of fluorescent techniques [60,145].The reported incidence of AFB smear positivity varies from 1.23% to12.19% (Table 30), and our results are similar to that of Rozati et al study [71].

**Table 30: Studies Showing Positive Results of AFB Smear in GTB**

| Author | Year | Country | Sample size | Positive cases % |
|---|---|---|---|---|
| Rozati et al [71 ] | 2006 | India | 65 | 5.2% |
| Bhanu et al [133 ] | 2005 | India | 25 | 1.6% |
| Abebe et al [ 150 ] | 2004 | Ethiopia | 25 | 4% |
| Namava et al [ 25 ] | 2001 | Iran | 46 | 12.19% |
| Misra et al [ 81 ] | 1996 | India | 162 | 1.23% |
| Present study | 2008 | India | 173 | 4.6% |

The acid fast bacilli (AFB) could be any pathogen or saprophytic mycobacteria [137]. Therefore, positive smear alone in not an adequate criteria and must be followed by culture to determine the species of mycobacterium [138].

In our study, none of the 8 samples that were AFB positive grew MTB on culture. However, in 3 cases NTM organisms were positive. In two of these cases NTM organisms were grown both in the endometrium and POD aspirate. In two other cases PCR was positive**.**

Absence of AFB does not exclude the diagnosis of female genital tract tuberculosis. Eisenach study showed that PCR could distinguish between tuberculosis and non tuberculous mycobacteria in cases of smear positive disease [145].In specimens positive for AFB, American Thoracic Society recommended to use PCR as additional evidence [146].

**Microbiological Culture**

Accurate identification of Mycobacterium tuberculosis through culture is presently the yardstick for diagnosis and remains the gold standard technique. However, the time required for the report and frequent negative results in paucibacillary specimens are important limitations.

Endometrial biopsy is the most popular method of obtaining samples for culture. In this study, culture was carried out in 173 premenstrual endometrial samples, 81 POD aspirate and 52 urine samples. Since these extra-pulmonary lesions are pauci bacillary in nature, their processing included milder decontamination and inoculation into multiple media. At the end of 8 weeks species of *MTB* was identified in positive cultures.

Blanie et al (2005) claimed that combined inoculation of liquid and solid medium increases the sensitivity and helps to identify the pathogen in extra-pulmonary specimens [250].

However, in spite of inoculation into multiple media, our study revealed that only six samples (3.5%) yielded microbiological proof of MTB. Interestingly, in our study, NTM was isolated in 41 cases.

Positive endometrial cultures for *MTB* observed in various studies have ranged from 2.05% to12%.TABLE 31

**Table 31: Positive endometrial culture reported by various authors:**

| Author | Year | Country | No. of cases | Positive cases |
|---|---|---|---|---|
| Roya et al [71 ] | 2006 | India | 65 | 7.8% |
| Bhanu et al [133 ] | 2005 | India | 25 | 3.2% |
| Abebe et al [150 ] | 2004 | Ethiopia | 25 | 12% |
| Manjari et al [ 163] | 1995 | India | 1124 | 2.05% |
| DeepJyoti et al[21] | 1990 | India | 200 | 6% |
| Chhabra [92] | 1986 | India | 150 | 7.3% |
| Present study | 2008 | India | 173 | 3.5% |

Abebe et al (2004) from Ethiopia has reported a prevalence of 12% on endometrial cultures. Indian studies have shown a lower prevalence rate [150].Failure to obtain tubercle bacilli is certainly not conclusive evidence of their absence in the lesion.

The possible reasons for the low incidence of culture positivity in endometrial tissue could be due to paucibacillary nature of the endometrial site and a substantial number of TB lesions of the genital tract are bacteriologically mute [133].The specimen of endometrial tissue used for culture may be unrepresentative. The low rate of positivity in culture may also be due to the presence of a bacteriostatic substance which inhibits the growth of the bacilli [159]. Similar negative results are obtained

when the patient has had previous ATT. Most of the infertile women in India are started on empirical ATT, and this could contribute to low pick up rate of *MTB* by culture.

Samples that show preponderant caseous granulomas have a higher percentage of culture positivity than samples which show non- caseating granulomas, as they are thought to have more number of bacilli [251].

It has been suggested that in order to increase the microbiological yield, the optimal time for sampling is at the end of the menstrual cycle or within 12 hours following the onset of menses to allow maximum time for the endometrial granulomas to develop [16]. In this study endometrial sampling was done between the $24^{th}$ to $28^{th}$ day of menstrual cycle.

Studies have also shown that culture of the menstrual blood yield greater number of positive results [167,168,169]. In a study from South Africa, of the 23 cases of GTB, culture was positive in 69.6% of menstrual blood and 17% of endometrial tissue [20]. Similarly in Margolis et al study in 1992, menstrual fluid culture proved to be the most reliable diagnostic procedure since it was positive in 11 patients in whom pre-menstrual endometrial culture was negative [ 75]. In the present study menstrual blood culture could not be done, because our women are reluctant and refuse to come for pelvic examination during periods.

In order to improve the diagnostic accuracy samples from other sites (POD aspirate -81samples, urine - 52 samples) were also taken for culture. However, none of the POD aspirate and urine samples were positive for mycobacterial organisms.

Various studies have evaluated combined histology and bacteriological methods in the diagnosis of endometrial tuberculosis and concluded that both the methods of diagnosis are complementary to each other and neither is completely dependable [160,161,162,163]. Authors have shown that a combination of

histopathology and microbiological evaluation increases the diagnostic rate by 10% to 14.5% [79,166].

Tuberculous endometritis can masquerade as a non-specific non-granulomatous lesion [64]. Bacteriological study was of greater value in doubtful cases where there was absence of granulomas or epitheloid cells but presence of inflammatory exudates. In our study, non-specific endometritis was reported on HPE in 6 cases, but none were positive by culture. However, PCR was positive in these cases. In Deep Jyoti et al study, in 4 cases where histology revealed chronic endometritis, the AFB culture was positive [21]. In Chakraborty et al study, by histology, 8 doubtful cases and 4 normal cases were picked by culture [166]. Similar observations were made by Kothadia et al. [165].

The value of culture lies not in the diagnosis of tuberculosis, but in the assessment of drug resistance and in distinguishing non tuberculous mycobacteria (NTM) from *MTB* [170]. NTM organisms are acid fast Mycobacteria, but do not cause tuberculosis. They have characteristics distinct from those of M. tuberculosis. NTM organisms are acquired from the environment. Exposure to contaminated water, injections, surgical procedures, and trauma has been linked to infection with NTM. Most NTM infections have been linked to cutaneous disease with manifestations such as painful popular lesions, erythematous nodular and ulcerating skin nodules. Histopathological studies of skin biopsy specimens have shown non-specific chronic inflammation and suppurative granulomas.

NTM organism has been cultured in extra-pulmonary sites such as lymph nodes [171]. Few studies have reported atypical mycobacterium in culture while investigating cases of infertility for GTB. In 1978, Khiancy et al reported 7 cases of atypical mycobacteria grown in culture [172]. In Chhabra et al study (1986) out of 150 endometrial samples 9 *MTB* and 2 atypical mycobacterium had grown in culture

[162]. In Chakraborty et al study, (1993) out of 1320 endometrial samples *MTB* was grown in 80 cases and atypical mycobacteria in 2 cases [166].

In our study in 41 (23.7%) cases NTM (previously known as atypical mycobacteria) had grown in culture. Among these, in 25 cases, the samples were analyzed by High Performance Liquid Chromatography (HPLC) to identify the Mycolic acid characteristics of various NTM organisms.

HPLC analysis showed that Mycobacterium chelonae / Mycobacterium abscessus was the predominant organism grown in 16 samples. Mycobacterium Fortuitum was grown in 4 samples, Mycobacterium Kansasii in 1 sample, and Mycobacterium simiae was positive in 2 samples, Mycobacterium Intracellulare and Mycobacterium marinum were grown in one sample each. Because of the high prevalence of NTM organisms in our study, to rule out the possibility of contamination from the environment, 10 samples were taken from the theatre environment as well as from the instruments which were used for curettage and laparoscopy. All the 10 samples were negative for NTM organisms.

Moreover, in 24 of the 41 cases laparoscopic findings showed tubal block and adhesions and these 24 cases were negative for *MTB*, gonococci and Chlamydia. In 10 cases, NTM organisms were positive both in the Endometrium as well as in the POD aspirate.

In 1996, Horsburgh noted clinically important nontuberculous mycobacteria which included M.chelonae, M.fortuitum, M.kansaii, M.simiae, M.avium and M.marinum. This raises an unanswered question whether NTM organisms are also responsible for tubal damage leading to infertility in southern parts of India. In the present scenario the significance of these findings is not known. Probably further study may enlighten whether NTM organisms could lead to tubal damage [172A].

**Histopathological Examination**

A definite diagnosis of genital tuberculosis is possible by the recovery of *MTB* from the genital tract or by the histological demonstration of tuberculous granulomas in tissue samples. Histo pathological examination is easy, quick and cheap and provides characteristic features of *MTB.* In female genital tuberculosis, the fallopian tube is the initial site of involvement, affected in almost all cases, followed by secondary extension to the endometrium in 50 to 90% of cases [18, 23,104,145,149].

It seems probable that tuberculous endometritis is almost 100% proof of tubal tuberculosis even without clinical and palpable evidence of adnexal disease [61].As the uterus is more accessible for taking biopsies, most studies use endometrial curettage to collect the material for the diagnosis of genital tuberculosis [25].

A diagnosis of endometrial TB made on histological grounds alone is reasonably dependable. The typical and almost pathognomonic lesion of the tuberculous endometritis in regularly menstruating woman is the non-caseating granulomas composed of epithelioid cells, giant cells and peripheral lymphocytes.

In our study, in 173 infertile women endometrial samples were taken by pre menstrual curettage for HPE and 7 (4.0%) of the 173 samples were positive for *MTB.* Another 6 specimens were reported as non specific endometritis.

The reported incidence of MTB by HPE varies from 0% to 4.92 %.

(TABLE 32) Our results are comparable with those reported by other authors.

**Table 32 HPE positivity of endometrium reported by various authors.**

| Author | Year | % Positive |
|---|---|---|
| Bazaz Malik [ 64 ] | 1983 | 2.3% |
| Manjari et al [ 163 ] | 1995 | 1.87% |
| Misra et al [ 81 ] | 1996 | 4.92% |
| Nagpal et al [88 ] | 2001 | 3.8% |
| Bhanu et al [ 133 ] | 2005 | 0% |
| Ojo et al [252] | 2008 | 0.45% |
| Present study | 2008 | 4% |

The low prevalence of *MTB* in endometrial biopsy may be due to various reasons. Due to the secondary nature of the genital tuberculosis, the infecting organisms are sparse in number, the sampled site may not represent the infected area and therefore, the infected site can be easily missed. In as many as 50% of cases, the infection may be limited to the fallopian tubes [20]. Moreover, due to the cyclical shedding of the endometrium, granulomas do not have enough time to form, so, the endometrium may not show evidence of tuberculosis in all the cycles [41]. HIV co-infection may not elicit adequate cell mediated immune response to produce changes in the endometrium. [71].

Due to the above reasons, to ensure maximum yield multiple specimens from several sources should be collected. The best time for examining the endometrium is in the premenstrual phase, at which time the tubercles reach their maximum growth. The portion of the endometrium most likely to show tubercles is in the region of the uterine cornua where the spread from the tube first occurs.

Granulomatous lesions can also be produced by other conditions such as acidosis and foreign body reaction; however, these conditions are rare in the

endometrium. Therefore, when a granulomatous lesion is present it is probably tuberculosis [38]. In those cases presenting with amenorrhoea, endometrial replacement with caseous granuloma is a predominant finding, because, there is ample time for the lesions to progress to full blown caseous granuloma [145,175].

The endometrium can almost be completely replaced by tuberculous granulation tissue. In one study the incidence of lost endometrium was reported to be 50.95% [176]. In our study, only fibro collagenous tissue was obtained in two cases.

In early lesions the epithelium of endometrial glands exhibit necrosis and the lumen contains exudates, often eosinophilic and inflammatory cells including polymorphs and macrophages. Histologically these lesions may appear normal [164]. In the absence of typical granuloma, dilatation of glands, active destruction of epithelium and inflammatory exudates in the lumen also suggest tubercular aetiology [64].

Tuberculous endometritis can masquerade as a non – specific non – granulomatous lesion [62, 64]. In our study; six specimens were reported as non-specific endometritis.

Number of studies has evaluated the value of microbiological culture in cases where normal endometrial histology or chronic endometritis was reported. Bacteriological study was of greater value in doubtful cases when there was absence of granuloma or epitheloid cells but presence of inflammatory exudates [166].

In DeepJyoti et al (1990) study, among the 200 infertile women, eight cases were diagnosed as tubercular endometritis by histopathology as well as culture of AFB. In yet another 4 cases, where histology revealed chronic endometritis, the AFB culture was positive [21]. In Chakraborty et al study, histologically 8 doubtful cases and 4 normal cases were picked by culture [166].Similar observations were made by Kothadia et al. [165]. In our study, none of the six specimens reported as non-specific

endometritis were positive for AFB smear or culture. However, five of them were positive for PCR reaction.

In one of the cases, besides the endometrium the endocervix also showed evidence of tuberculosis. Bobhate et al reported that the tuberculosis of cervix was mostly observed in endocervical region, seen in 84.6% of cases [48]. When tuberculosis of the cervix is diagnosed, it is almost certain that TB spreads to the cervix from the Endometrium [49].

Though endometrial curettage is the commonest procedure to obtain endometrial tissues, studies have looked at endometrial aspiration cytology for the diagnosis of genital tuberculosis. Endometrial aspiration cytology has been claimed to be simple, inexpensive and less traumatic, can be repeated more than once and can be done in the out-patient departments (OPD) , as well as the accuracy of endometrial aspiration cytology has been reported to be 100% [108,178]. However, endometrial aspiration cytology was not done in this study.

**The Polymerase chain reaction (PCR) in the diagnosis of genital tuberculosis**

In recent years PCR technique is the best known and most widely used Nucleic Acid Amplification (NAA) test and has evolved as a rapid, sensitive and specific molecular biological method for detecting mycobacterial DNA in both pulmonary and extra-pulmonary samples from patients suspected of suffering from tuberculosis [182].

Many studies have shown PCR to be the most sensitive and rapid method to detect extra-pulmonary mycobacterial tuberculosis [186,187,188].

In recent years, PCR technique has been found to be useful in confirming the diagnosis in a substantial number of cases of tuberculosis of female genital tract [216,217,218,219].

Several target sequences have been described to detect *MTB.* The most widely used primers to detect M.tuberculosis in clinical specimens in PCR are from the insertion element IS 6110. Report by Negi et al (2007) suggests that the presence of IS6110 correlates more closely with the diagnosis of clinical tuberculosis than that of 65kDa, 38kDa and 85B proteins [199]. However, Narayanan et al. (1997) had reported that 40% of the strains of MTB isolated from patients in Chennai had only a single copy of IS 6110 and 4% did not carry even a single copy of IS 6110 [194].

Since IS 6110 based PCR for diagnosis may in some cases lead to false negative results, a new target for PCR using repetitive element called pTRC $_4$ was developed at the Tuberculosis Research Centre, Chennai [194]. Study by Narayanan et al (2000) showed that PCR with both probes IS 6110 and TRC $_4$ can be used as a fast and sensitive adjunct to other conventional techniques in the diagnosis of extra-pulmonary tuberculosis [200].

Therefore, in our study, PCR technique was carried out on 160 endometrial samples using both IS 6110 and TRC$_4$ probes. The PCR was positive in 45 (28.1%) of the endometrial samples using either or both probes.

Several studies have shown PCR positivity in endometrial samples ranging from 22.5% to 57.35% (TABLE 33)

**Table 33 Studies showing PCR positivity in endometrial samples**

| Sl.No. | Author | Year | Country | Sample size | % Positivity |
|---|---|---|---|---|---|
| 1 | Gupta et al[105] | 2007 | India | 40 | 22.5% |
| 2 | Rozati et al[71] | 2006 | India | 65 | 43% |
| 3 | Banu et al[133] | 2005 | India | 25 | 53.3% |
| 4 | Abebe et al[150] | 2004 | Ethiopia | 25 | 48% |
| 5 | Himanshu et al[57] | 2003 | India | 150 | 57.35 |
| 6 | Present study | 2008 | India | 160 | 28.1% |

Our results are comparable with those from Gupta et al (2007), reporting 22.5% PCR positivity in endometrial biopsy samples from 40 infertile women. However, other authors have reported higher rates of PCR positivity in endometrial samples.

One of the advantages of PCR is that, it can be applied to sterile fluids like peritoneal fluid where the culture is difficult due to low bacterial load [182]. Therefore, in our study, in order to improve the diagnostic accuracy, fluid from the POD was also aspirated in 81 patients for various diagnostic tests. Among the 81 samples PCR was positive in 16 (19.8%) samples. Similar results were reported by Bhanu et al in 2005, where 16% of POD aspirate were positive for PCR [133].

In this study 4 of the 52 urine samples were also positive for PCR reaction.

## EVALUATION OF DIAGNOSTIC TESTS IN ENDOMETRIAL SAMPLES

The results of various diagnostic tests done on endometrial samples were analyzed. It was seen that, AFB smear was positive in 4.6% of cases, culture in 3.5%, HPE in 4% of cases and PCR in 28.1% of cases.

Rozati et al. (2006), investigated the value of different diagnostic techniques for the diagnosis of genital tuberculosis in 65 infertile women, suspected of having genital tuberculosis on clinical grounds. In this study *MTB* was diagnosed by AFB smear in 5.2%, by positive culture in 7.8%, by histopathology in 11.05% and by PCR in 43.1% of 65 suspected cases [71].

Table 34 shows the results of specific diagnostic tests reported by various authors. Our results are similar to that of other authors except the PCR positivity which is higher in other studies.

**Table 34: Studies showing results of specific diagnostic tests on endometrial samples**

| Diagnostic test | Bhanu et al [133 ] | Rozati et al [ 71 ] | Present study |
|---|---|---|---|
| AFB smear | 1.6% | 5.2% | 4.6% |
| Culture | 3.2% | 7.8% | 3.5% |
| HPE | 0% | 11.05% | 4% |
| PCR | 56% | 43.1% | 28.1% |

**Evaluation of Urinary System**

10% of females with genital tuberculosis also show evidence of renal involvement [51]. While investigating a woman with infertility, investigations for genitourinary tuberculosis should be initiated in the presence of unexplained urinary symptoms, haematuria, persistent pyuria or abacteriuric pyuria [40]. In this study only one patient presented with haematuria. A non invasive way to diagnose GTB could be by testing of urine samples for *MTB* by various tests. Therefore, three first morning voided midstream urine specimens were sent for AFB stain and culture in 52 cases. Specimens were also tested for the presence of PCR DNA.

In this study, none of the urine samples were positive for *MTB* on culture and six samples were positive for NTM organisms. Four samples showed a positive reaction to PCR. Therefore, testing of urine samples may not be helpful in diagnosing GTB. Similar to our results; in Namavar et al study urine culture was negative in 41 cases of proved genital tuberculosis [25]. In Marna et al study, (1992) in an infertile patient, genital tuberculosis was diagnosed by isolating MTB only from the urine [135].

**IDENTIFICATION OF POSITIVE CLINICAL CRITERIA TO SUSPECT GTB**

The diagnosis of GTB is more challenging. Therefore, there is always a dilemma as to whether all infertile women are to be investigated for GTB? ; And which reliable diagnostic test to be used in clinical practice to diagnose GTB? Most clinicians, in India, however treat infertile women with ATT based on a probable or presumptive diagnosis of GTB.

The possibility of tuberculous infection should always be considered in a patient from an area in which the infection is common and endemic [16]. Diagnosis of GTB is difficult, as the disease has varied clinical presentation or may remain asymptomatic [68]. Therefore, a high degree of suspicion is necessary to diagnose the condition.

Published studies have shown various ways of diagnosing GTB. A presumptive diagnosis of genital tuberculosis can be arrived at with the following criteria: [16, 22,100].

- Presence of infertility for which no other cause can be found
- Chronic pelvic inflammatory disease refractory to standard antibiotic therapy
- A past history of definitely documented tuberculosis
- History of close contact with a family member suffering from tuberculosis
- Chest X-ray showing evidence of active or past infection

- Ultrasound evidence of disease
- Typical HSG findings
- A positive IgG, IgM against A60 mycobacterial antigens
- Positive Mantoux test

Jindal et al, in 2006 has used a simplified algorithm looking for a presumptive evidence of GTB. Based on clinical suspicion and a battery of laboratory tests a presumptive diagnosis of GTB was made on 7.2% of infertile women and were treated with ATT [82].

There are no accepted guidelines for diagnosing extra-pulmonary tuberculosis, in view of the low sensitivity of bacteriological tests and the poor specificity of most immunological and serological investigations [253,254]. Therefore, an attempt was made to identify a group of clinical parameters which would indicate the need for further evaluation by specific diagnostic tests to confirm the diagnosis.

The following parameters were identified as positive indicators to suspect GTB.

**Definite past history of tuberculosis:**

In this study in 10 of the 11 cases with past history of tuberculosis there was evidence to suspect GTB as other diagnostic parameters were also positive.

**Presence of active disease:**

Presence of active disease, especially in the presence of abdominal tuberculosis one would expect genital organs also to be involved.

**Positive Mantoux test:**

In our study 37 of 173 cases that showed positivity to Mantoux test, one or more other diagnostic parameters were also positive. The Mantoux test also showed a sensitivity of 85.7% and the specificity of 81.3% in diagnosing GTB when evaluated against the conventional methods

**Elevated ESR**

Among the 27 cases in which ESR was elevated, laparoscopy was suggestive of TB in 21 cases, PCR was positive in 14 cases and Mx test was positive in 20 cases. Conventional methods of diagnosis, the culture and HPE were positive in six cases, and one case was positive by HPE alone. When the efficiency of ESR in diagnosing GTB was evaluated against the conventional methods, the sensitivity of ESR was found to be 71.4% and the specificity was 86.7%.

**Positive Findings at USG**

Of the 23 cases in whom the USG findings were suggestive of TB, in 13 of them specific diagnostic tests were positive.

**Characteristic features on HSG**

Of the 22 cases with characteristic features of tuberculosis on HSG, 18 were positive by PCR and another one was positive by both HPE and culture.

**Positive findings at Laparoscopy**

As laparoscopic findings are included as one of the important criteria to diagnose genital tuberculosis, measures were taken to rule out other organisms such as Chlamydia and Gonococci which can also result in tubal blocks, adhesions and tubo-ovarian (TO) masses. Therefore, positive findings at laparoscopy in the absence of gonococcal or Chlamydia infection may suggest tuberculosis.

**EVALUATION OF SPECIFIC DIAGNOSTIC TESTS**

Cultivation of *MTB* is considered the Gold standard for the diagnosis of tuberculosis. However, this gold standard lacks sensitivity and is negative in specimens from majority of paucibacillary cases.

In genital tuberculosis one cannot use a gold standard technique to evaluate a diagnostic test as the detection rate of mycobacterial culture and HPE are very low.

This poses great dilemma for comparing gene amplification methods which are vastly more sensitive but have danger of false positivity due to contamination.

***In conditions , where there is no gold standard technique to evaluate a diagnostic test is available, one may have to develop and justify a combination of clinical profile and criteria against which the new test has to be assessed*** [211].

Therefore, in order to evaluate the specific diagnostic tests, based on the results of clinical profile and laparoscopic evaluation a diagnostic criterion was derived to suspect GTB in infertile women.

In the past authors have suspected GTB clinically and evaluated various specific diagnostic tests against the clinically suspected cases [71]. Bhanu et al have used laparoscopic findings to suspect GTB and to evaluate the diagnostic tests [133].

A woman was said to be suspected of having genital tuberculosis if she has had findings suggestive of tuberculosis by laparoscopy with one or more of the following findings: A definite past history of tuberculosis, presence of active extra genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux and evidence of calcification / complex adnexal mass by scan.

Of the 173 samples that were tested for Chlamydia trachomatis & Neisseria gonorrhoea infection, 13 were found to be positive for Chlamydia and 3 were found to be positive for gonococcal infection. Therefore, these 16 cases were excluded from the study.

In the remaining 157 cases, in 153 endometrial samples the results of all the three specific tests were available and therefore taken for further evaluation.

Based on the newly derived clinical criterion cases were divided into two groups.

**Group A: Those with suspicion of tuberculosis 61/153**

**Group B: Those in whom tuberculosis was not Suspected 92/153**

In order to identify to a suitable test which can be used in clinical practice, **the specific diagnostic tests: AFB smear, culture, HPE and PCR were evaluated against the newly derived diagnostic criteria.**

By bivariate analysis, the sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated for the above diagnostic tests and the results were as follows.

**Table 35: Comparative evaluation of various diagnostic tests**

| Test | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| PCR | 44.3% | 80.4% | 60% | 68.5% |
| HPE | 8.2% | 100% | 100% | 62.1% |
| Culture | 6.6% | 100% | 100% | 61.7% |
| AFB smear | 6.7% | 98.9% | 80% | 61.5% |

In this study, PCR showed a sensitivity of 44.2% which was higher compared to the other diagnostic tests. However compared to other authors the sensitivity of PCR in diagnosing GTB is low.

In Bhanu et al study, the sensitivity of PCR in both endometrial biopsy and endometrial aspiration samples were 76.9% [133]. Similarly, Rozati et al in 2006 reported a sensitivity of 96.4% for PCR in diagnosing GTB in 65 cases of suspected GTB [71].

**Evaluation of PCR test in the diagnosis of GTB**

This study has shown that the sensitivity of PCR is higher as compared to the other diagnostic methods. Therefore, it was evaluated further for false negative and false positive results.

**False negative PCR results**

The PCR results were negative in 34 patients who had positive clinical and laparoscopic features of genital TB. This could indicate the possibility of false negative PCR results. When comparing PCR results with the conventional methods of diagnosis, in the five cases that were positive by either culture or HPE, PCR was positive only in three cases. Similar observation was also made by Rozati et al in 2006. In their study of 65 clinically suspected GTB, four of the seven histology positives were not supported by PCR [71].

The possible explanation for these false negative results of PCR could be due to paucibacillary nature of the specimen, and the portion of the specimen taken for PCR would not have had any *MTB.* The analyzed specimen may also contain inhibitors of PCR. A thorough DNA purification is of the greatest importance in testing blood containing samples.

Blood containing specimens are characterized by the presence of numerous PCR inhibitors. Restrepo et al (2006) have shown that mycobacterial DNA amplification was compromised when the human: bacterial genome ratio was at least 190:1. Separation of the specimen into bacterial and host – rich fractions prior to DNA extraction improved mycobacterial DNA detection by 30% [255]. As endometrial samples are always mixed with blood, this could possibly explain the

false negative results in this study. The inhibitory activity of clinical material can be decreased by additional purification of DNA by gel filtration on microcolums. Several strategies have been used to improve the sensitivity; such as, use of immunomagnetic beads and capture resins [209,210].

False negative result can also occur when there is contamination of the sample with heparin which is a known PCR inhibitor. [123]. However, in this study heparin was not used at any stage of the study. Bhanu et al study also reported that, 63.9% of their samples were negative for the mpt 64 gene in spite of the positivity based on the laparoscopy. They concluded that, the involvement of the genital tract in TB could be generalized or localized and the technique of sample collection is blind, there is a possibility of missing the infected site [133].

In our study, among the 34 cases with possible false negative results, in 11 samples NTM organisms were grown in culture. Whether NTM organisms could have resulted in inflammatory changes in the pelvis giving rise to positive findings at laparoscopy and negative PCR results should be ascertained. In the present scenario the significance of these findings is not known. Probably further study may enlighten whether NTM organisms could lead to tubal damage.

**False positive PCR results**

In 18 women, PCR was positive and the clinical profile was negative. This raises the possibility of false positive PCR results by way of contamination, dead bacilli or previous infection

During this study stringent precautions were taken to avoid the problem of false positivity by way of strict discipline about collection and processing of the specimen and handing of reagents. Preparation of PCR reagents, addition of template DNA and analysis of amplified products was done in three different rooms to avoid carryover contamination. Reagents were aliquoted and each aliquot was used only once. Wax beads were added to minimize non-specific amplification. Moreover,

during the procedure, in order to monitor the false positive results, each PCR run was controlled by adding concurrent negative control (without templates). No positive bands were noted in these controls and all were PCR negative.

Therefore, it is unlikely that positive PCR reaction and a negative clinical profile is a case of false positivity. But PCR has probably detected the early disease with low number of bacilli or with latent infection when women are still asymptomatic and before structural damage to the tube has taken place.

During early stages of infection, subtle clinical manifestation of GTB may be overlooked at laparoscopy. The pelvic structures and the covering peritoneum may appear normal; however, there may be an outpouring of bacilli from the tube into the uterine and peritoneal cavity.

The problem of false positivity can also be minimized by way of multiple areas of sampling and repetitive sampling. In our study, in 13 of the 18 cases false positive results can be ruled out as the PCR was positive both in the endometrium and POD aspirate and in 2 cases urine samples were also positive for PCR. As multiple areas of sampling have given positive results, it is possible that PCR has picked up an early disease or a latent infection.

In order to enhance the sensitivity of PCR, repeat sampling was done in 10 cases. Though it was not possible to prove the reliability of the test by re sampling a large population, within the available small number of cases there was concordance in the results PCR between the first and second visit in 9 of the 10 cases ; a crude agreement of 90%.

Bhanu et al study also suggested that multiple sampling and repeat sampling from the patient will enhance the sensitivity of PCR as a diagnostic tool [133].

Also, in six of the 18 cases, the HPE findings on the endometrium were reported as chronic endometritis. Studies have shown that chronic endometritis

without typical granulomas could be the earliest finding in endometrial tuberculosis [64].

When a histopathologic diagnosis of chronic granulomatous inflammation is reported which may be due to Mycobacterium tuberculosis, foreign body reaction, sarcoidosis and brucellosis, TB-PCR should be performed to enable a definitive diagnosis of tuberculosis [205,206]. American Thoracic Society recommended using PCR as additional evidence in specimens smear positive for AFB [212]. In this study PCR was positive in 2 of the 8 smear positive cases.

**Analysis of PCR in Multiple Samples**

PCR study was carried out on the endometrium, POD aspirate and urine samples.28.1% of the 160 endometrial samples, 19.8% of 81 POD aspirate and 7.7% of 52 urine samples were positive for PCR.

In 81 women, both endometrium and POD aspirate were available for PCR study. An attempt was made to analyze the concordance in PCR results in the endometrial and POD aspirate samples. (Table 21) The analysis showed that out of 16 cases that showed positivity in POD aspirate, 13 of them were positive in the endometrial samples also. In 66 (81.5%) of the 81 samples there was agreement in the results and the Kappa statistics (52%) showed a good agreement.

The above findings points out that PCR is a useful tool in diagnosing genital tuberculosis, as there is a good chance corrected agreement ( Kappa = 52%) between the samples taken from different sources from the same patients.

Our study has shown that, false positive results are possibly true positives as shown by the results of multiple sampling and concordance of results with HPE. Therefore, as PCR is able to pick up latent and early diseases, a positive PCR result should be given due importance and treatment initiated.

However, as the false negative results are high, a negative PCR result does not rule out GTB, therefore, further investigations are necessary before a definite diagnosis is made. It is not known whether NTM organisms could have caused inflammatory changes in the pelvis to give a positive finding at laparoscopy, therefore PCR is negative for *MTB.*

As the pick up rate of *MTB* by PCR in the endometrial sample is almost similar to that of POD aspirate, one need not resort to laparoscopy to collect material to confirm diagnosis. Endometrial sampling alone, a less invasive procedure, can be used for the diagnosis. However, if the woman is subjected to laparoscopy for various reasons, then POD aspirate can also be taken for PCR studies which will increase the accuracy of diagnosis.

**Evaluation of two sets of primers in PCR technique:**

IS 6110 primers are widely used in the diagnosis of *MTB.* Studies have shown that use of more set of primers along with IS6110 will increase the sensitivity of PCR. Therefore, along with IS6110 primers we have used another primer called $TRC_4$ primer in this study [200].

On evaluating PCR using two sets of primers, the agreement between the results of two chance corrected sets of primers was marginal (Kappa = 15.8%). The sensitivity of IS6110 primer in diagnosing genital tuberculosis was 14.8 %, and the sensitivity of $TRC_4$ was 36.1 %.However, simultaneous use of two sets of primers namely, IS6110 and $TRC_4$ probes has increased the sensitivity of PCR to 44.2%. The false negative rate of IS 6110 probe was more (85.2%) compared to the false negative rate of $TRC_4$ primer. (63.9%) Therefore, use of IS 6110 primer alone may miss the diagnosis in some of the cases. Use of more than one set of primer would enhance the pick up rate of GTB.

**Reliability of PCR in Diagnosing GTB**

Reliability of PCR in diagnosing GTB was checked by repeat sampling on 10 women.

- In 9 of the 10 repeat samples, there was concordance in the results of first sample and repeat samples. This validates the PCR test and the quality control of the laboratory in which the tests were done.
- In two cases, where the clinical criteria were negative, the PCR results were consistently positive. In one of these cases, the initial PCR was positive in the endometrium, POD fluid and the urine sample and the repeat endometrial sample was also positive. PCR being positive in multiple samples and repeat samples shows that PCR is able to detect early disease in these cases.
- In four cases, where the clinical criterion was positive for GTB, the results of PCR in the initial sample and repeat samples were consistently negative. The common infections to cause tubal damage are gonococci, Chlamydia and *MTB*. In this study, cases which were gonococci/Chlamydia positive were not included in the evaluation, and PCR for *MTB* has been consistently negative in these four cases. Therefore, one should be looking for other organisms which can cause tubal damage. In this study NTM has been cultured in a proportion of cases which needs further evaluation.
- The result of re-testing of samples showing consistency in 18 of the 19 samples (10 negative and 9 positive samples) once again validates the PCR test and the quality control of the laboratory in which the tests were done.

**TREATMENT**

Early diagnosis with culture positive for AFB, but before the development of histologic evidence of disease may be associated with a more favourable outcome if the disease is treated promptly and adequately [222]. Even extensive genital and

intraperitoneal tuberculosis resolve almost completely with ATT [256]. Treatment of genital tuberculosis with a 9 month short course Chemotherapy has been shown to be effective [82,108,154].

Though tubal patency may have been restored following ATT, the tubes may remain rigid and beaded in most cases. Despite the advances in chemotherapeutic treatment, pregnancy after treating genital tuberculosis is rare and when it does occur is more likely to be an ectopic or result in spontaneous miscarriage [225,226,227].

In our study, the seven cases who were positive for GTB either by culture or by HPE were treated with short course of chemotherapy for 9 months. The patients are under follow up for the last two years. No conception has occurred so far. The possible reason could be that, they were all diagnosed at an advanced stage of the disease with extensive adhesions, distal tubal blocks with hydrosalpinx, the tubes damaged beyond recovery. The possible options available for these unfortunate women would be IVF-ET and adoption.

In Vitro fertilization with embryo transfer remains the most effective method of treating associated infertility [257]. However, among patients treated with IVF-ET, patients with GTB are a less favourable subset among other tubal infertility cases. Even IVF-ET cannot be attempted in one case as part of the endometrial cavity was replaced by calcification [43].

Among 97 cases of infertile women with GTB, Tripathy et al (2002) reported a conception rate of 19.2% and a live birth rate of 7.2% [228]. Sotskaia (1998) reported that in severe genitoperitoneal tuberculosis, timely combined antituberculous treatment concurrently with surgical intervention yields good results for recovery of reproductive function [258].

# CHAPTER VII

## SUMMARY

This is a prospective study conducted at the Fertility Research clinic of the Institute of Obstetrics and Gynaecology, Chennai. This study is a comprehensive attempt to arrive at a presumptive diagnosis of GTB as well as to evaluate the efficiency of specific diagnostic tests namely; AFB smear, culture, HPE and PCR in the diagnosis of GTB. Based on the historical, clinical, laboratory investigations and results of gynaecological procedures a presumptive diagnosis of GTB was arrived at and the results of specific diagnostic tests were evaluated against this presumed criterion.

This study included 173 infertile women in whom infertility was due to tubo-peritoneal factors and cases of unexplained infertility. Cases with other causes of infertility were excluded from this study.

In these 173 women, detailed history was taken; careful clinical examination was carried out. Laboratory investigations for tuberculosis and specific gynaecological procedures were also performed on these women. Premenstrual endometrial tissue, POD fluid and urine samples were collected and submitted for direct AFB smear, culture for *MTB* and PCR testing for *MTB* DNA.

The above test results were evaluated against the presumed diagnostic criteria.

**SECTION A:**

**CLINICAL PROFILE**

- The age of the women included in this study ranged between 20 and 37 years. Majority of them 124 (71.7%) were aged less than 30 years.
- The duration of marital life ranged between 2 and 15 years. 94 (54.3%) women were married for more than 5 years.

- One hundred and sixty one (93.1%) patients sought medical advice for primary infertility and the remaining 12 (6.9%) were investigated for secondary infertility. Women who presented with secondary infertility have had miscarriages in the past.
- Of the 173 cases, 56.6% of women did not have any gynaecological symptoms other than infertility.
- Forty eight women (27.7%) presented with menstrual disturbances and oligomenorrhoea was the predominant menstrual disturbance observed in 34 (70.8%) of them. In 20 of the 34 cases, who presented with oligomenorrhoea, the duration of infertility was more than 6 years.
- The other predominant symptoms noted were dysmenorrhoea and chronic pelvic pain seen in 27 (15.6%) and 9 (5.2%) cases respectively.
- A definite past history of TB was available in 11 (6.4%) cases. In these women TB was diagnosed 2 to 15 years earlier and were treated with ATT for 9 months to 2 years. In 10 of these cases, one or more of the diagnostic parameters, namely laparoscopy, Mx test, ESR, culture, HPE and ESR were positive to suggest GTB.
- In seven (4%) cases there was a history of close contact with family members who were treated for tuberculosis in the past.

**SECTION B:**

**EXAMINATION FINDINGS**

- Anaemia was seen in 8(4.6%) cases, and scar in the neck region due to TB cervical adenitis was seen in one case.
- Only one case presented with ascites and adnexal mass.
- BMI was less than 19 in 30 women (17.4%). 75.7% of women had normal BMI and 6.9% had above normal BMI.

**SECTION C:**

**INVESTIGATIONS**

- The haemoglobin values ranged between 6 Gms and 13 Gms /dl and in eight (4.6%) patients the levels were less than 11 Gms %.
- The total count and the differential counts were within normal limits in all the cases.
- The ESR was elevated more than 15 mm at one hour in 27 (15.6%) of cases. Among these 27 cases, in 20 of them there was also corroborative evidence of TB from other test results. When the results of ESR were evaluated against the conventional methods of diagnosis, the sensitivity of ESR was 71.4% and the specificity was 98.63% in diagnosing GTB.
- Only in four patients (2.3%) chest X-ray showed evidence of old healed lesions with fibrous scars.
- HIV testing in all the subjects and their partners were found to be negative.
- A positive Mantoux test with an induration of more than 10 mm was seen in 37 (21.4%) cases. In these 37 cases with positive Mx test, there was also corroborative evidence of TB from other test results. In 29 of them laparoscopy showed evidence of TB, PCR was positive in 18 cases, HPE and culture were positive in 6 cases each. When the results of Mx test was evaluated against the conventional methods of diagnosing GTB; namely culture and HPE , the sensitivity of Mx was 85.7% and specificity was 81.3% in diagnosing GTB.
- In this study 105 (60.7%) women showed evidence of BCG vaccination.

**SECTION D:**

**SPECIFIC GYNAECOLOGICAL PROCEDURES**

- An abdominal USG was carried out in all the 173 cases. Findings suggestive of TB were seen in 23 (13.3%) cases. Adnexal masses and cysts were seen in 17 cases, ascites in 3 cases, calcification in 2 cases and endometrial fluid in one case. Among these 23 cases HPE, culture and PCR were positive in 13 of them.
- Tubal evaluation by HSG was carried out in 131 cases. Among them, in 57 (43.5%) cases, HSG showed abnormal findings. In 22 of them characteristic features of GTB were seen. Among the 57 cases with abnormal findings in 31 of them other diagnostic parameters such as Mx, ESR, HPE, culture and PCR were also positive.
- In all the 173 cases, by laparoscopy, a systematic and thorough evaluation of pelvis and abdominal cavity was carried out for evidence of GTB. Among the findings were suggestive of TB in 80 (46.2%) cases. In 20 of the 80 cases there was definite evidence of TB with findings such as granuloma, caseation, calcification and tubercles. In another 18 cases, there was probable evidence of tuberculosis with findings such as hydrosalpinx, dilated retort shaped tubes and loculated ascites. In the remaining 42 cases, there was suspicion of TB with minimal adhesions, cornual blocks and fluid in the POD.
- Evaluation of uterine cavity by hysteroscopy was available only in 7 cases. In 2 cases who presented with secondary amenorrhoea, hysteroscopy showed intra uterine adhesion in one case and calcification in another case. Another two case showed white patchy lesion and tubercles within the uterine cavity. All the above four cases were positive by PCR. However, culture and HPE were negative.

**SECTION E:**

**RESULTS OF SPECIFIC DIAGNOSTIC TESTS FOR GTB**

- From 173 infertile women, 173 endometrial samples, 81 POD fluid sample and 52 urine samples were available for specific diagnostic tests.
- Direct smear for AFB, culture for *MTB*, histology and PCR for *MTB* DNA were carried out on the above samples.

Results of AFB smear

- 8(4.6%) of the 173 endometrium and 5 (6.2%) of the 81 POD fluid were positive for AFB smear. Among the 8 cases that were positive in endometrial samples none were positive for *MTB* in culture, but NTM organisms were grown in 3 cases.
- Among the 5 samples that were positive for AFB in POD fluid culture for *MTB* was negative in all the 5 samples, however, NTM organisms had grown in 2 cases.

Results of culture for *MTB*

- Culture was carried out in 173 endometrial samples, 81 POD aspirate and 52 urine samples.
- Culture was positive only in 6 (3.5%) of 173 endometrial samples. None of the POD aspirate and urine samples were positive by culture.
- However, NTM organisms were grown in 41 (23.7%) of 173 endometrial samples and 16 (19.8%) of POD fluid and 6 (11.5%) of urine samples.
- Contamination by NTM organisms in the theatre was ruled out by culture of 10 samples taken from the theatre environment and instruments. All were proved to be negative for NTM organisms.

- HPLC analysis of NTM organisms showed that Mycobacterium chelonae / Mycobacterium abscessus was the predominant organism grown in 16 of the 25 samples tested.

Results of HPE examination

- HPE of the endometrium showed that only 7 (4%) of the 173 samples were reported positive for *MTB.*
- Another 6 specimens were reported as non specific endometritis. In two cases only collagenous tissue was obtained.
- In one cases with active abdominal tuberculosis, tubal and peritoneal biopsy also showed evidence of TB.
- In yet another case, besides the endometrium, the endocervix also showed histological evidence of TB.
- In another case who presented with T-O mass, along with the endometrium, the ovarian tissue also showed evidence of TB.

Results of polymerase chain reaction

- The PCR using IS 6110 and TRC4 primers was carried out on endometrial, POD aspirate and urine samples to identify *MTB.*
- Using either or both probes PCR was positive in 45(28.1%) of the 160 endometrial samples, 16(19.8%) of the 81 POD fluid samples and 4(7.7%) of the 52 urine samples.
- The endometrial samples showed higher PCR positivity compared to the POD aspirate and urine samples.

Results of specific diagnostic tests on endometrial samples

- On analyzing the results of specific diagnostic tests performed on the Endometrium, it was seen that the AFB smear was positive in 4.6% of cases,

Culture was positive in 3.5%, HPE was positive in 4% and PCR was positive in 28.1% of cases.

Results of specific diagnostic tests on 81 pod fluid samples

- On 81 POD fluid samples AFB smear was positive in 6.2% of cases, culture was negative in all the samples and PCR was positive in 19.8% of cases.

Results of specific diagnostic tests on 52 urine samples

- In 52 urine samples culture was negative for *MTB* in all the cases, and PCR was positive in 7.7% of cases.

**SECTION F:**

**EVALUATION OF SPECIFIC DIAGNOSTIC TESTS ON 153 ENDOMETRIAL SAMPLES**

- As the detection rate of *MTB* by culture and HPE are low one cannot use them as gold standard to evaluate diagnostic tests.
- Therefore, a combination of clinical profile and criteria were identified against which the diagnostic tests were evaluated.
- The diagnostic criteria was derived based on the historical, clinical and results of laboratory and Gynaecological procedures.
- A woman was said to be suspected of having genital tuberculosis if she has had findings suggestive of tuberculosis by laparoscopy with one or more of the following findings: A definite past history of tuberculosis, presence of active extra genital tuberculosis, characteristic features on HSG, elevated ESR, positive Mantoux and evidence of calcification / complex adnexal mass by scan.
- Sixteen cases were excluded from the 173 cases as they were positive for Chlamydia and gonococcal infection diagnosed by PCR testing.

- In the remaining cases, in 153 cases results of all diagnostic parameters were available for further evaluation.
- Based on the newly derived diagnostic criteria to suspect GTB, in 61 cases GTB was suspected, and in 92 cases GTB was not suspected.
- By bivariate analysis, the sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated with 95% confidence interval (CI) for the specific diagnostic tests (AFB smear, culture, HPE and PCR).
- Based on the evaluation the sensitivity of AFB was 6.7%, culture was 6.6%, HPE was 8.2% and PCR was 44.3%.
- The specificity for the above test results were 98.9%, 100%, 100% and 80.4% respectively.
- Among the four diagnostic tests PCR showed a higher sensitivity in diagnosing GTB.

**SECTION G:**

**EVALUATION OF PCR TEST IN THE DIAGNOSIS OF GTB**

False negative PCR

- Out of the 153 cases, in 34 patients there were positive clinical and laparoscopic features of genital TB but the PCR results were negative. This could indicate the possibility of false negative PCR results.
- PCR was positive only in three of the five cases who were positive by either or both conventional methods. (HPE, culture) (IS 6110 was positive in one case and $TRC_4$ was positive in two cases).
- Among the 34 cases with possible false negative results, in 11 samples NTM organisms were grown in culture. False negative PCR in these cases could be

the result of presence of NTM organism which shows the specificity of the test.

False positive PCR

- Of the 153 women, in 18 of them PCR was positive but the clinical profile was negative. This raises the possibility of false positive PCR results.
- In our study, in 13 of the 18 cases with possible false positive results, PCR was positive both in the endometrium and POD aspirate and in 2 cases urine samples were also positive for PCR. (multiple sources have given positive results) This indicates that PCR could be the early indicator of TB in the endometrium.
- In six of the 18 cases, the HPE findings on the endometrium were reported as chronic endometritis.

Analysis of Endometrial and POD Aspirate Samples

- The PCR results were analyzed from samples taken from different sources on the same patient.
- In 81 women PCR results were available from samples taken from both endometrium and POD aspirate.
- In 66 (81.5%) of the 81 samples there was (crude) agreement in the positive and negative results. The Kappa statistics of chance corrected agreement was 52% which indicates good agreement.

Evaluation of two sets of primers in PCR technique

- To increase the sensitivity of PCR, along with IS6110 primers we have also used another primer called $TRC_4$ primer in this study.
- On evaluation of IS 6110 and clinical criteria there was agreement between the results in 61.4% of cases. The false negative rate of IS 6110 was 85.2%.

- On evaluation of $TRC_4$ and clinical criteria the crude agreement between the results was 66.0%. The false negative rate of $TRC_4$ was 63.9%.
- On evaluating the PCR results using two sets of primers against the clinical criteria, it was seen that PCR with $TRC_4$ primers had a higher sensitivity (36.1%) than PCR with IS6110 primers (14.8%) in detecting clinically positive GTB.
- There was agreement between the results of the two primers among 114 of 153 samples. The crude agreement was 74.5%, and the chance corrected agreement (Kappa) was 15.8. There was marginal agreement between the two methods.

Reliability of PCR in diagnosing GTB.

- In 10 patients repeat sampling was done and PCR test was carried out. In 9 of the 10 cases tested, there was agreement in the results of PCR between the first visit and the second visit.
- Retesting of samples was also carried out in 19cases, and the results were consistent in 18 of them. This validates the PCR test and the quality control of the laboratory in which it was done.

**SECTION H:**

**TREATMENT**

Seven cases were positive by conventional methods, either by culture or HPE. They were treated with standard Anti tuberculosis treatment (ATT) with short course of chemotherapy for 9 months; initial two months with isoniazid, rifampicin and ethambutol followed by isoniazid and rifampicin for the next 7 months.

No pregnancies have been reported in two years of follow up.

# CHAPTER VIII

# CONCLUSION

The diagnosis of GTB is difficult and challenging. A high degree of suspicion aided by intensive investigations is important in the diagnosis of the disease. In areas where TB is endemic, while investigating an infertile patient, the possibility of GTB should always be considered and evaluated further.

Clinical parameters such as past or contact history of tuberculosis, positive Mantoux test, elevated ESR, positive findings on X-ray, USG, HSG, and laparoscopy would suggest the possibility of tuberculosis, but have their own limitations and are not sufficient criteria to diagnose GTB.

However, in an infertile woman, when the above test results are positive (positive clinical criteria) it indicates the need for further investigations to confirm /exclude GTB by specific diagnostic tests.

In this study, conventional methods of diagnosis namely HPE and AFB smear and culture have shown low pick up rates.

Given the low sensitivity of AFB smear, culture and HPE, and the other diagnostic parameters do not provide conclusive evidence; PCR with its comparatively higher sensitivity may be a useful tool in diagnosing GTB in clinical practice.

However, the sensitivity and specificity of PCR are not high enough to use as a single definite test for screening infertile women for GTB.

Based on the results of PCR, our study has shown that a positive PCR result should be given due importance. In 18 cases, where clinical criteria were negative, the PCR results were positive. Though this finding raises the possibility of false positive results, adequate precautions were taken to avoid the problem of false positivity by

way of strict discipline about collection and processing of the specimen and handling of reagents and measures were taken to avoid carryover contamination. Wax beads were added to minimize non-specific amplification.

Moreover, during the procedure, in order to monitor the false positive results, each PCR run was controlled by adding concurrent negative control (without templates). No positive bands were noted in these controls and all were PCR negative.

There is also evidence from the test results to show that the false positive results are in fact true positives. In the above 18 cases, not only in the endometrial samples, but, the PCR results were also positive in 13 of the POD aspirate. Moreover, in 6 cases, the HPE of the endometrium also showed additional evidence by the presence of non-specific endometritis which could be the early lesions of tuberculous endometritis.

A positive test in a patient with reasonably high pre-test possibility is fairly confirmatory of tuberculosis. Therefore, in clinically suspected cases, in the presence of positive PCR results, an infertile woman should be considered as having GTB and should be treated.

The high false negative result is an important limitation in this study. A negative PCR may result in missing the diagnosis in a few cases. Therefore, when GTB is suspected clinically, but the PCR results are negative, it indicates the need for further evaluation using other diagnostic tests and repeat testing to confirm/ exclude diagnosis.

In an infertile woman with advanced stage disease, the outcome of pregnancy is not optimistic in most cases. Assisted Reproductive techniques (ART) are the only option available to them to bear a child. However, the ART procedures are not available and affordable to most Indian women. As well as the success rate of ART is less in these cases due to the intra uterine synechiae caused by endometrial tuberculosis.

Therefore, the main aim should be to diagnose the disease early or in the latent form; the stage at which treatment would provide complete cure, thereby restoring fertility.

Our study has shown that PCR is able to pick up early/ latent GTB. Therefore, in women with unexplained infertility, where major causes of infertility (anovulation, male factors, tubal causes, sexual dysfunction) have been ruled out, even in the absence of positive clinical criteria, PCR of the endometrium should be carried out and if positive, should be treated.

This study has also shown that the sensitivity of PCR can be improved by using more than one set of primers.

**The strength of this study is that;**

- In a large series of 173 patients with suspected GTB, clinical, histology, microbiology and molecular biological features have been evaluated.
- This study has also evaluated the results of diagnostic tests on largest number of samples from multiple sources. (173 endometrial samples, 81 POD aspirates and 52 urine samples)
- This study is one of the first ones where PCR has been repeated in follow up patients and in 9 out of 10 patients the results are repeatable.
- In 13 out of 18 cases, false positive results were ruled out by multiple specimens processing from the same patient.
- This study has shown that in a high proportion of samples, the supposedly false positive results are not true because, PCR could be detecting *MTB* in paucibacillary cases which the conventional tests like culture fail to detect due to low sensitivity.
- This study has also shown that, a proportion of the false negative results of PCR could be due to NTM and not due to *MTB.*

- Based on the clinical profile this study has made an attempt to derive at a diagnostic criterion to suspect GTB.
- This study has shown that PCR has contributed significantly for the diagnosis of early disease.

**The limitation of this study is that;**

- The diagnostic parameters have been evaluated against the assumed diagnosis of GTB which may be subjective. However, we are probably justified in this action as there is no 'gold standard technique available' to evaluate the diagnostic tests for GTB.
- It is not known whether a proportion of the false negative PCR results are due to NTM infection giving positive findings at laparoscopy, therefore PCR for *MTB* is negative.
- It is also not known whether some of the false negative results are due to the presence of inhibitors.

PCR is a very sensitive and specific test. If executed with proper care it can be used to detect tuberculosis in the genital tract. By treating the patients who are positive by repeat PCR early intervention is possible before there is complete destruction of the endometrium.

**Future Research**

Further research is required to see whether NTM organisms are responsible for a significant number of infertility cases. As past history of tuberculosis is not available in most cases, whether one should look for genital tuberculosis in male partners for the possibility of sexual mode of transmission.

Also, there should be research on areas to simplify and reduce the cost of PCR to make it available for clinical use.

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## ANNEXURE I

## PROFORMA FOR DATA COLLECTION

ID NO: FRC NO: IP NO: DATE:

**I.DEMOGRAPHIC DETAILS**:

1. NAME : Husband's Name:

2. Age : Husband's Age :

3. Address :

4. Religion :

5. Education : Wife Husband :

6. Occupation : Wife Husband :

7. Income : Wife Husband :

8. Type of Family : Joint/Nuclear/Extended

9. Diet : Vegetarian/Non-Vegetarian

**II.HISTORY**:

Married : Years

Menarche : $^{th}$ Year

MH : Regular / Irregular
Duration of flow :
Amount of flow :

LMP :

Main Complaint : Primary infertility / Secondary Infertility

**OTHER PROBLEMS**:

1. Menstrual Irregularity

    a) Secondary Amenorrhoea Yr:
    b) oligo – Amenorrhoea
    c) Menorrhagia
    d) Metrorrhagia
    e) Hypomenorrhoea

2. Dysmenorrhoea – Congestive/Spasmodic

3. Dyspareunia

4. Discharge

5. Chronic pelvic pain

6. Abdominal pain/bloating

7. Bowel Symptoms

8. Urinary Symptoms

9. Previous Surgery – appendicectomy/ovarian cyst.

**General Complaints**:

1. Loss of weight
2. Loss of appetite
3. Evening rise of temperature

**PAST HISTORY**:

1. PID Yrs

2. Tuberculosis
    - PC
    - Cervical nodes
    - Abdominal tuberculosis
    - Others

3. Treated for how long?

4. Treated when and where?

**Family History**:

a) Tuberculosis in family members
b) Tuberculosis in neighbors

**Obstetric History**:

a) Previous abortions
b) Delivery

**III. Examination:**

**GENERAL:**

1. Build : Obese/emaciated/normal

2. Height

3. Weight

4. BMI

5. Anaemia

6. Cervical nodes/scars

7. Respiratory System

8. Evidence of previous Disease

**ABDOMINAL EXAMINATION**:

Scars/Mass/Distension/Ascites/Doughy feel

**VAGINAL EXAMINATION**:

a) Fixity of the uterus
b) T-O mass
c) Irregularity in POD
d) Tenderness

**IV. LABORATORY INVESTIGATIONS**:

| | **Value** | **Date** |
|---|---|---|
| 1. Hb% | | |
| 2. TC | | |
| 3. DC | | |
| 4. ESR | | |
| 5. Mx | | |
| 6. X-Ray Chest | | |
| 7. X-Ray Abdomen | | |
| 8. HIV Status : Husband | Wife | |
| 9. Chest Physician's opinion | | |
| 10. USG - a) T-O mass<br>b) Cysts<br>c) Calcification | | |
| 11. HSG - a) Normal study<br>b) Cornual block<br>c) Fimbrial block<br>d) Beaded appearance | | |
| 12. X-ray abdomen | | |
| 13. Laparoscopy Findings: | | |

**V. Source of Material for HPE, Culture & PCR studies**.

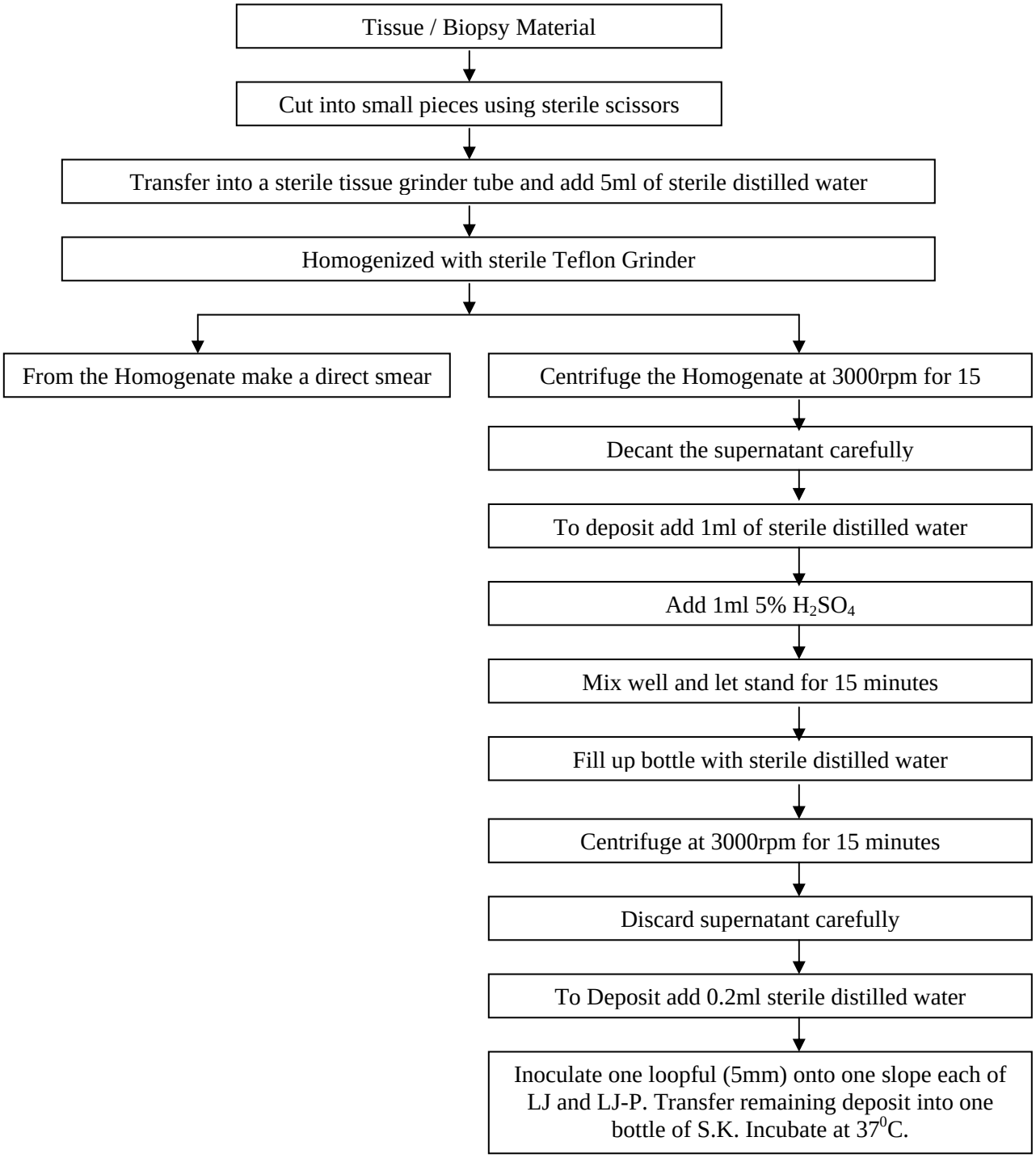
| | Endometrium | Tubal Wall | OVARIAN LESIONS | PERITONEAL FLUID | MENSTRUAL FLUID |
|---|---|---|---|---|---|
| HPE | | | | | |
| CULTURE | | | | | |
| PCR | | | | | |

**VII. FOLLOW-UP**:

| Date | General Well being | Weight | Symptoms | Pregnancy |
|---|---|---|---|---|
| | | | | |

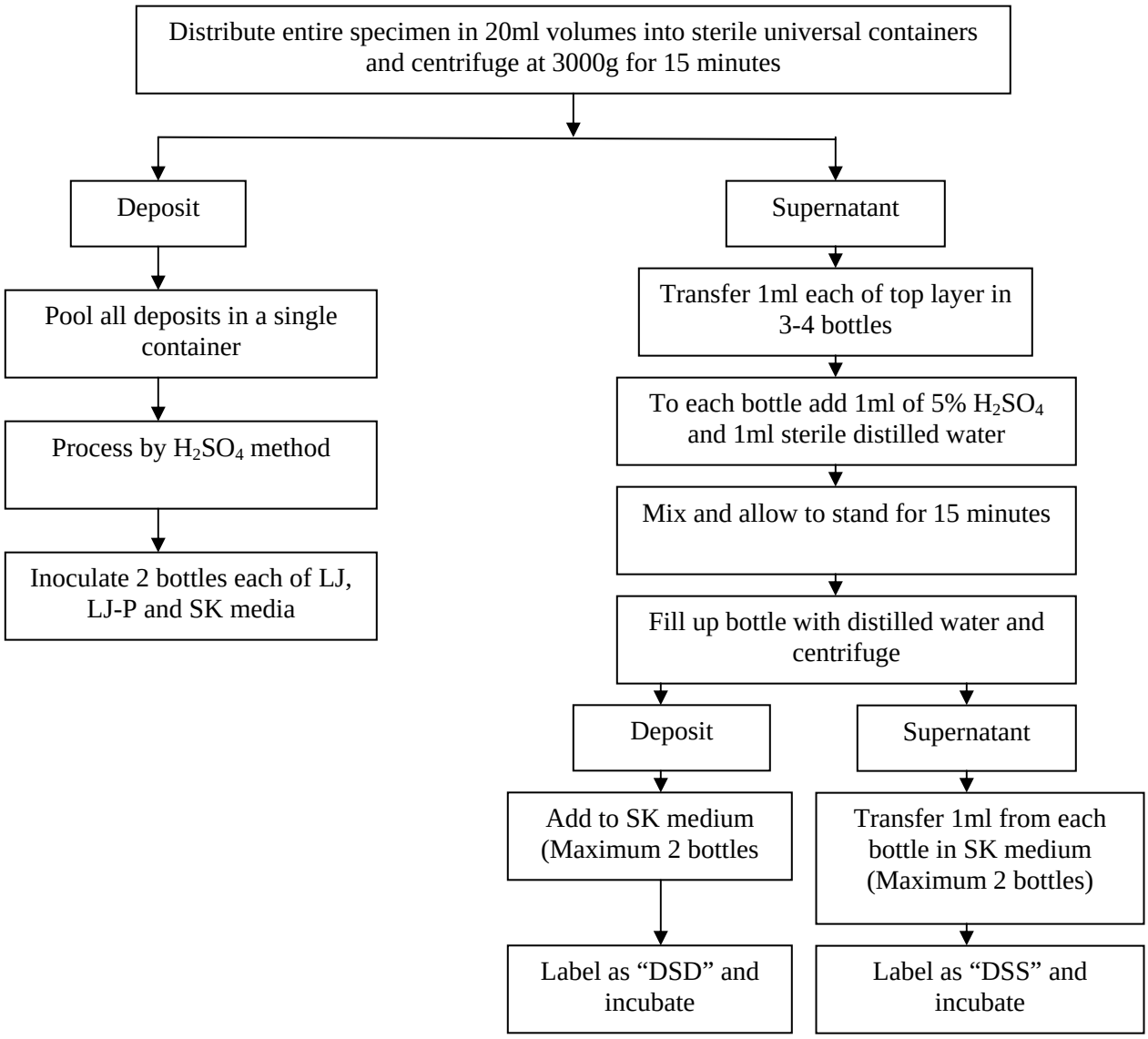
## ANNEXURE IA

## CULTURE METHOD FOR TISSUE / BIOPSY SPECIMEN



## ANNEXURE I B

## CULTURE METHOD FOR URINE SAMPLES



# ANNEXURE II

## HUMAN ETHICAL COMMITTEE

IOG and GOVT. Hospital for Women and Children
Egmore, Chennai- 600 008

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MEMBERS

Chairman: Prof. A. Sundaravalli

Dr. K. Mathiharan  Dr. T. S. Vijayalakshmi

Mrs. Sudha Ramalingam  Dr. S. Devambigai  Dr. A. V. Shanthi

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The Ethical Committee of the Institute of Obstetrics and Gynaecology, Egmore, Chennai, 600 008 met on the 11th October 2003 and the following Thesis Projects were discussed.

1) Thesis project submitted by Dr. T. Radha Bai Prabhu entitled ' Diagnosing Genital Tuberculosis in Female Infertility by Clinico- pathological, Culture and PCR studies'

This Thesis Project was approved by the Ethical Committee and the following recommendations were made.

a) To apply for external funding for the project
b) To consider giving incentives for the participants
c) To consider whether indemnity cover would be applicable

2) ICMR Project submitted by Dr. Shymala, Senior Research Officer , ICMR, IOG, Chennai , entitled 'Prevalence of Gestational Diabetes Among Antenatal Population' .

This Project was approved by the Ethical Committee.

Prof. A. Sundaravalli  Dr. K. Mathiharan  Mrs. Sudha Ramalingam

Dr. Shanthi  Dr. Devambigai  Dr. Vijayalakshmi

## ANNEXURE III

### அரசு தாய் சேய் நல பொது மருத்துவமனை – எழும்பூர், சென்னை.

### நோயாளிகள் அறிவிப்பு படிவம்

குழந்தையின்மைக்கு மூன்று முக்கிய காரணங்கள் உள்ளன. அவை

1. ஆண்விந்து சம்பந்தப்பட்ட பிரச்சனைகள்
2. கருமுட்டை வெளிவருவதில் தடங்கல், மற்றும்
3. கருக்குழாயில் அடைப்பு ஆகியவையாகும்.

பலவிதமான நோய்க்கிருமிகளால் கருக்குழாய் அடைப்பு ஏற்படுகின்றது. இவ்வாறு ஏற்படும் கருக்குழாய் அடைப்பு 30 சதவிகித குழந்தையின்மைக்கு காரணமாக உள்ளது. குழந்தையின்மைக்கு காரணத்தை கண்டறிய பலவிதமான பரிசோதனைகள் செய்யப்படுகின்றது.

கருக்குழாய் அடைப்பை கண்டறிய எக்ஸ்ரே (HSG) மற்றும் லேப்பராஸ்கோப்பி (Laparoscopy) ஆகிய பரிசோதனைகள் செய்யப்படுகின்றன. இவ்வாறான பரிசோதனைகளின் போது சிறு அளவில் திசுக்களோ அல்லது நீரோ கர்ப்பப்பை மற்றும், வயிற்றின் உள்பாகத்தில் இருந்து பரிசோதனைக்கு எடுக்கப்படும். நம்முடைய மருத்துவமனையில், குழந்தையின்மை ஆராய்ச்சி பகுதியில், காசநோய் எந்த அளவுக்கு கருக்குழாய் அடைப்பிற்கும், குழந்தையின்மைக்கும் காரணமாக உள்ளன என்பதை கண்டறிய ஆராய்ச்சி நடத்தப்பட்டு வருகின்றது.

இந்த ஆராய்ச்சியில் எப்பொழுதும் எடுக்கப்படும் திசுக்களின் ஒரு பகுதியை சில முக்கியமான பரிசோதனைகளுக்கு (Culture மற்றும் PCR) அனுப்பப்படும். இந்த ஆராய்ச்சியின் விளைவால், காசநோயை மிக ஆரம்பத்திலே கண்டறிந்தால், மருத்துவ உதவியுடன் கருக்குழாய் அடைப்பை தவிர்த்து குழந்தையின்மையை நீக்க முடியும்.

**ANNEXURE IV**

# PATIENT INFORMATION SHEET

## GOVT. HOSPITAL FOR WOMEN & CHILDREN, EGMORE, CHENNAI.8.

## FERTILITY RESEARCH CLINIC

The major causes of infertility include defects in male factors, ovarian factors and tubal factors. Tubal blocks account for 30% of infertility cases. Tubal block can be caused by various infections such as gonococal infection, chlamydial infection and tuberculous infection. If tubal block in suspected you will be subjected to investigations such as HSG and laparoscopy to see whether the tubes are blocked or not. During these investigations tissue samples will be taken from the uterus, peritoneum or tubal wall for histo-pathological testing.

In the Fertility Research Clinic of Govt. Hospital for women & children, Egmore, research is being carried out to see whether tuberculosis could be the cause of tubal block. In this research, from the tissues that are normally taken during the investigative procedures, a part of it will be sent for special investigations such as culture and PCR studies.

This research will help us to identify the disease in its early stage so that treatment will result in complete cure of the disease, so that tubal damage and tubal block can be prevented.

## ANNEXURE-V

**அரசு தாய் சேய் நல பொது மருத்துவமனை – எழும்பூர், சென்னை.**
**ஒப்புதல் படிவம்**

குழந்தை இல்லாத பெண்களின் இனப்பெருக்க உறுப்புகளில் காணப்படும் காச நோய் பற்றிய ஆராய்ச்சி.

**நோய்க்குறியியல், நுண்ணுயிர் வளர்ப்பு, மூலக்கூறு ஆய்வு மற்றும் உடல் சோதனை மூலம் பரிசோதித்தல்**

. . . . . மாவட்டம் . . . . . வட்டம் . . . . . .
. . . . . கிராமம் . . . . . தெரு . . . . . . . . வீட்டு
எண்ணில் வசிக்கும் . . . . . அவர்களின் மனைவி . . . . . ஆகிய நான், எனக்கு அளிக்கப் போகும் சிகிச்சை தொடர்பான முழுவிவரங்களையும் மருத்துவர்கள் மூலம் தெளிவாக அறிந்து கொண்டேன். குழந்தை இன்மையின் காரணத்தை அறிய கர்ப்பப்பை சுத்தம் செய்தல் (D&C) மற்றும் கருக்குழாயில் அடைப்பு உள்ளதா என்பதை கண்டறிய கர்ப்பப்பை எக்ஸ்ரே (HSG), லேப்பராஸ்கோப்பி (Laparoscopy) ஆகிய சில பரிசோதனைகளை நான் செய்து கொள்ள வேண்டும் என்பதையும் மருத்துவர்கள் மூலம் நான் தெளிவாக அறிந்து கொண்டேன்.

அவ்வாறு பரிசோதனை செய்யும் போது சிறு திசுப்பகுதியோ அல்லது திரவப்பகுதியோ எடுத்து பரிசோதனைக்கு அனுப்பப்படும் என்பதை தெரிவிக்கப்பட்டது. அவ்வாறு எடுக்கப்படும் திசு மற்றும் திரவப்பகுதியின் ஒரு பகுதியை, என்னுடைய குழந்தையின்மையின் காரணம் காசநோயா என்பதை கண்டறிய ஆராய்ச்சிக்கு (Culture & PCR) அனுப்பப்படும் என்பதை எனக்கு அறிவிக்கப்பட்டது. இவ்வகை ஆய்வைப்பற்றி நான் தெளிவாக அறிந்து கொண்டதால் சதை மற்றும், திரவப்பகுதியை ஆராய்ச்சிக்கு கொடுத்து ஒத்துழைக்க நான் முழு மனதுடன் சம்மதிக்கிறேன். இவ்வகை பரிசோதனைகள் ஆய்வுக்காக செய்யப்படுகின்றன என்பதை அறிந்து விரும்பி பங்கேற்கின்றேன்.

இந்த ஆய்விலிருந்து நான் எப்போது வேண்டுமானாலும் விரும்பி விலகிக்கொள்ளலாம் என்றும், அவ்வாறு விலகும்போது எனக்கு அளிக்கப்படும் மருத்துவ உதவியில எந்த விதமான மாறுதலும் இருக்காது என்பதையும் அறிந்து கொண்டேன்.

சாட்சிகள் :

1. ஒப்பம் :
2. நாள் :

**ANNEXURE VI**

**GOVT. HOSPITAL FOR WOMEN & CHILDREN,**
**EGMORE, CHENNAI.8.**

## CONSENT FORM

**Prevalence of genital tuberculosis in female infertility**

**Clinico-pathological, culture and PCR study**

I..... w/o..... Residing at ..... have understood and read the information about the study. I fully understand that I have to undergo a series of investigations including invasive procedures such as D&C, Hystero-salpingogram and laparoscopy for my problem of infertility. I have been explained that during these procedures small tissues or fluid samples will be taken and sent for testing. In order to know whether tuberculosis could be the cause for my infertility, I was explained that a portion of these samples will be sent for special tests such as culture and PCR studies. I fully understand the purpose of this research and I am willing to give tissue samples required for this study.

I understand that the records of my participation in this study are to be used only for the purpose of this research project.

I consent to participate as volunteer in this study and understand that I have the right to withdraw from the study at anytime without any prejudice to my further medical care at the hospital.

Witness: 1. Signature of Volunteer

2. Date:

| ID | Name | Age | Married in yrs | Type infert | Mens dist | Dysmeno | Dyspareunia | Discharge | Pelvic pain | Abd bloating | Bowel symp | Urinary symp | Active disease | Previous Surgery | PID | TB | Dur treat | Family History | Contact History | BMI | Anaemia | Cervical node | RS | Abd exam | Vginal exam | Hb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Rameswari | 22 | 2 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $13.00 |
| 2 | Malar | 30 | 10 | PR | POLY | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 3 | Pachiammal | 25 | 7 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | YES | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $13.00 |
| 4 | Selvi | 30 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 5 | Neelavathy | 35 | 8 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | YES | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $12.00 |
| 6 | Yeshoda | 27 | 12 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | YES | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 7 | Usharani | 20 | 5 | PR | MD | NO | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $13.00 |
| 8 | Bhuvaneswari | 29 | 5 | PR | NONE | NO | YES | YES | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | TEN | $12.00 |
| 9 | Subha | 29 | 4 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | AX | 9 | NO | YES | 18 | NO | YES | neg | nor | nor | $12.00 |
| 10 | Sooriyakumari | 25 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $13.00 |
| 11 | Thangammal | 32 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 12 | Alice | 30 | 6 | PR | SA | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 13 | Kavitha | 25 | 3 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 14 | Rameswaree | 26 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $13.00 |
| 15 | Sarasu | 22 | 5 | SE | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $13.00 |
| 16 | Kalaiselvi | 25 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 17 | Sumathy | 22 | 4 | SE | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 18 | Suguna | 33 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 26 | NO | NO | neg | nor | nor | $12.50 |
| 19 | Mala | 30 | 13 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | ABD | 15 | NO | NO | 22 | NO | YES | neg | nor | nor | $12.00 |
| 20 | Dharani | 25 | 12 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.50 |
| 21 | Tamilselvi | 23 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $13.00 |
| 22 | Jeya | 27 | 6 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $13.00 |
| 23 | Josephine | 27 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $13.00 |
| 24 | Senbagam | 35 | 20 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $12.50 |
| 25 | Kala | 22 | 4 | SE | NONE | NO | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $13.00 |
| 26 | Revathy | 22 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | YES | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.50 |
| 27 | Jayanthy | 30 | 9 | PR | OL | YES | YES | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $13.00 |
| 28 | Gandhimathy | 33 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $13.00 |
| 29 | Deepa | 24 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 30 | Sarasu | 25 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.00 |
| 31 | Rajeswari | 28 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $13.00 |
| 32 | Stella | 28 | 4 | PR | OL | YES | NO | YES | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |

| TC | ESR | XRay Ch | XRay Abd | HIV status H | HIV status W | Chest opinion | USG | HSG | Lap | EM HPE | EM AFB | EM cul | EM Is6110 | EM TRC4 | POD AFB | POD cul | POD Is6110 | POD TRC4 | UR AFB | UR cul | UR IS6110 | UR TRC4 | Mantoux | CH/GC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| nor | nor | nor | ND | neg | neg | nor | cy | crb | mod | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 2 | |
| nor | EL | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | |
| nor | EL | nor | ND | neg | neg | nor | nor | ND | mld | NSE | neg | neg | pos | pos | ND | ND | ND | ND | ND | ND | ND | ND | 10 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | cy | crb | svr | pos | neg | pos | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 20 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 6 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | |
| nor | nor | nor | ND | neg | neg | nor | cy | crb | svr | neg | neg | NTM | pos | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | |
| nor | EL | ABN | CAL | neg | neg | nor | cal | cal | svr | pos | neg | pos | pos | neg | ND | ND | ND | ND | ND | ND | ND | ND | 11 | |
| nor | EL | nor | ND | neg | neg | nor | cy | ND | mld | neg | neg | NTM | pos | neg | ND | ND | ND | ND | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | pos | NTM | neg | neg | ND | ND | ND | ND | 5 | |
| nor | nor | nor | ND | neg | neg | nor | cy | ND | nor | neg | neg | cont | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | CH |
| nor | EL | ABN | ND | neg | neg | nor | cy | ND | mld | neg | pos | neg | pos | pos | ND | ND | ND | ND | ND | ND | ND | ND | 12 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | NTM | neg | pos | neg | NTM | neg | pos | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | pos | NTM | neg | neg | pos | NTM | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | NTM | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | svr | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 14 | |
| nor | EL | nor | ND | neg | neg | nor | cy | ND | nor | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | mld | neg | neg | neg | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | svr | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | NSE | neg | neg | neg | pos | neg | neg | neg | pos | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | cy | ND | mld | neg | neg | neg | pos | neg | ND | ND | ND | ND | ND | ND | ND | ND | 2 | |
| nor | EL | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | EL | nor | ND | neg | neg | nor | nor | nor | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | Alamelu | 25 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $10.00 |
| 34 | Gomathy | 23 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $12.00 |
| 35 | Kavitha | 25 | 5 | PR | NONE | YES | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $10.00 |
| 36 | Lakshmi | 25 | 8 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 37 | Tamilselvi | 33 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 38 | Revathy | 27 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 39 | Sharjula | 24 | 3 | PR | NONE | NO | NO | NO | YES | NO | NO | YES | NO | NO | NO | NONE | 0 | YES | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 40 | Thenmozhi | 30 | 5 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.50 |
| 41 | Dhanalakshmi | 29 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 42 | Geetha | 21 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 43 | Ezhilarasi | 24 | 6 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 44 | Rani | 35 | 20 | PR | NONE | NO | NO | NO | YES | YES | YES | NO | YES | NO | NO | NONE | 0 | NO | NO | 18 | YES | NO | neg | DIS | TO | $6.00 |
| 45 | Malarvizhi | 24 | 5 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 46 | Priya | 23 | 5 | PR | ME | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $12.00 |
| 47 | Banupriya | 36 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $10.00 |
| 48 | Krishnaveni | 22 | 4 | PR | NONE | YES | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 49 | Uma | 23 | 4 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 27 | NO | NO | neg | nor | nor | $13.00 |
| 50 | Manjula1 | 26 | 2 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 51 | Thilaga1 | 23 | 4 | SE | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 52 | Sivagami | 27 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $11.00 |
| 53 | Velankanni | 29 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | ABD | 4 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 54 | Latha | 25 | 4 | SE | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $15.00 |
| 55 | Lalitha | 25 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | ABD | 12 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 56 | Manjula2 | 23 | 2 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | CER | 2 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 57 | Prema | 30 | 8 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $13.00 |
| 58 | Amudha | 28 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 59 | Alamelu | 34 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 60 | Nalini | 26 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $13.00 |
| 61 | Shanthy | 34 | 4 | PR | OL | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $11.00 |
| 62 | Hemalatha | 36 | 4 | PR | NONE | YES | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 63 | Parimala | 24 | 6 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $13.00 |
| 64 | Lakshmi | 30 | 10 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 65 | Sharmila | 33 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 66 | Cristee | 26 | 3 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 67 | Sumathi | 30 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | YES | NO | neg | nor | nor | $9.00 |
| 68 | Devi | 27 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 69 | Suganthi | 24 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.50 |
| 70 | Nalini | 29 | 11 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 71 | Bhoopathy | 27 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.50 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| nor | EL | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | cy | ND | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | EL | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 13 | |
| nor | nor | nor | ND | neg | neg | nor | cy | crb | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | EL | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | EL | nor | ND | neg | neg | nor | cy/as | ND | mod | pos | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | |
| nor | EL | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | pos | pos | ND | ND | ND | ND | ND | ND | ND | ND | 15 | |
| nor | EL | nor | ND | neg | neg | nor | cy | crb | mld | neg | neg | neg | pos | pos | ND | ND | ND | ND | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | NTM | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 10 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | NTM | neg | pos | neg | NTM | pos | pos | ND | ND | ND | ND | 15 | |
| nor | EL | nor | ND | neg | neg | nor | nor | crb | mld | neg | pos | NTM | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 15 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | neg | neg | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 7 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | mld | neg | neg | neg | pos | pos | neg | NTM | neg | neg | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | neg | neg | neg | neg | neg | neg | neg | pos | ND | ND | ND | ND | 0 | |
| nor | EL | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | 15 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | pos | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | NTM | pos | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | neg | pos | neg | neg | neg | neg | neg | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | b | mld | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | b | mod | NSE | neg | neg | neg | pos | neg | NTM | neg | pos | ND | ND | ND | ND | 11 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | NSE | neg | neg | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 72 | Bhuvaneswari | 36 | 2 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 73 | Deepa | 25 | 10 | PR | NONE | NO | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 74 | Selvi | 25 | 9 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | YES | NO | neg | nor | nor | $9.00 |
| 75 | Ezhilarasi | 27 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | YES | NO | neg | nor | nor | $9.00 |
| 76 | Khalija | 23 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.00 |
| 77 | Kavitha | 25 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 78 | Nimala | 31 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 79 | Indra | 32 | 8 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 80 | Mumtajbegam | 33 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 27 | NO | NO | neg | nor | nor | $13.00 |
| 81 | Sudha | 30 | 13 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 82 | Shanthi | 30 | 12 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $13.00 |
| 83 | Parvathy | 27 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 84 | Radha | 27 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.00 |
| 85 | Stella | 29 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 86 | Menaka | 23 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $13.00 |
| 87 | Sumathy | 24 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 17 | NO | NO | neg | nor | nor | $12.00 |
| 88 | Malar | 37 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $10.50 |
| 89 | Selivi | 33 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 90 | Sagumbunisha | 24 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $10.50 |
| 91 | Boomadevi | 34 | 2 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 92 | Rejinameri | 35 | 10 | SE | ME | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 93 | Suseela | 27 | 12 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 94 | Dhanalakshmi | 26 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 95 | Hemavathy | 35 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 96 | Meera | 29 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $11.00 |
| 97 | Jothilakshmi | 25 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $12.00 |
| 98 | Thulasi | 28 | 9 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 99 | Saritha | 24 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 100 | Rajeswari | 28 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $12.00 |
| 101 | Radika | 28 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 26 | NO | NO | neg | nor | nor | $12.00 |
| 102 | Muthulakshmi | 24 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $10.00 |
| 103 | Kalpana | 22 | 5 | PR | OL | YES | NO | YES | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 104 | Jayanthi | 25 | 5 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | YES | NO | neg | nor | nor | $9.00 |
| 105 | Bhuvneswari | 26 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 106 | Andal | 26 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 25 | NO | NO | neg | nor | nor | $12.00 |
| 107 | Jayanthi | 23 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $10.00 |
| 108 | Rajammal | 23 | 10 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 109 | Sarasvathy | 34 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 110 | Nandeeswari | 25 | 2 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |

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| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | cy | b | mod | neg | pos | neg | neg | pos | neg | neg | neg | pos | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | NTM | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | pos | neg | neg | pos | ND | NTM | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | pos | neg | neg | neg | neg | NTM | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | pos | neg | neg | neg | pos | neg | ND | neg | pos | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | TO | b | mld | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 13 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | pos | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | mld | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | pos | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | NTM | neg | pos | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | NTM | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | NTM | neg | pos | neg | neg | pos | neg | ND | neg | neg | neg | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | pos | neg | pos | neg | pos | ND | ND | ND | ND | ND | neg | neg | neg | 1 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | neg | pos | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | NTM | neg | neg | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | pos | neg | neg | neg | neg | neg | ND | NTM | neg | neg | 3 | |

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| 111 | Sreedevi | 22 | 12 | PR | OL | YES | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | YES | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 112 | Bhagiyalakshmi | 28 | 10 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | YES | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 113 | Premila | 27 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $11.00 |
| 114 | Valli | 23 | 5 | PR | OL | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 17 | NO | NO | neg | nor | nor | $10.50 |
| 115 | Indra | 20 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 116 | Uma | 27 | 7 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $10.00 |
| 117 | Lakshmi | 27 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 118 | Geetha | 30 | 3 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 119 | Uma | 27 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 120 | Manjula | 28 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 121 | Vijaya | 20 | 3 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 122 | Uma Mahe | 26 | 7 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 123 | Menaka | 25 | 9 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 124 | Govindammaal | 27 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 18 | NO | NO | neg | nor | nor | $12.00 |
| 125 | Karpagam | 30 | 7 | PR | OL | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 126 | Jayalakshmi | 24 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | YES | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 127 | Parvathy | 26 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 128 | Kasthuri | 34 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $10.00 |
| 129 | Malar | 25 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 17 | NO | NO | neg | nor | nor | $10.50 |
| 130 | Venkatammal | 25 | 7 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $10.50 |
| 131 | Shunmugavalli | 36 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 132 | Sudha | 24 | 8 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | DIS | nor | $11.00 |
| 133 | Uma | 27 | 6 | PR | OL | NO | NO | NO | NO | YES | NO | NO | NO | NO | YES | PT | 10 | YES | NO | 19 | NO | NO | neg | DIS | nor | $11.00 |
| 134 | Prabavathy | 26 | 4 | PR | ME | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 135 | Shanthy | 25 | 4 | SE | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.00 |
| 136 | Sivakumari | 32 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 137 | Savithri | 28 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 138 | Valarmathy | 23 | 2 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | PT | 1 | NO | NO | 18 | YES | NO | neg | nor | nor | $8.00 |
| 139 | Chithra | 23 | 2 | PR | ME | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 140 | Jayasudha | 26 | 3 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 141 | Sasikasla | 28 | 4 | PR | HYPO | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | PT | 7 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 142 | Vijaya | 26 | 4 | PR | ME | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 143 | Vimala | 30 | 6 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 144 | Shanthy | 24 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 145 | Darani | 32 | 18 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 23 | NO | NO | neg | nor | nor | $12.00 |
| 146 | Angeline Grace | 33 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $12.00 |
| 147 | Sundari | 27 | 2 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | YES | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 148 | Mahalaxmi | 24 | 2 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 149 | Janaki | 30 | 10 | PR | OL | YES | NO | NO | NO | NO | NO | NO | NO | yes | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| nor | EL | nor | ND | neg | neg | nor | cy | crb | svr | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 19 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 5 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | pos | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 5 | |
| nor | EL | nor | ND | neg | neg | nor | nor | crb | mld | NSE | neg | neg | pos | pos | neg | neg | neg | neg | ND | NTM | neg | neg | 15 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | nor | NSE | neg | NTM | neg | pos | neg | neg | neg | pos | ND | neg | neg | neg | 12 | |
| nor | nor | nor | ND | neg | neg | nor | cy | crb | svr | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 6 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | neg | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | NTM | neg | neg | neg | neg | neg | pos | ND | neg | neg | neg | 12 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | NTM | neg | neg | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | nor | neg | neg | NTM | neg | neg | neg | neg | neg | neg | ND | NTM | neg | pos | 5 | |
| nor | nor | nor | ND | neg | neg | nor | cy | FBB | mld | neg | neg | NTM | neg | pos | neg | NTM | neg | neg | ND | neg | neg | neg | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | mld | neg | neg | NTM | neg | neg | neg | NTM | neg | neg | ND | neg | neg | pos | 11 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mld | neg | neg | NTM | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 10 | |
| nor | EL | nor | ND | neg | neg | nor | as | crb | svr | NSE | neg | neg | neg | pos | neg | neg | pos | pos | ND | neg | neg | neg | 10 | |
| nor | EL | ABN | ND | neg | neg | nor | as | ND | svr | pos | neg | pos | neg | pos | neg | neg | neg | pos | ND | ND | ND | ND | 15 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | mld | neg | cont | cont | neg | neg | cont | cont | neg | neg | ND | ND | ND | ND | 4 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | no r | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | nor | neg | neg | NTM | neg | neg | neg | NTM | neg | neg | ND | ND | ND | ND | 5 | |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 4 | |
| nor | EL | ABN | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | cont | neg | neg | ND | ND | ND | ND | 15 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 3 | |
| nor | nor | ABN | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | EL | nor | ND | neg | neg | nor | nor | FBB | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 13 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 2 | |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 6 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 4 | |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 150 | Kalpana | 26 | 3 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $12.00 |
| 151 | Lakshmi | 32 | 7 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 152 | Rani | 28 | 8 | PR | NONE | NO | NO | YES | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $12.00 |
| 153 | Shanthy | 28 | 7 | PR | SA | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $12.00 |
| 154 | Bujammal | 30 | 17 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 155 | Kavitha | 21 | 2 | PR | ME | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 24 | NO | NO | neg | nor | nor | $11.00 |
| 156 | Rajathy | 29 | 10 | SE | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 157 | Fathima | 25 | 3 | PR | NONE | NO | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $11.00 |
| 158 | Anjalai | 35 | 20 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $10.00 |
| 159 | Punitha | 23 | 6 | PR | NONE | NO | NO | NO | YES | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | $12.00 |
| 160 | Indra | 34 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | $11.00 |
| 161 | Kousalya | 30 | 10 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 162 | Mohana | 30 | 15 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | $10.00 |
| 163 | Indrani | 30 | 17 | SE | HYPO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $10.00 |
| 164 | Latha | 29 | 12 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | PT | 1 | NO | NO | 19 | NO | NO | neg | nor | nor | $11.00 |
| 165 | Bhuvaneshwari | 29 | 8 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | PT | 15 | NO | NO | 18 | NO | NO | neg | nor | nor | $11.00 |
| 166 | Selvi | 30 | 13 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | PT | 3 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 167 | Mala | 27 | 2 | PR | HYPO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 168 | Sakuntala | 28 | 12 | SE | ME | NO | NO | YES | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | $11.00 |
| 169 | Malar | 22 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | 11 |
| 170 | Pachiammal | 25 | 8 | PR | NONE | YES | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 19 | NO | NO | neg | nor | nor | 11.5 |
| 171 | Ramawari | 24 | 4 | PR | OL | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 22 | NO | NO | neg | nor | nor | 10 |
| 172 | Sudha | 32 | 5 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 21 | NO | NO | neg | nor | nor | 11 |
| 173 | Clarance Mary | 22 | 4 | PR | NONE | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NONE | 0 | NO | NO | 20 | NO | NO | neg | nor | nor | 10 |

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| nor | nor | nor | ND | neg | neg | nor | nor | crb | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 0 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | svr | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | nor | nor | ND | neg | neg | nor | cy | crb | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | |
| nor | EL | nor | ND | neg | neg | nor | cal | ND | svr | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 6 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 5 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 2 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | mod | neg | pos | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 7 | CH |
| nor | EL | nor | ND | neg | neg | nor | nor | FBB | svr | pos | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 18 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 8 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | mod | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 4 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 3 | CH |
| nor | EL | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 4 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 4 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | nor | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 6 | GC |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 11 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | mod | neg | pos | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 10 | |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 15 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | MOD | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 8 | CH |
| nor | nor | nor | ND | neg | neg | nor | nor | ND | nor | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 5 | CH |
| nor | nor | nor | ND | neg | neg | nor | cy | ND | svr | pos | neg | pos | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 20 | |
| nor | EL | nor | ND | neg | neg | nor | nor | b | mod | neg | neg | neg | neg | neg | neg | neg | neg | neg | ND | ND | ND | ND | 14 | |
| nor | nor | nor | ND | neg | neg | nor | nor | crb | mod | neg | neg | neg | neg | pos | neg | neg | neg | neg | ND | ND | ND | ND | 3 | |
| nor | nor | nor | ND | neg | neg | nor | nor | nor | mld | neg | neg | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | 6 | GC |
| nor | nor | nor | ND | neg | neg | nor | nor | FBB | mod | neg | neg | neg | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 5 | GC |

**Figure : 8**

# HSG picture showing calcified ovary and Para-aortic nodes

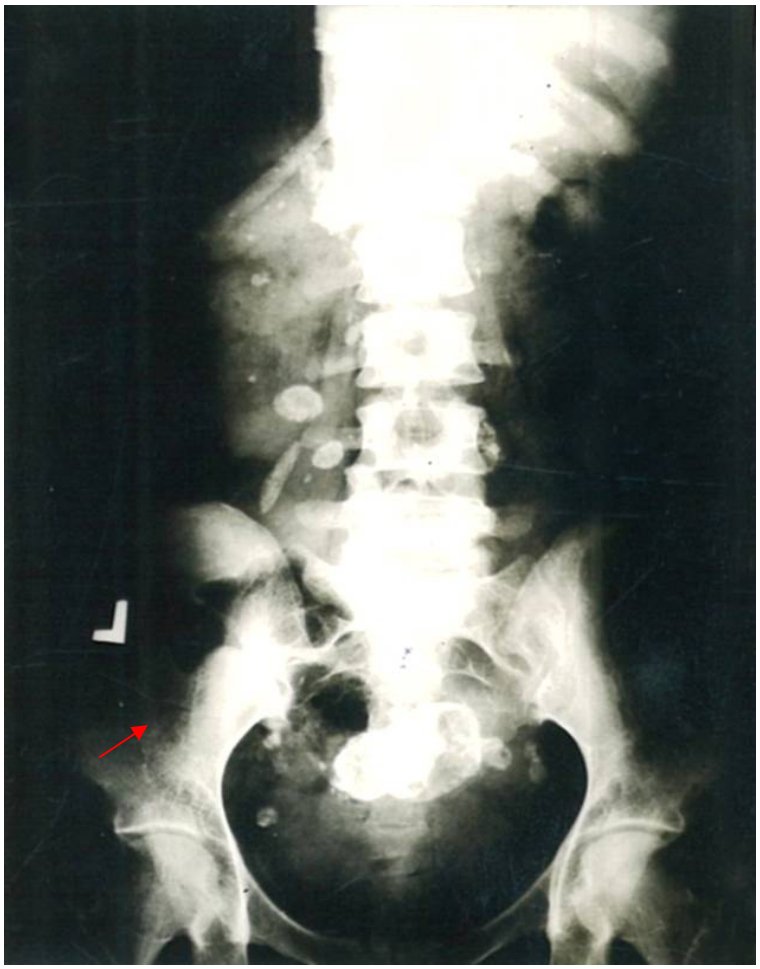


**Figure : 9**

## HSG picture showing distorted uterine cavity

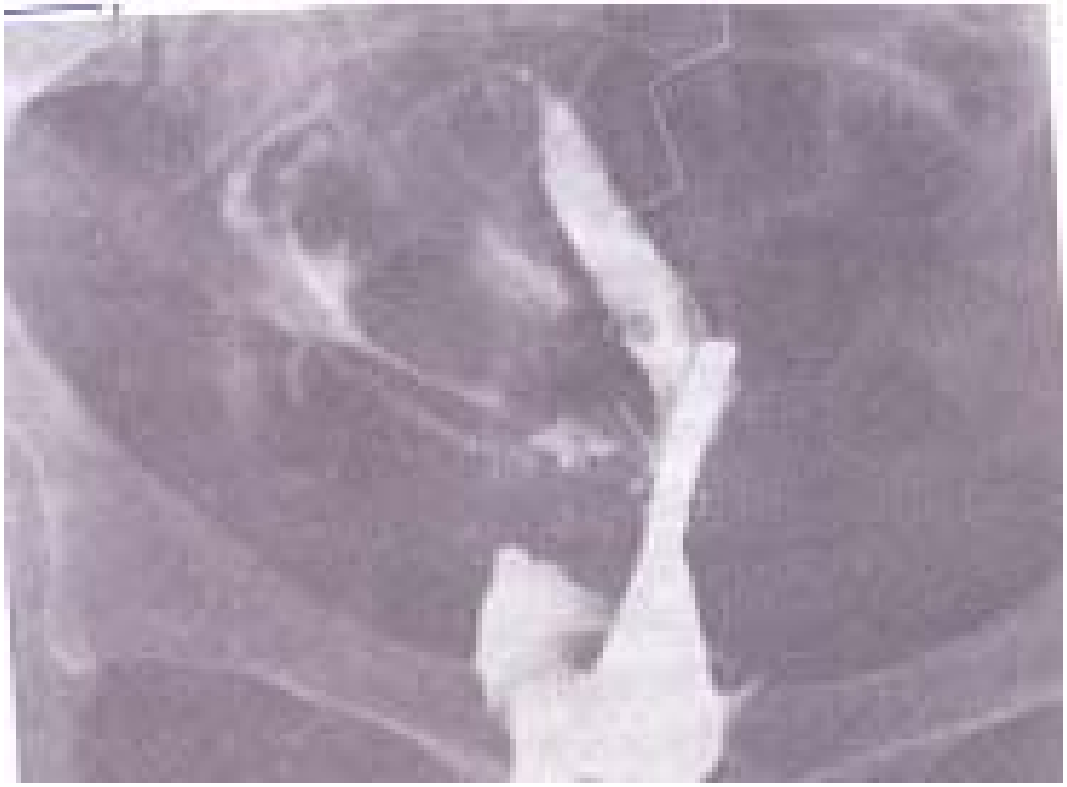


**Figure : 10**

# HSG picture showing intravasation of dye

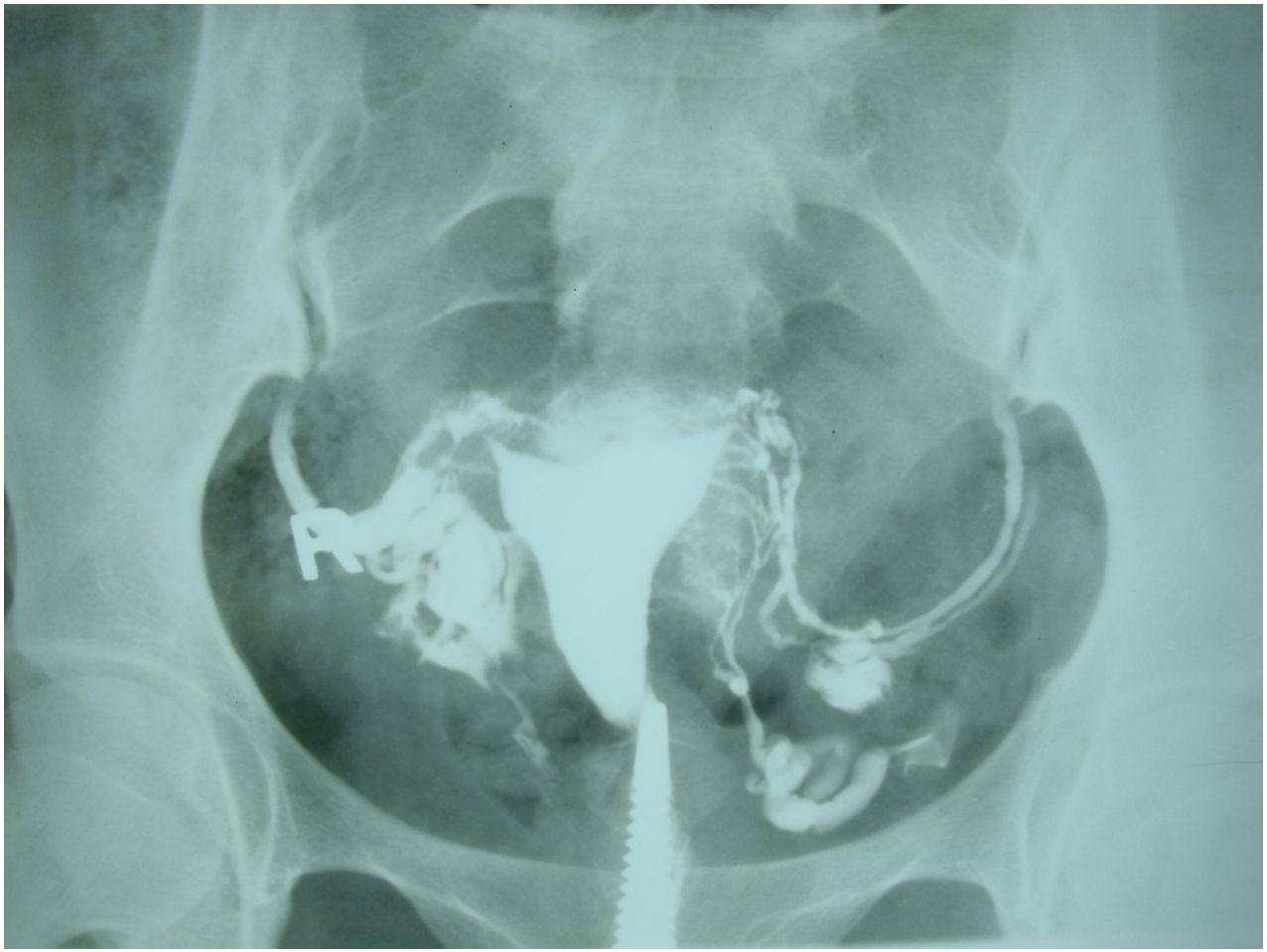


**Figure : 10 A**

## HSG picture showing intravasation of dye into the parametrium

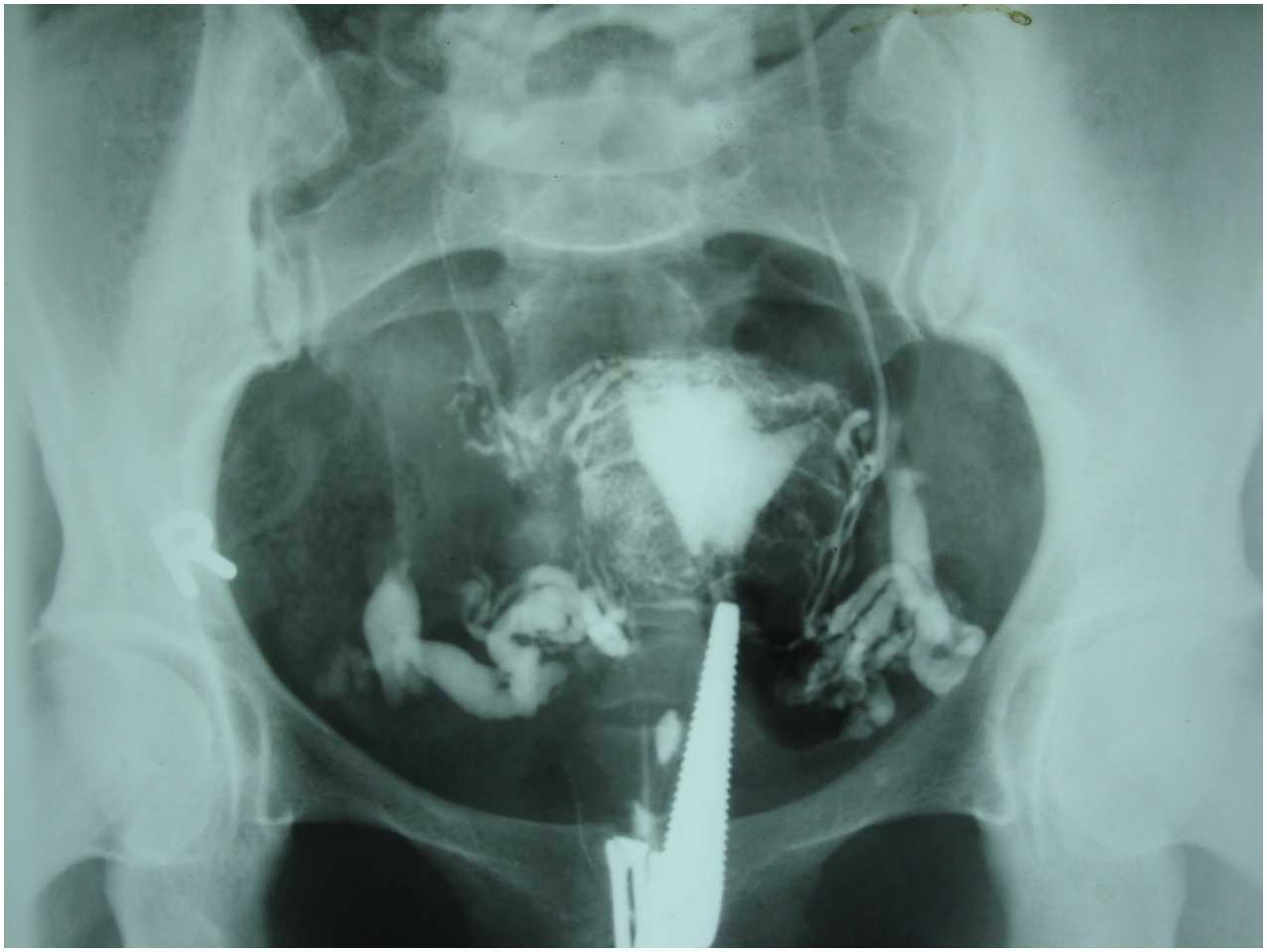


**Figure : 11**

# Bilateral Mid Tubal Block

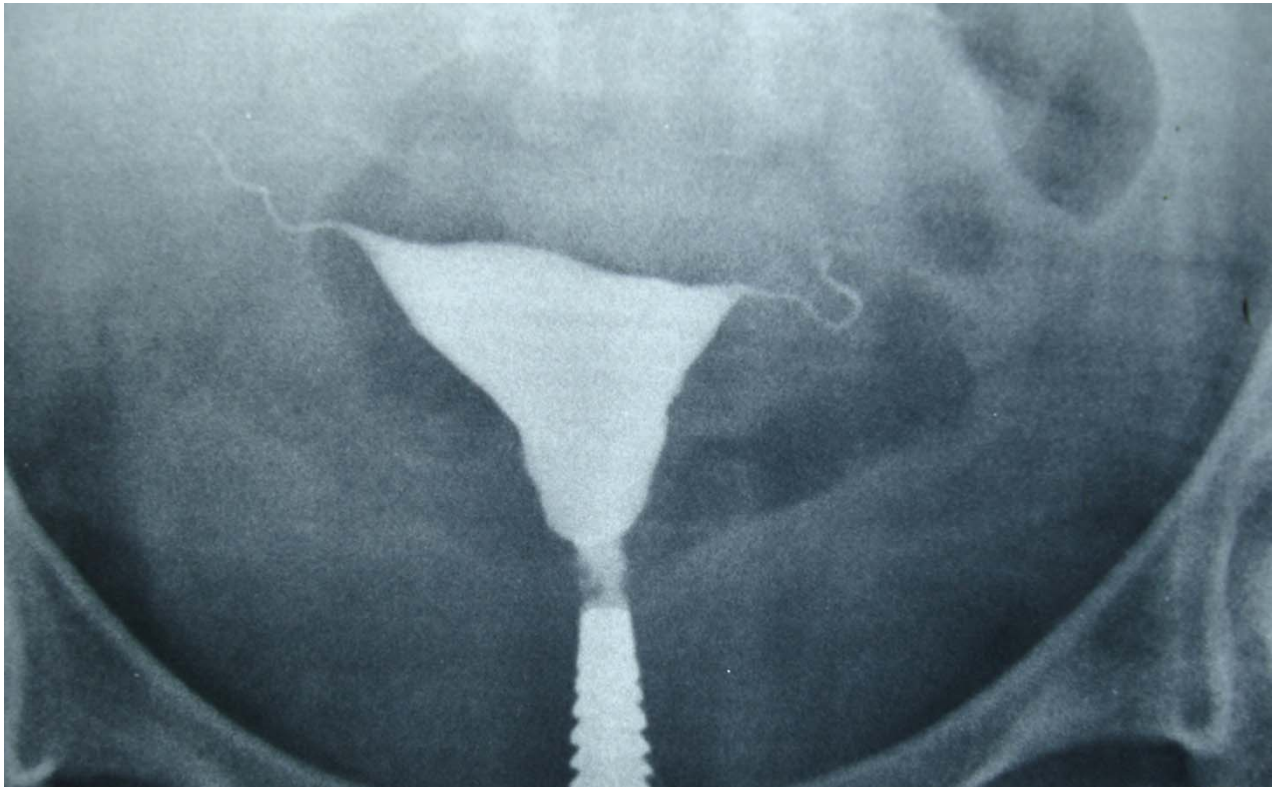


**Figure : 12**

# Left cornual block with right hydrosalpinx

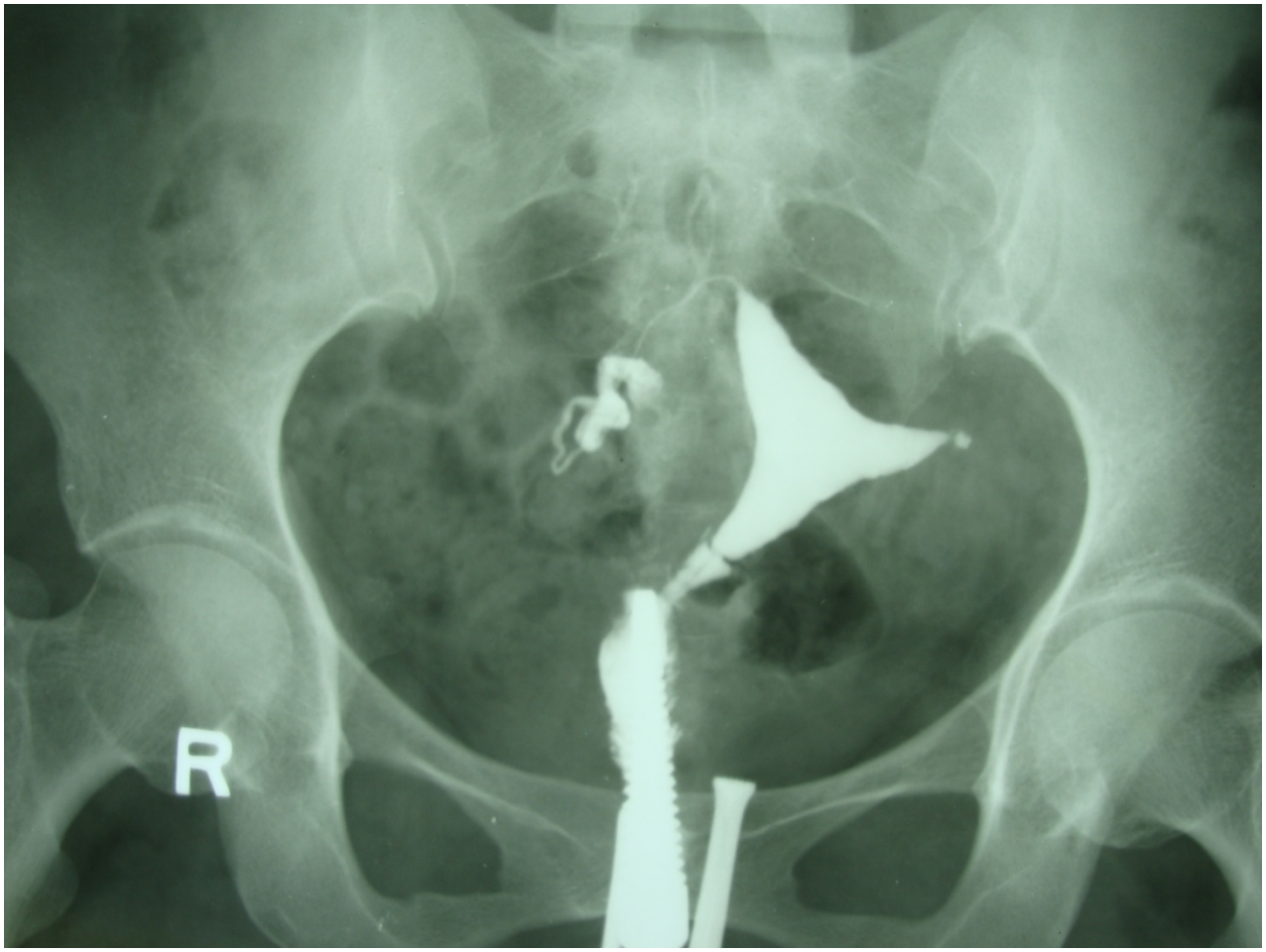


**Figure : 13**

# HSG Picture Showing Bilateral Hydrosalpinx

**Figure : 14**

# HSG picture showing bilateral cornual block

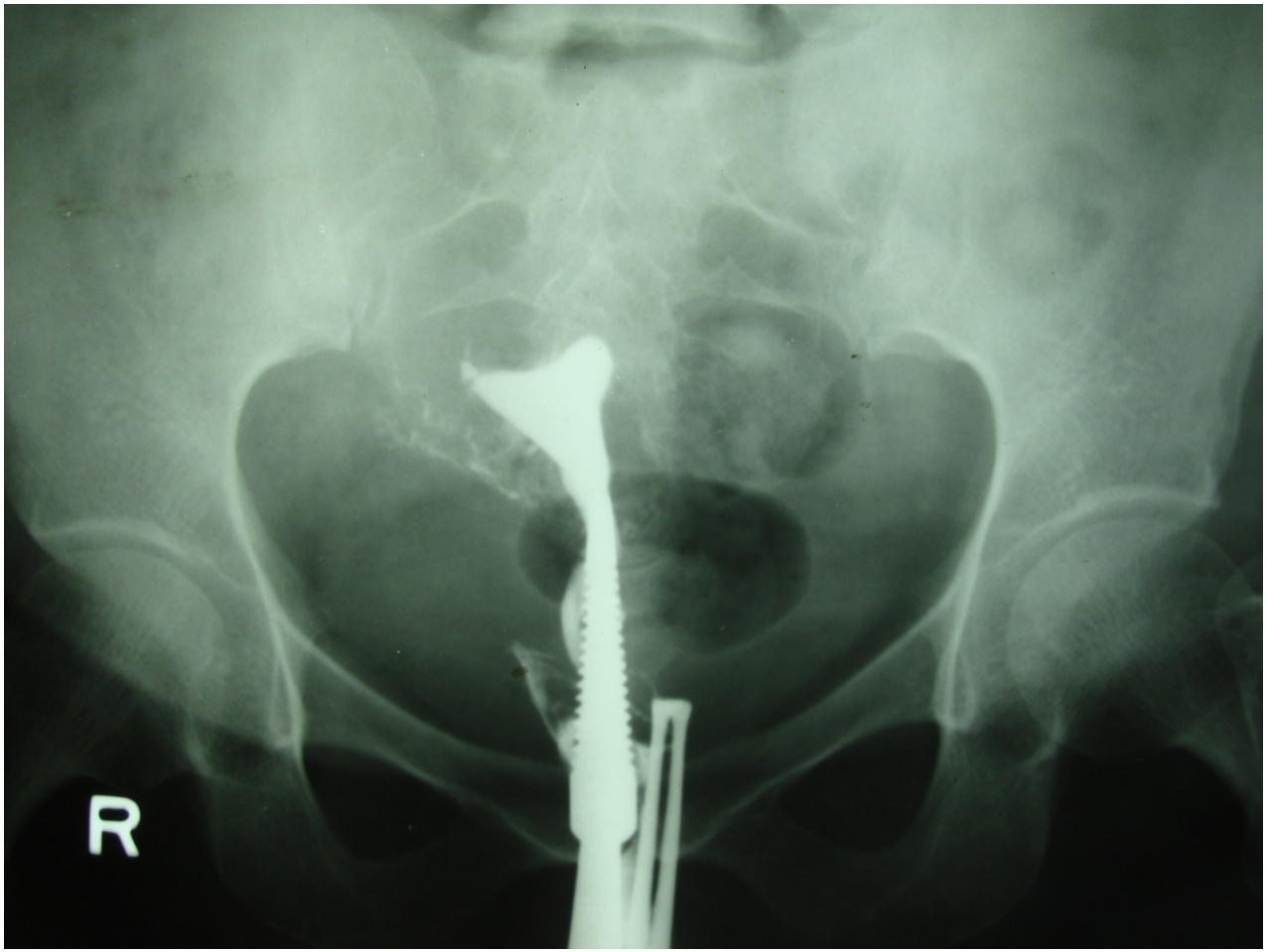


**Figure : 15**

# Left Cornual Block With Distorted Uterine Cavity

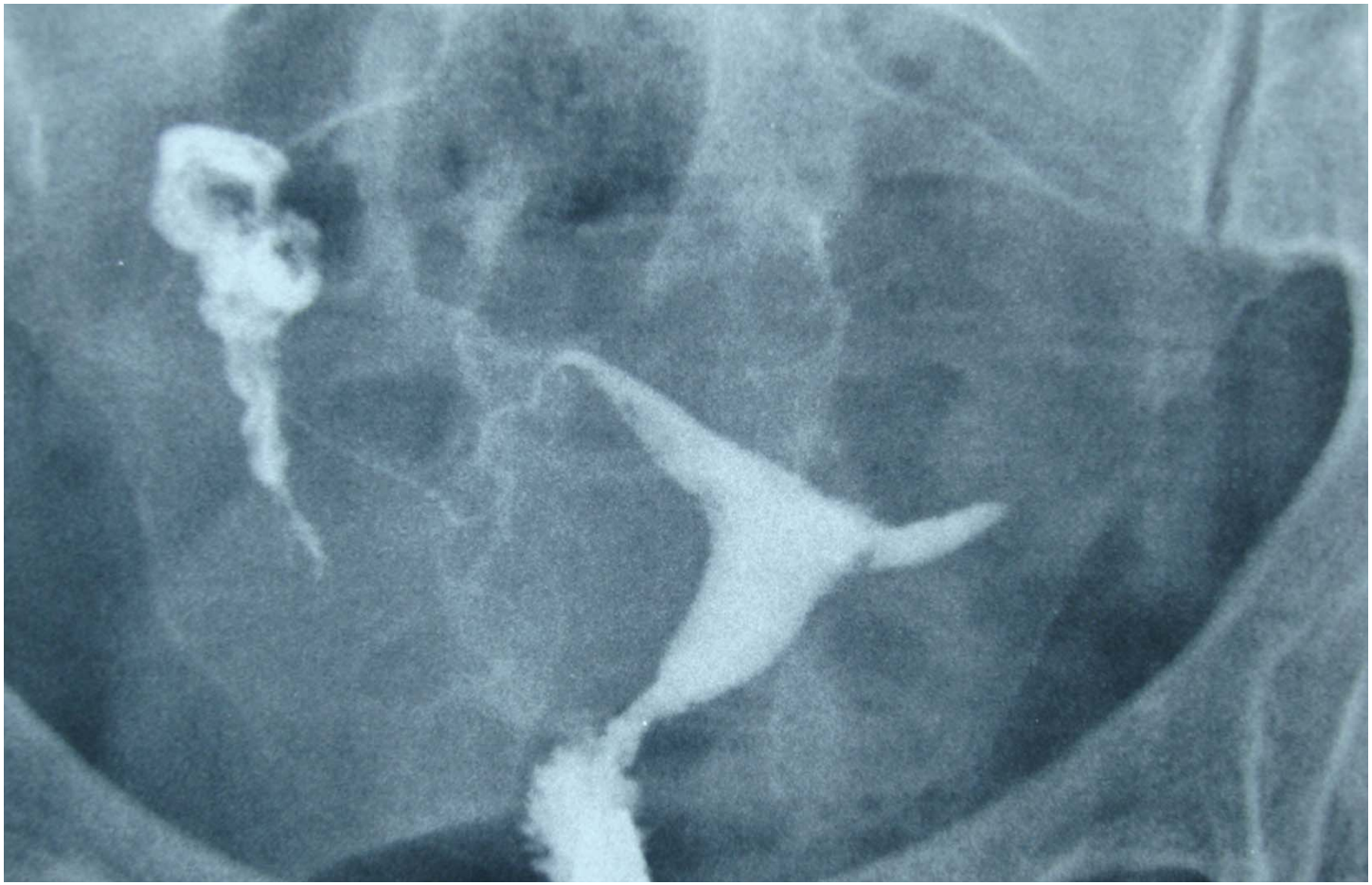


**Figure : 16**

# Laparoscopic picture showing granuloma in the adnexal region

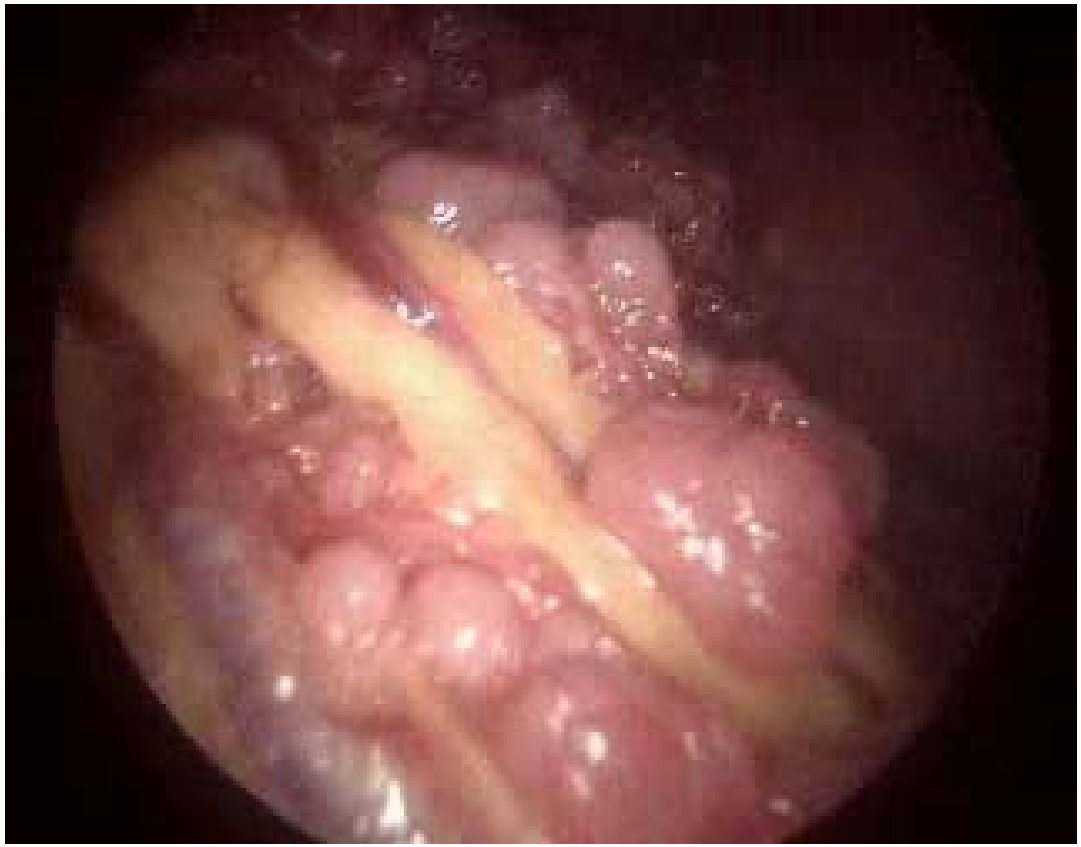


**Figure : 17**

## Laparoscopic picture showing calcified lesion in the adnexal region

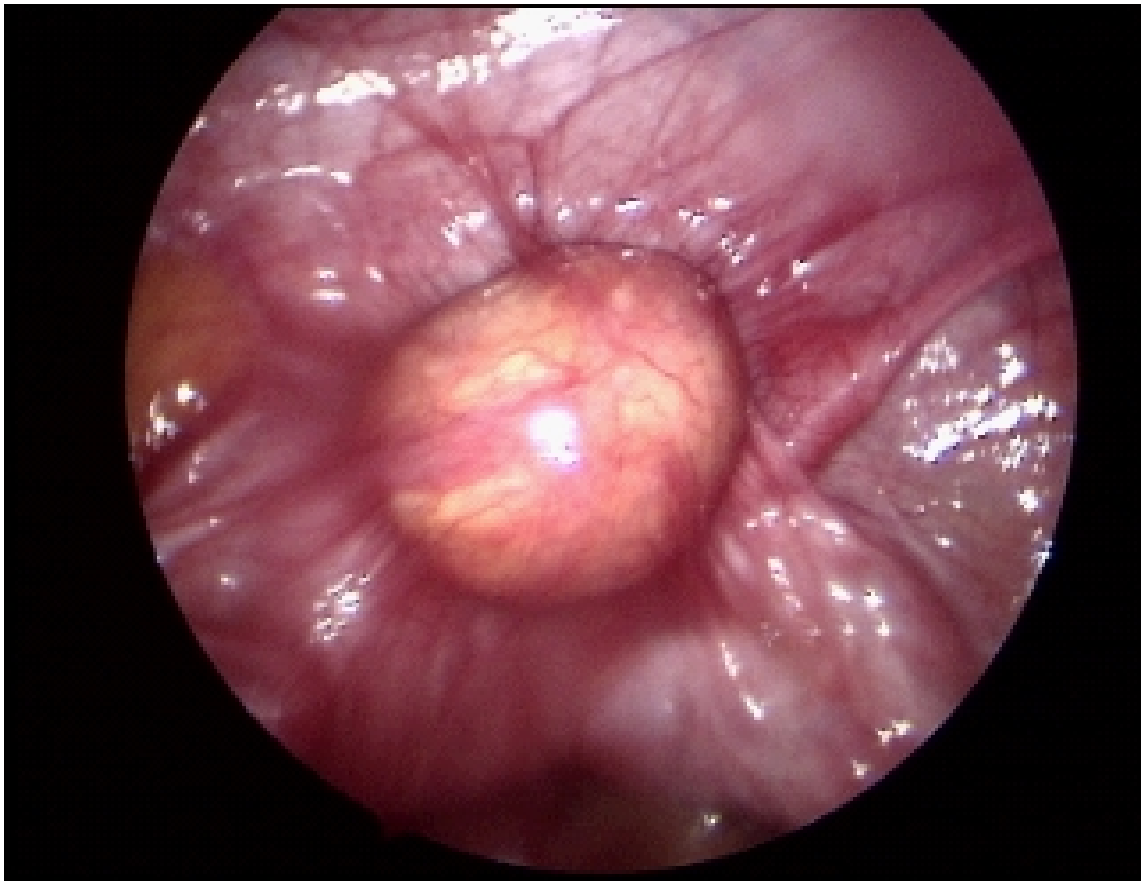


**Figure : 18**

## Laparoscopic picture showing enlarged ovary, distended tubes with white tubercle on the left side

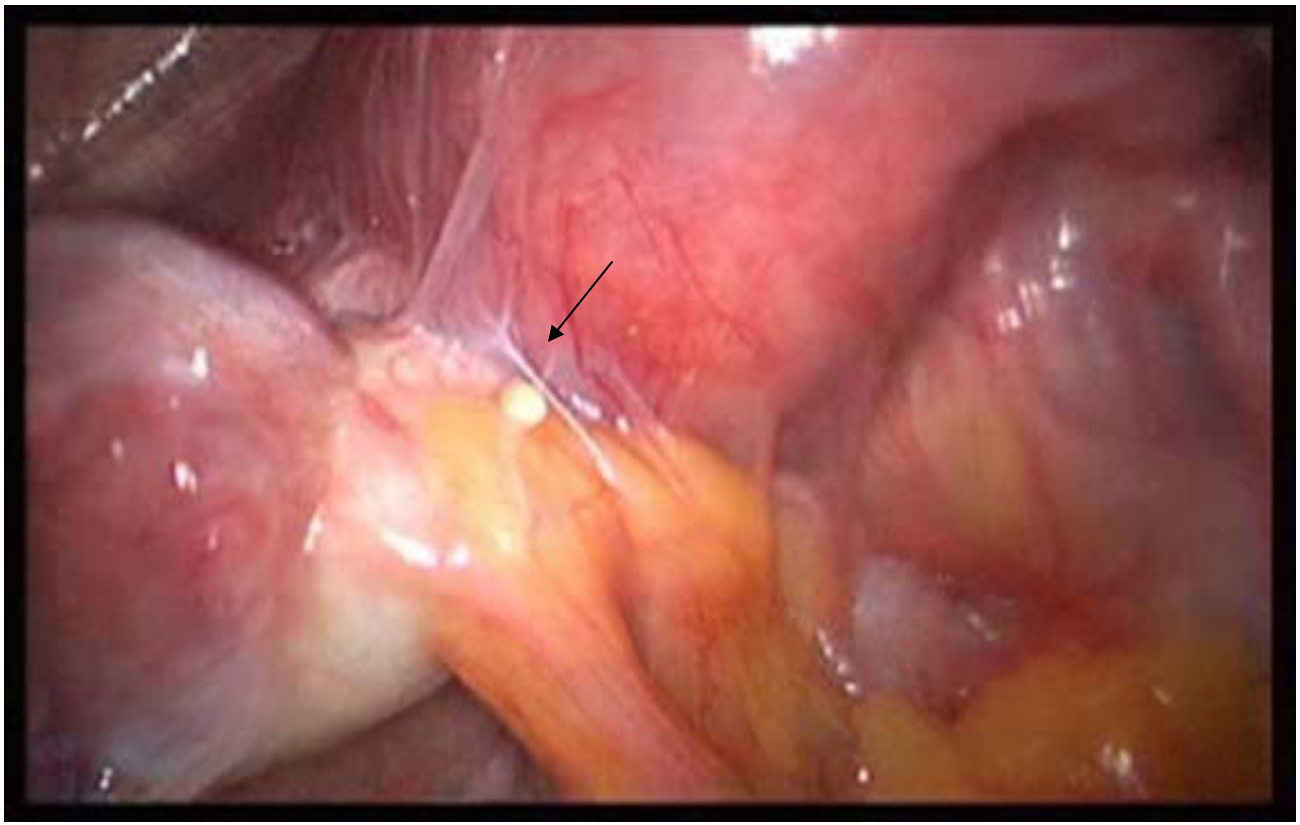


**Figure : 19**

## Laparoscopic picture showing tubercle over bladder peritoneum

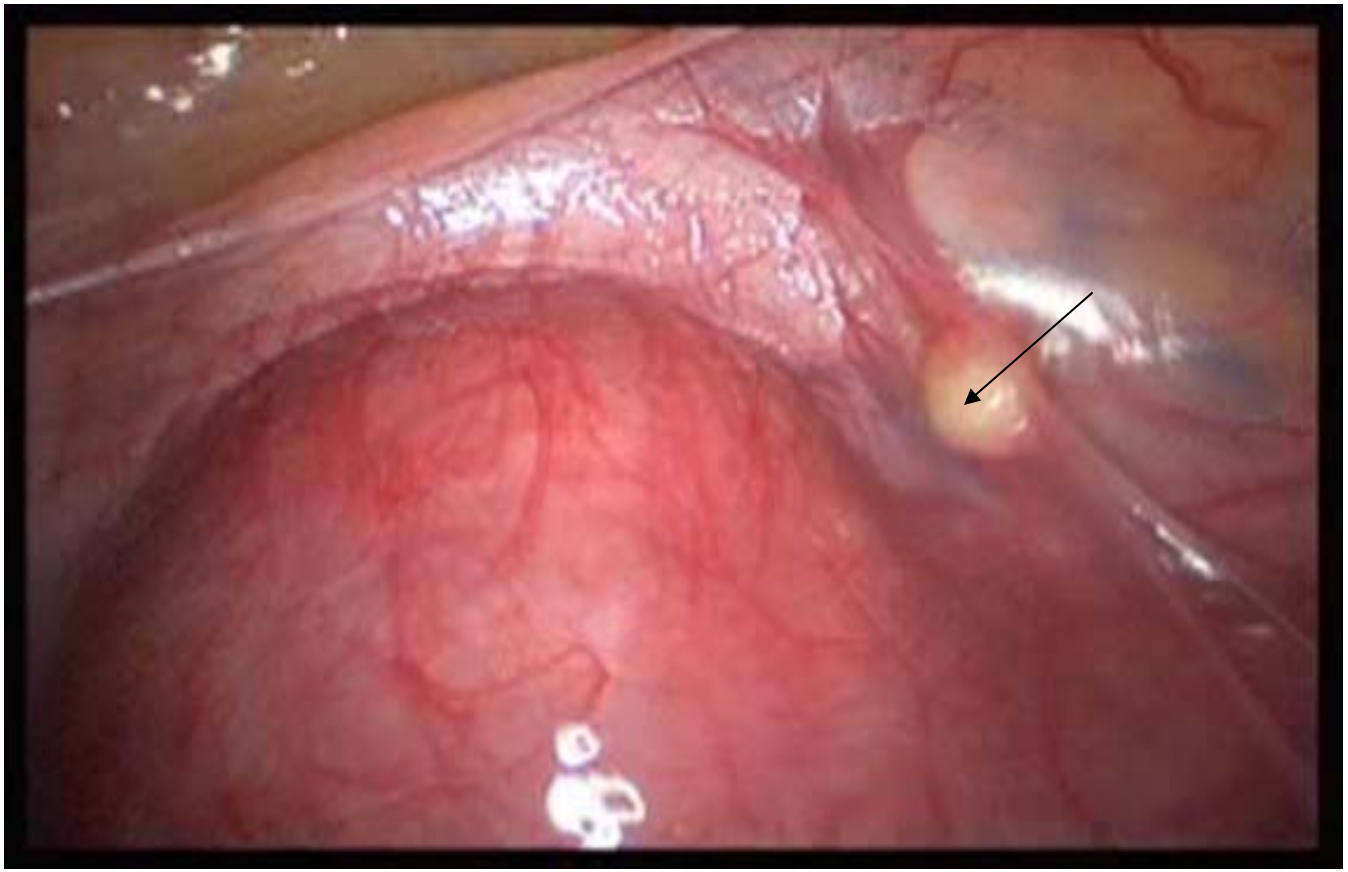


**Figure : 20**

**Laparoscopic picture showing hydrosalpinx with tubercle**

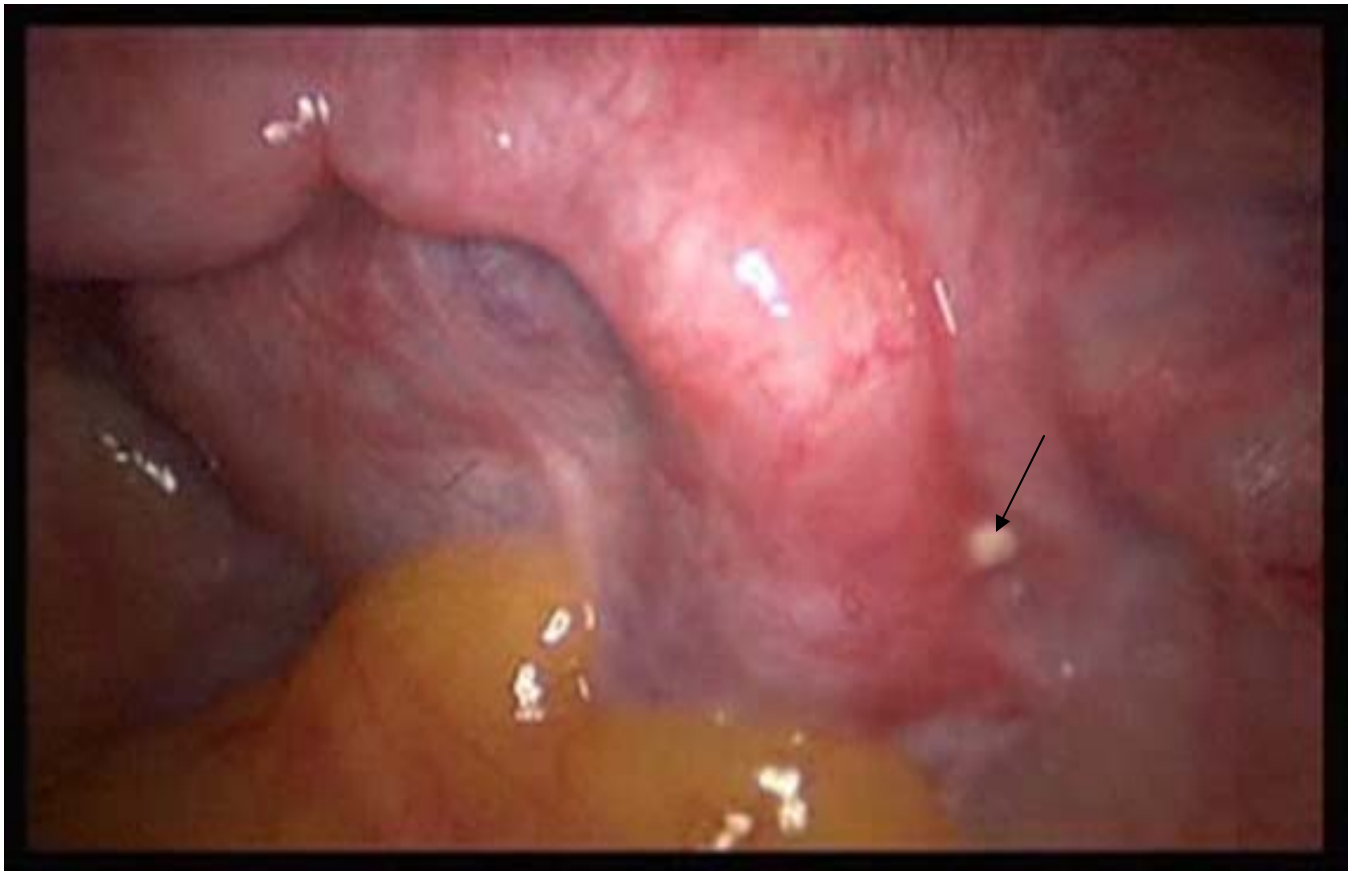


**Figure : 21**

**Laparoscopic picture showing tubercle over parietal peritoneum**

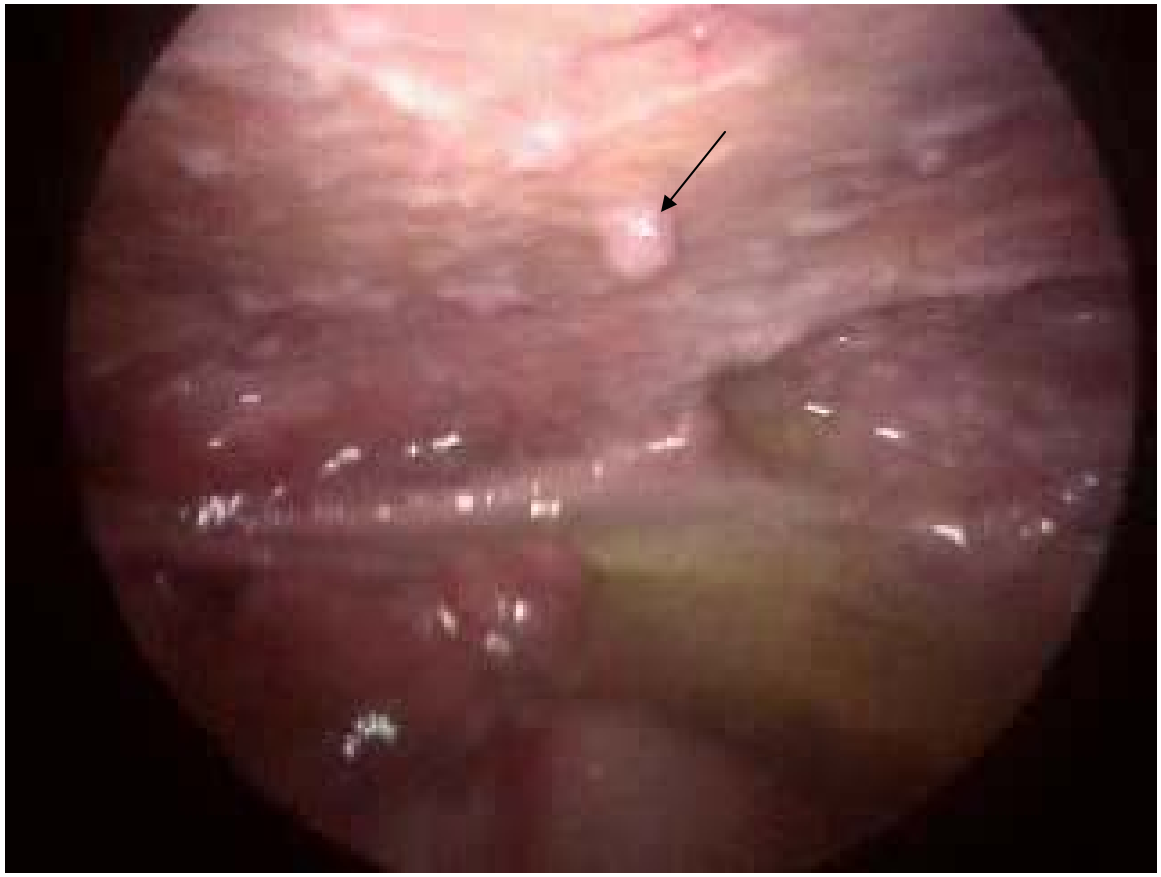


**Figure : 22**

## Laparoscopic picture showing hydrosalpinx distended with methylene blue

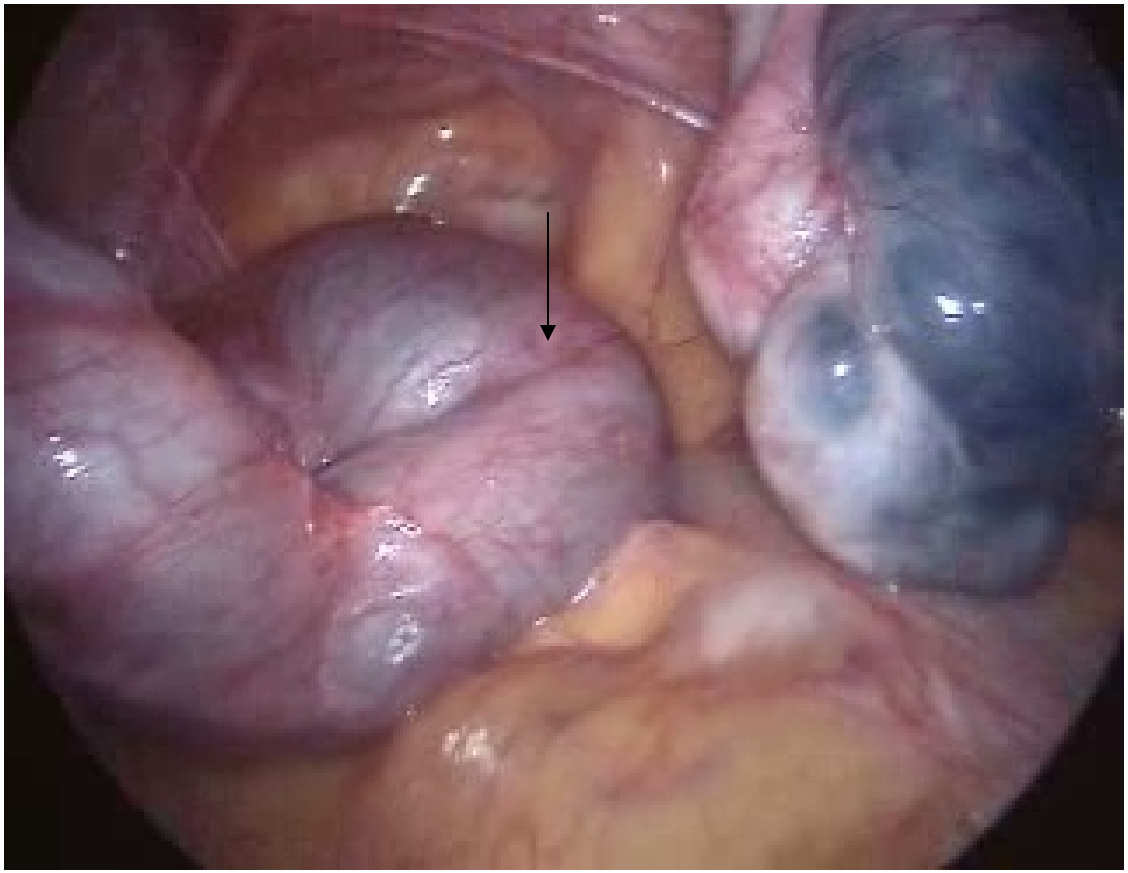


**Figure : 23**

**Laparoscopic picture showing hydrosalpinx with adhesions in POD**

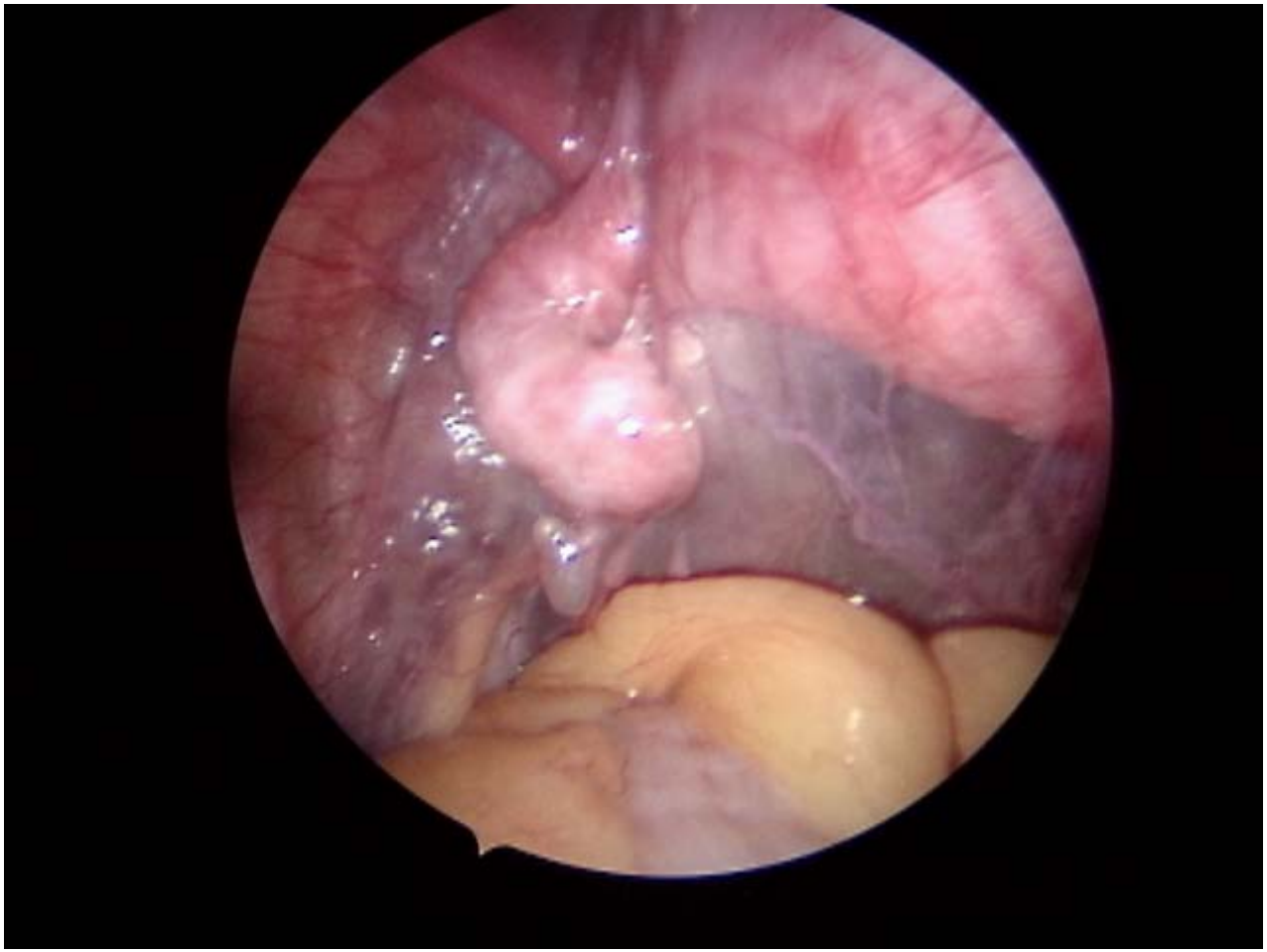


**Figure : 24**

## Laparoscopic picture showing dilated retort shaped tube

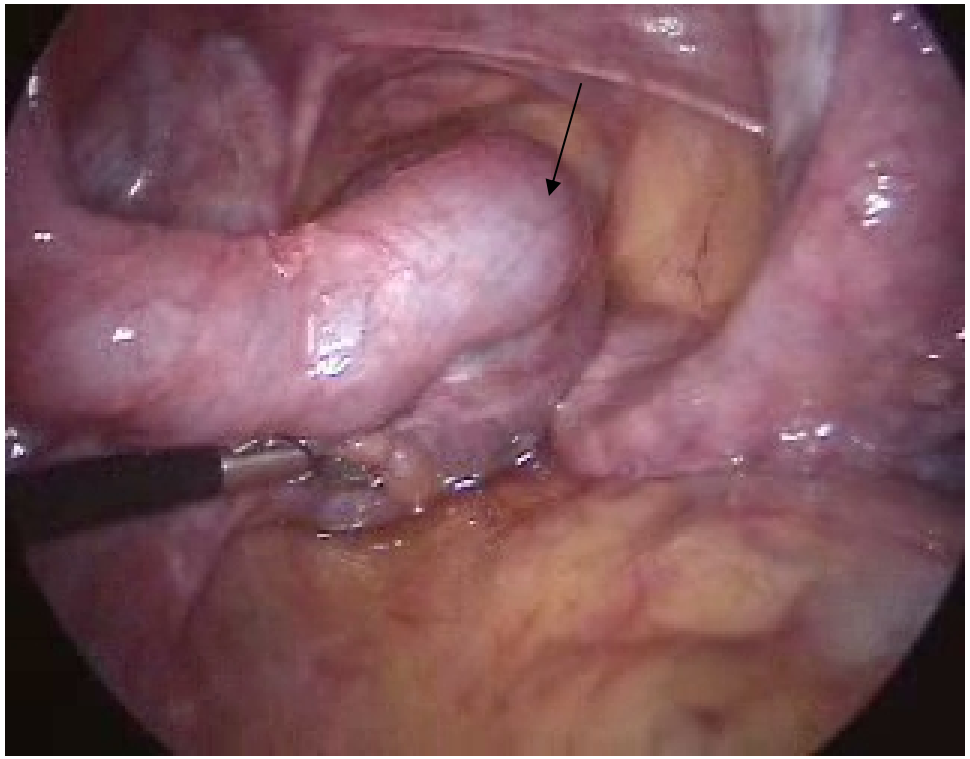


**Figure : 25**

## Laparoscopic picture showing adhesions between uterus, POD and bowel

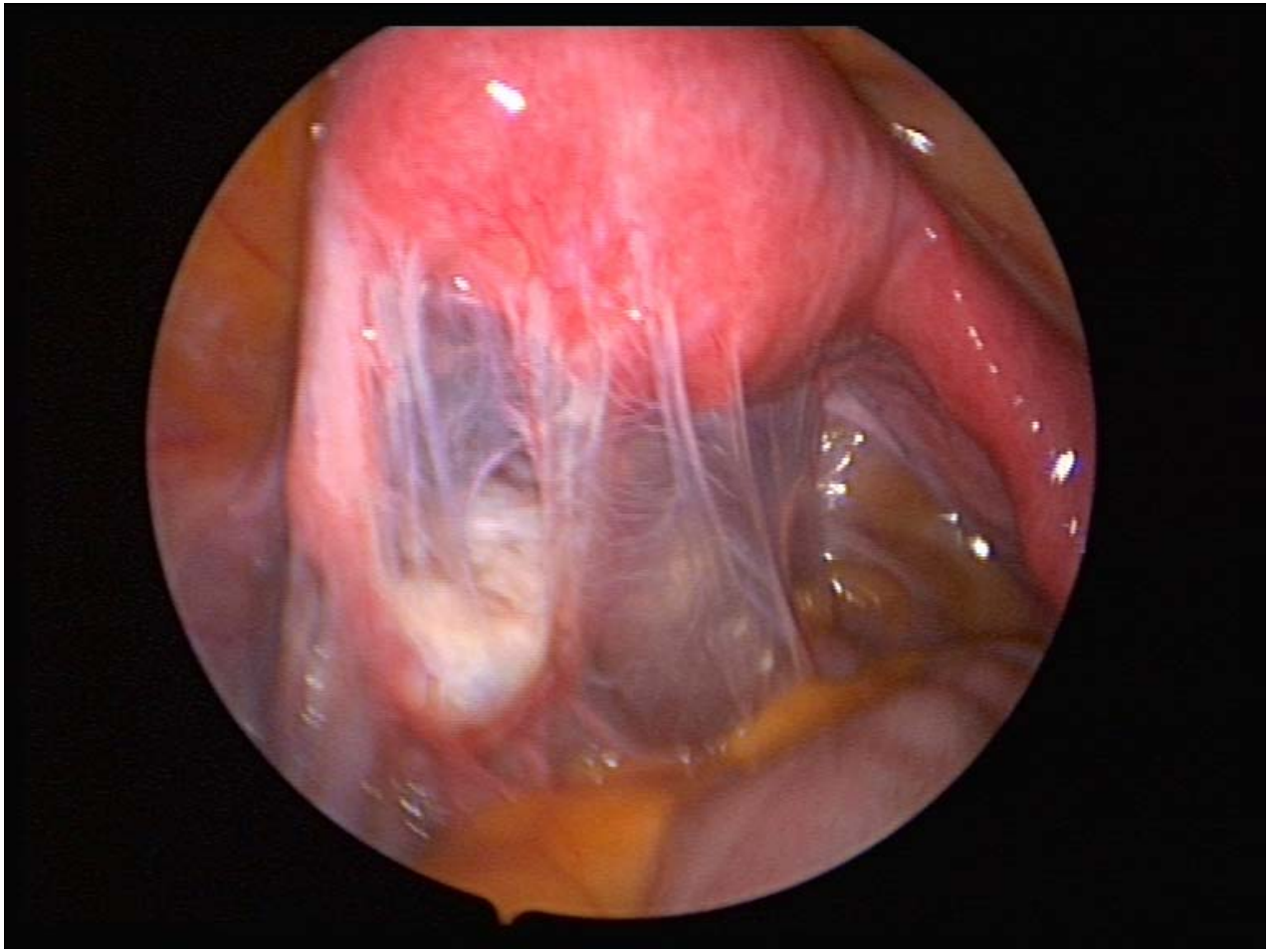


**Figure : 26**

## Laparoscopic picture showing parietal adhesions

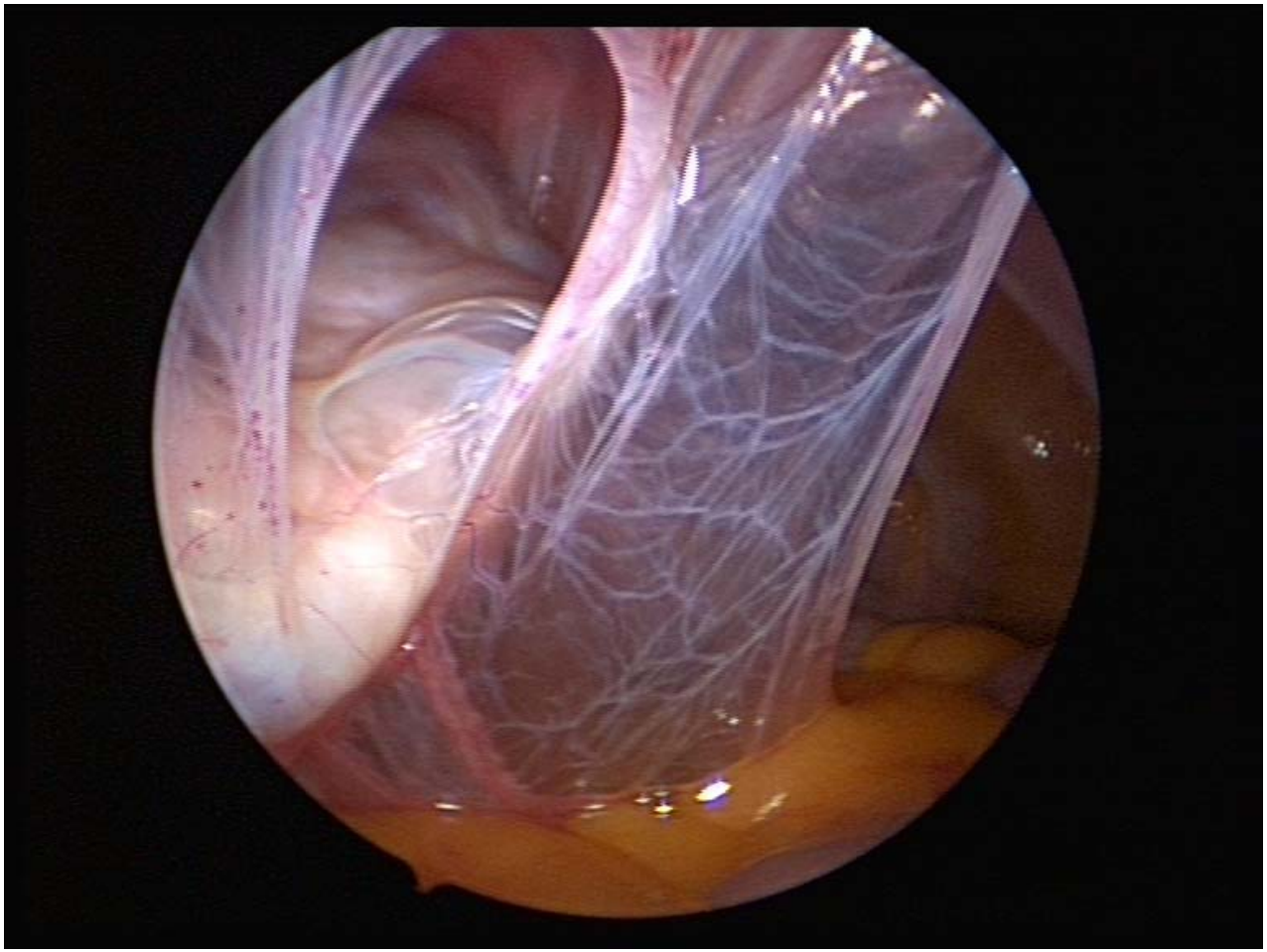


**Figure : 27**

**Laparoscopic picture showing dense adhesions between the uterus, bowel in POD**

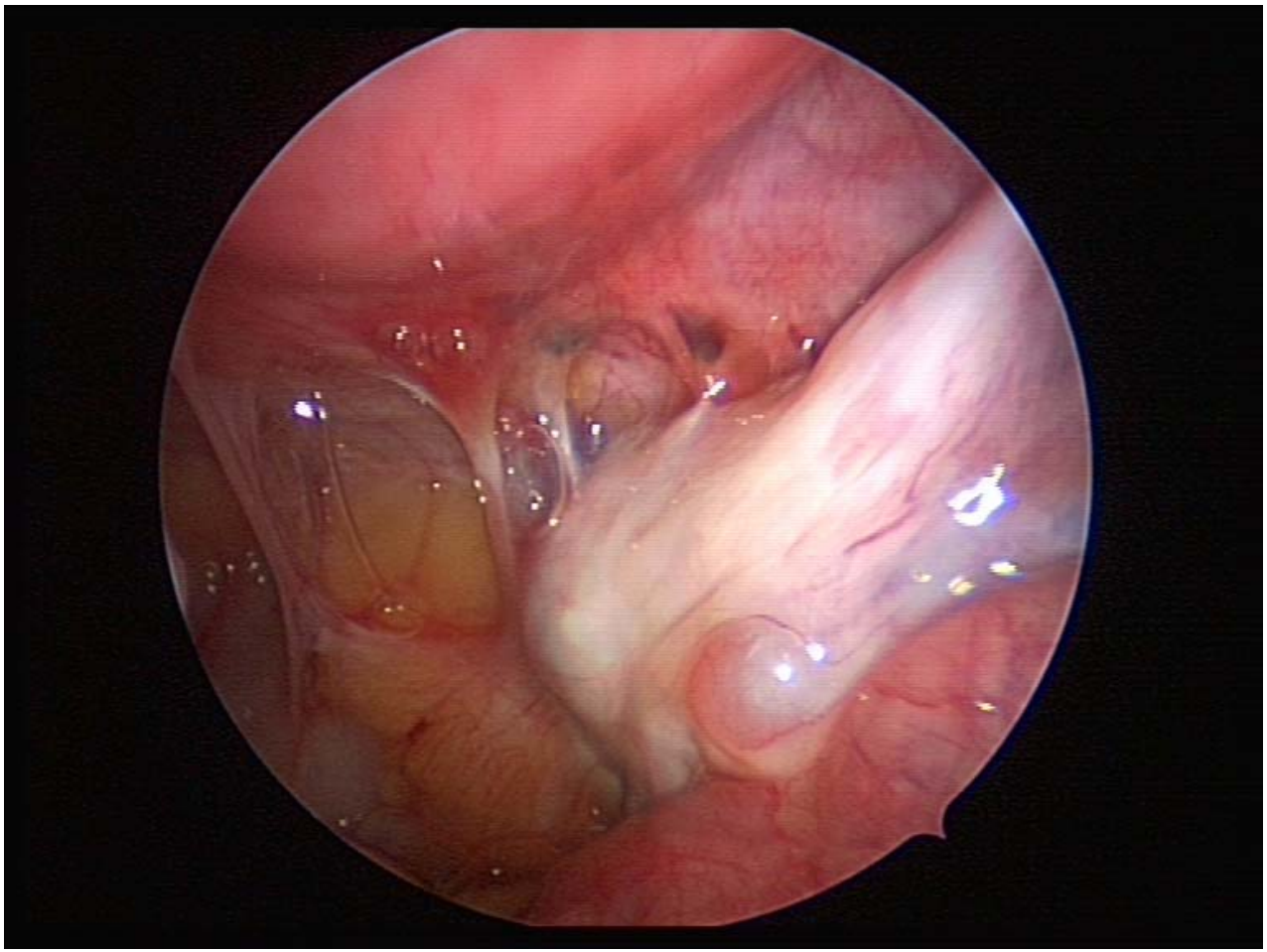


**Figure : 28**

# Hysteroscopy picture showing intrauterine calcification

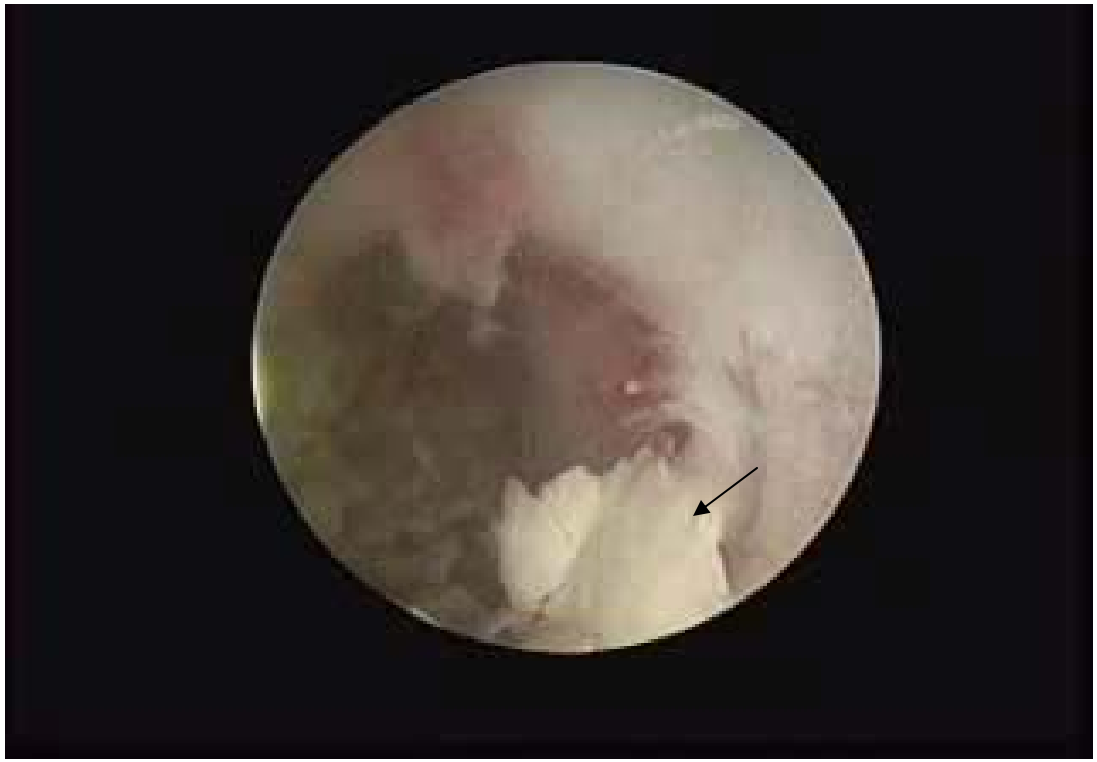


**Figure : 29**

# Hysteroscopy picture showing white patchy lesion

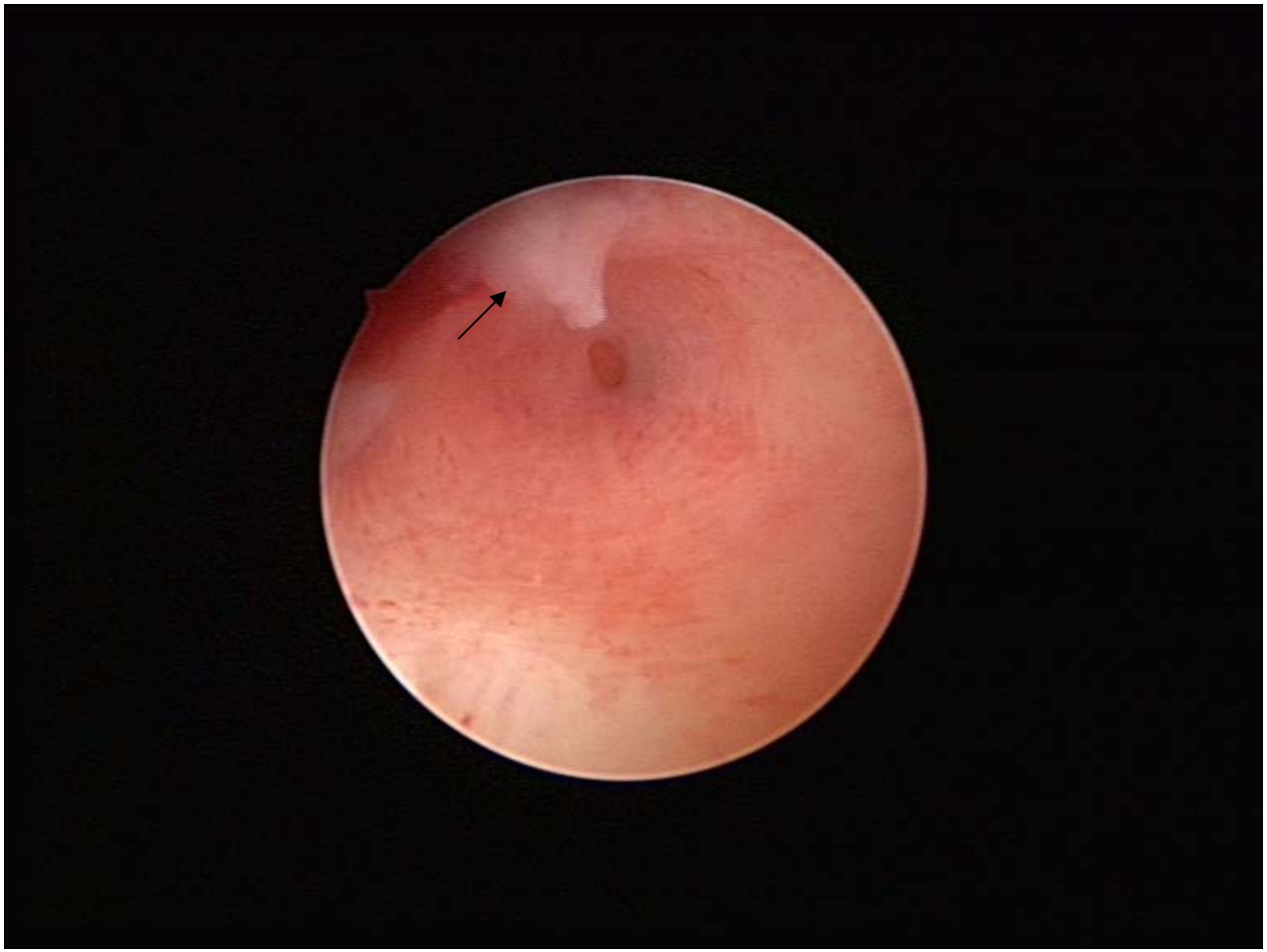


**Figure : 30**

# Hysteroscopy picture showing lesion within the cornua

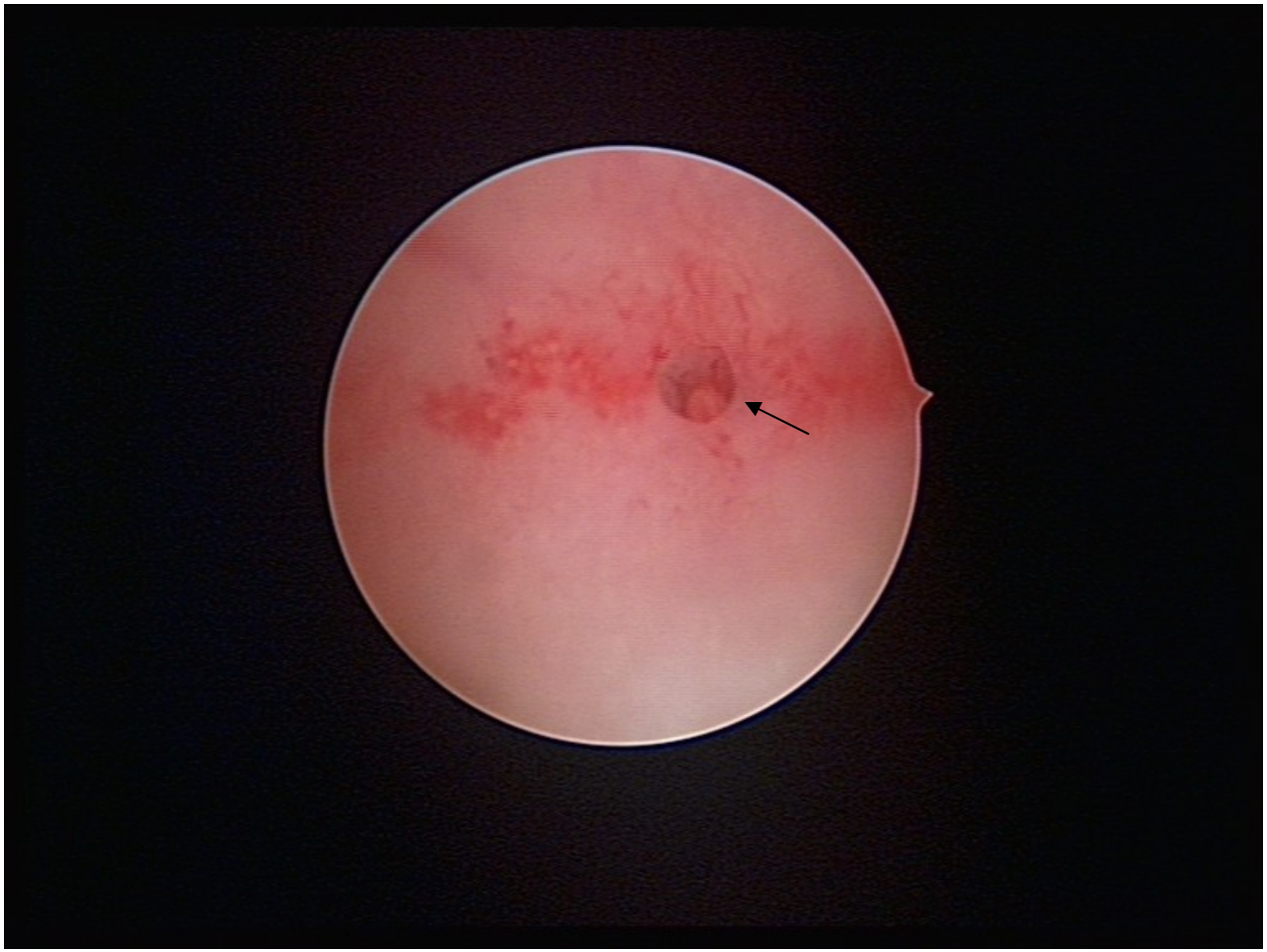


**Figure : 31**

**NTM growth – smooth morphology, mucoid colonies with coloured appearance**

**Figure : 32**

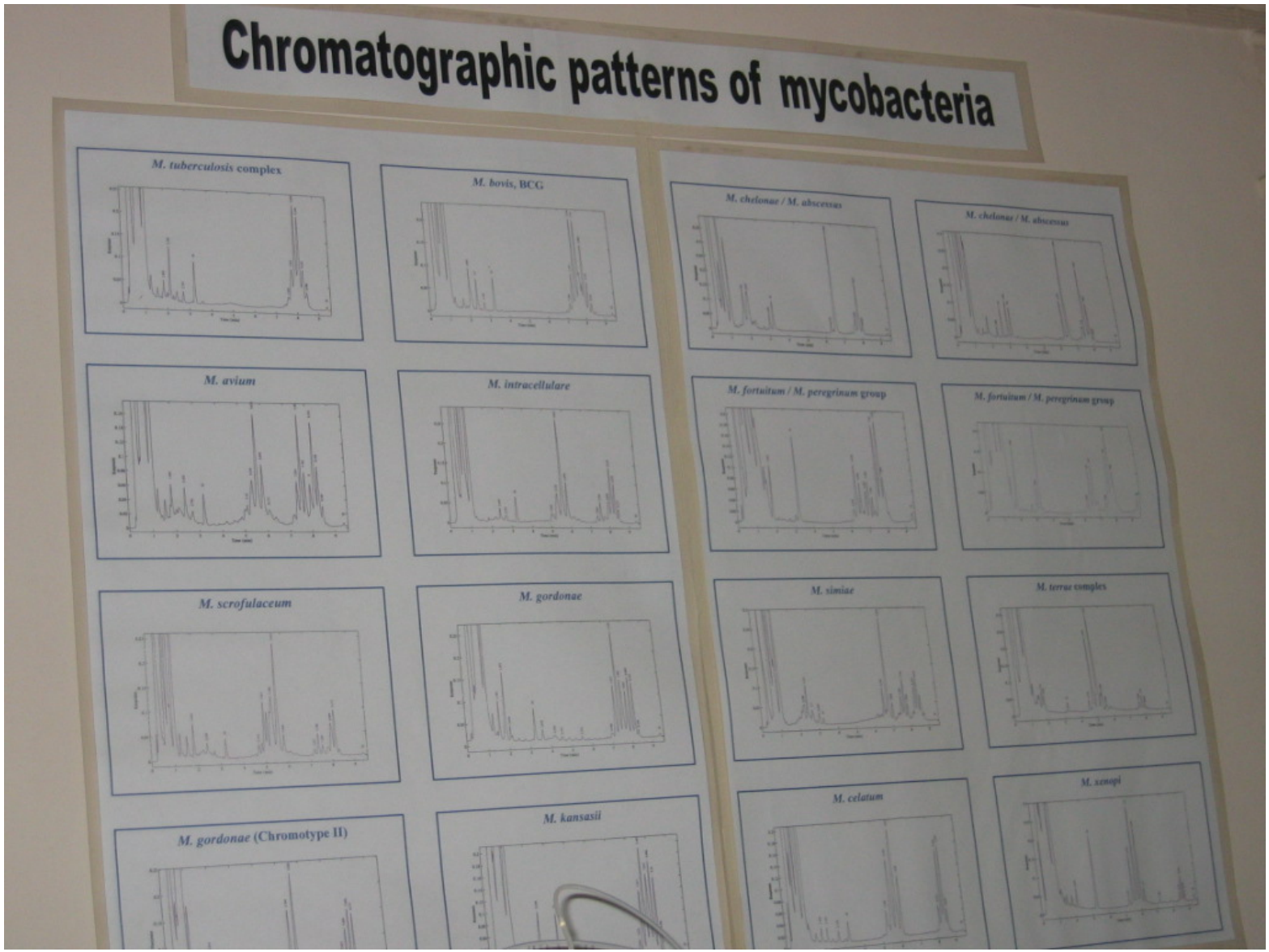
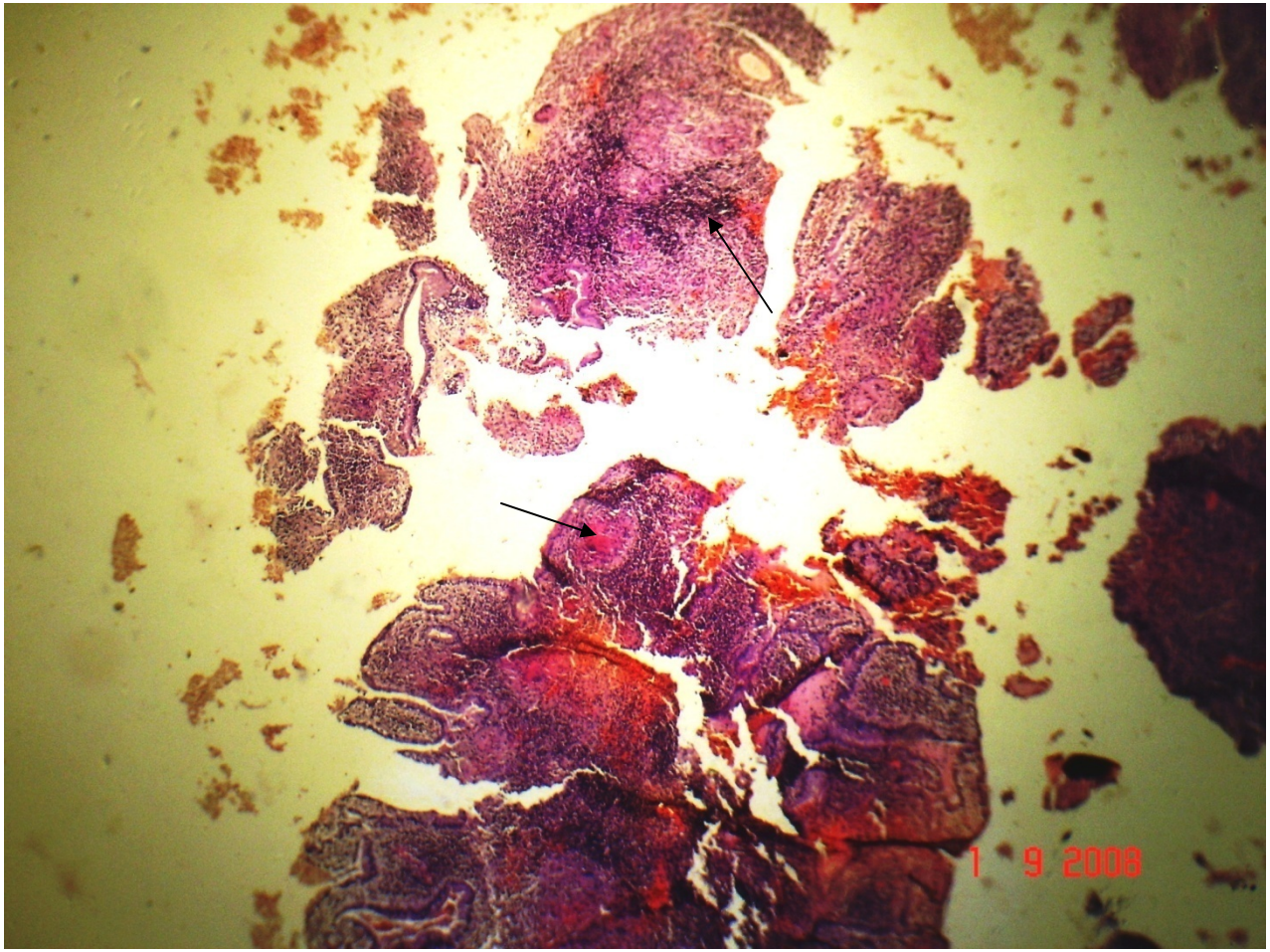

Chromatographic patterns of mycobacteria
M. tuberculosis complex
M. bovis, BCG
M. chelonae / M. abscessus
M. chelonae / M. abscessus
M. avium
M. intracellulare
M. fortuitum / M. peregrinum group
M. fortuitum / M. peregrinum group
M. scrofulaceum
M. gordonae
M. simiae
M. terrae complex
M. gordonae (Chromotype II)
M. kansasii
M. celatum
M. xenopi

**Figure : 33**

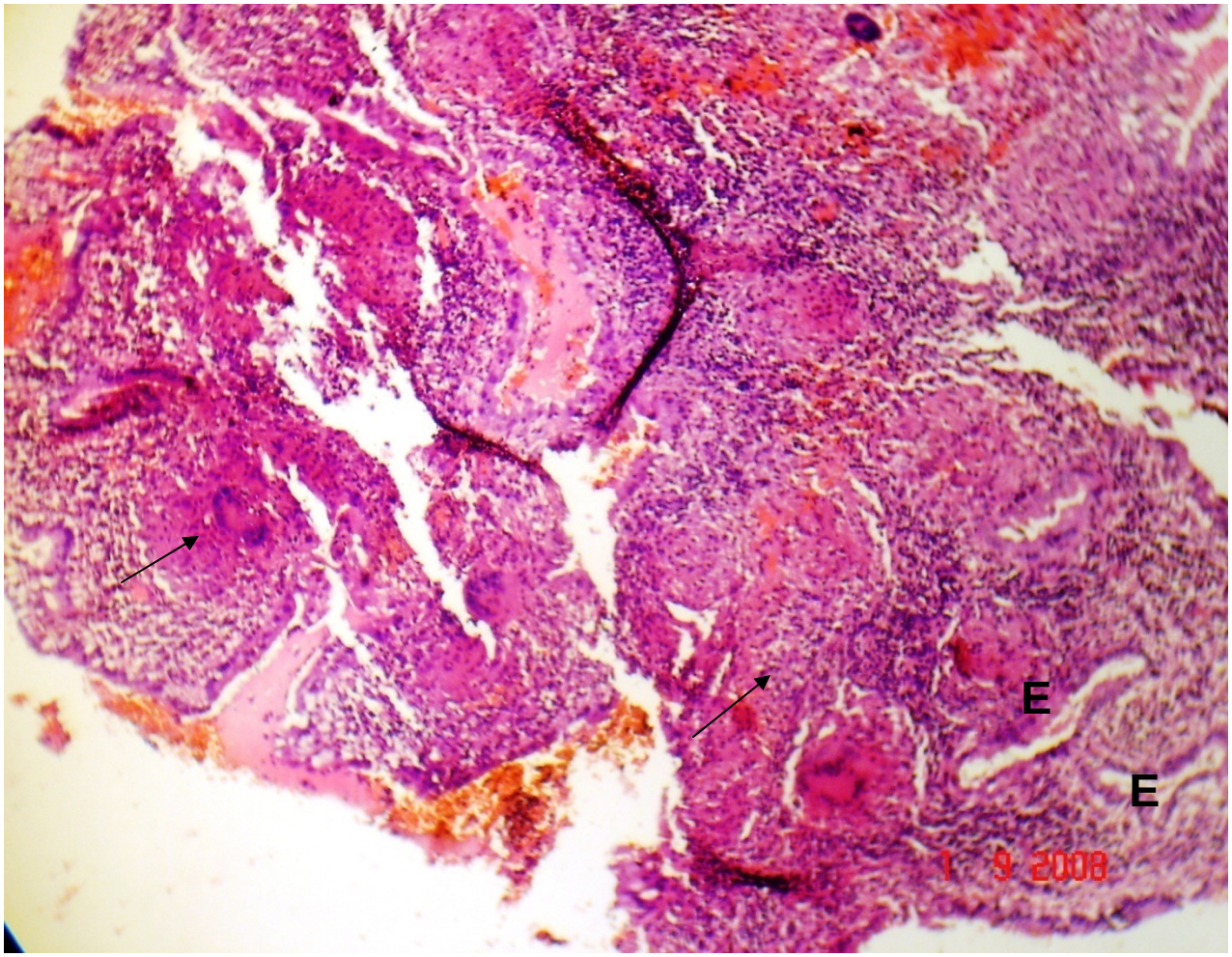
# TB Endometrium  H&E (10X)




Arrows showing Granuloma

**Figure : 34**

**HPE –TB endometritis** H&E (10X)

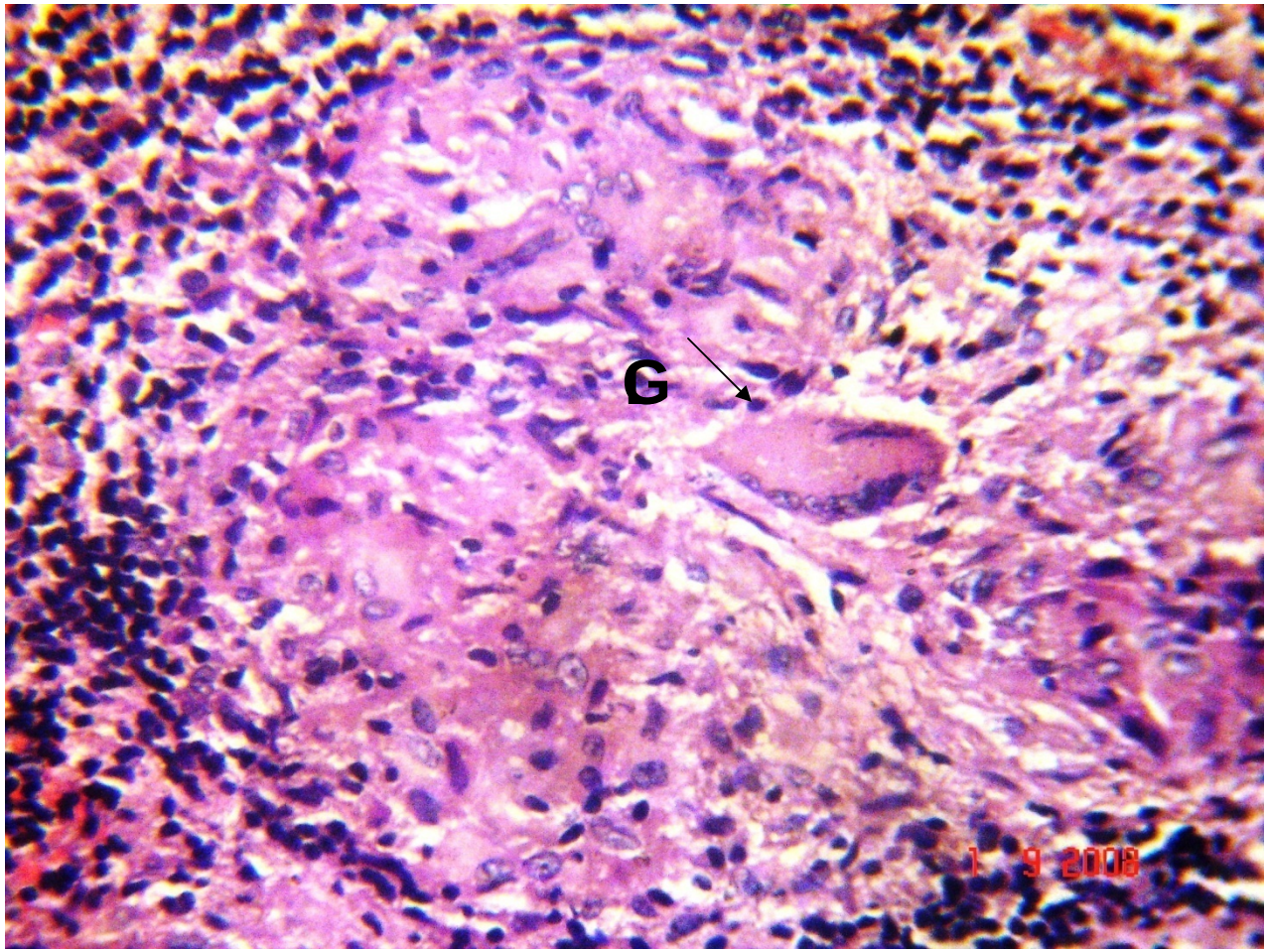



Arrows showing Granuloma

E-Endometrial glands

**Figure : 35**

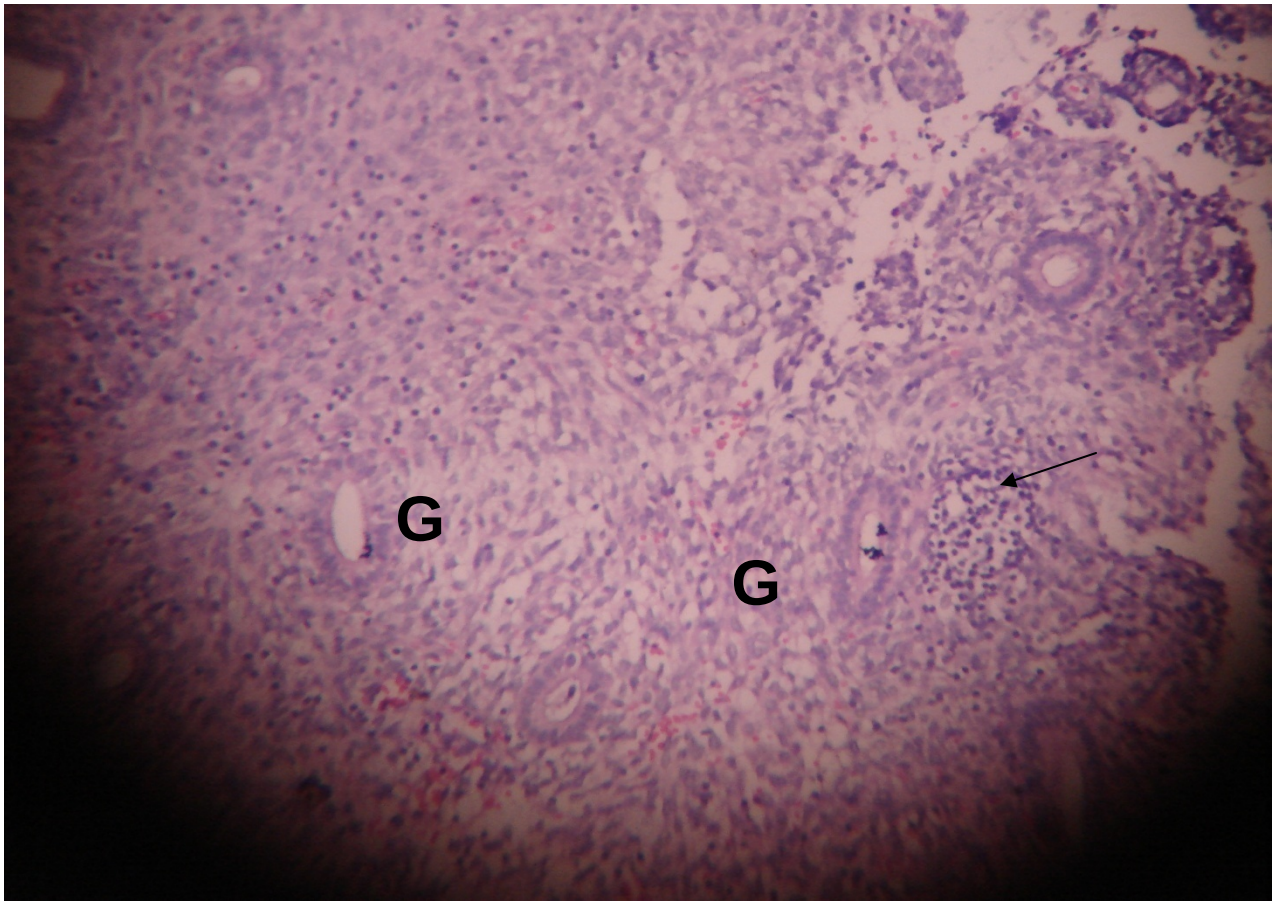
## Granuloma Showing Langhan Giant Cell Surrounded By Epithelioid Cells And Lymphocytes H&E (40X)




**Arrow showing Giant cell**

**Figure : 36**

## Non specific endometritis
## H&E (10X)

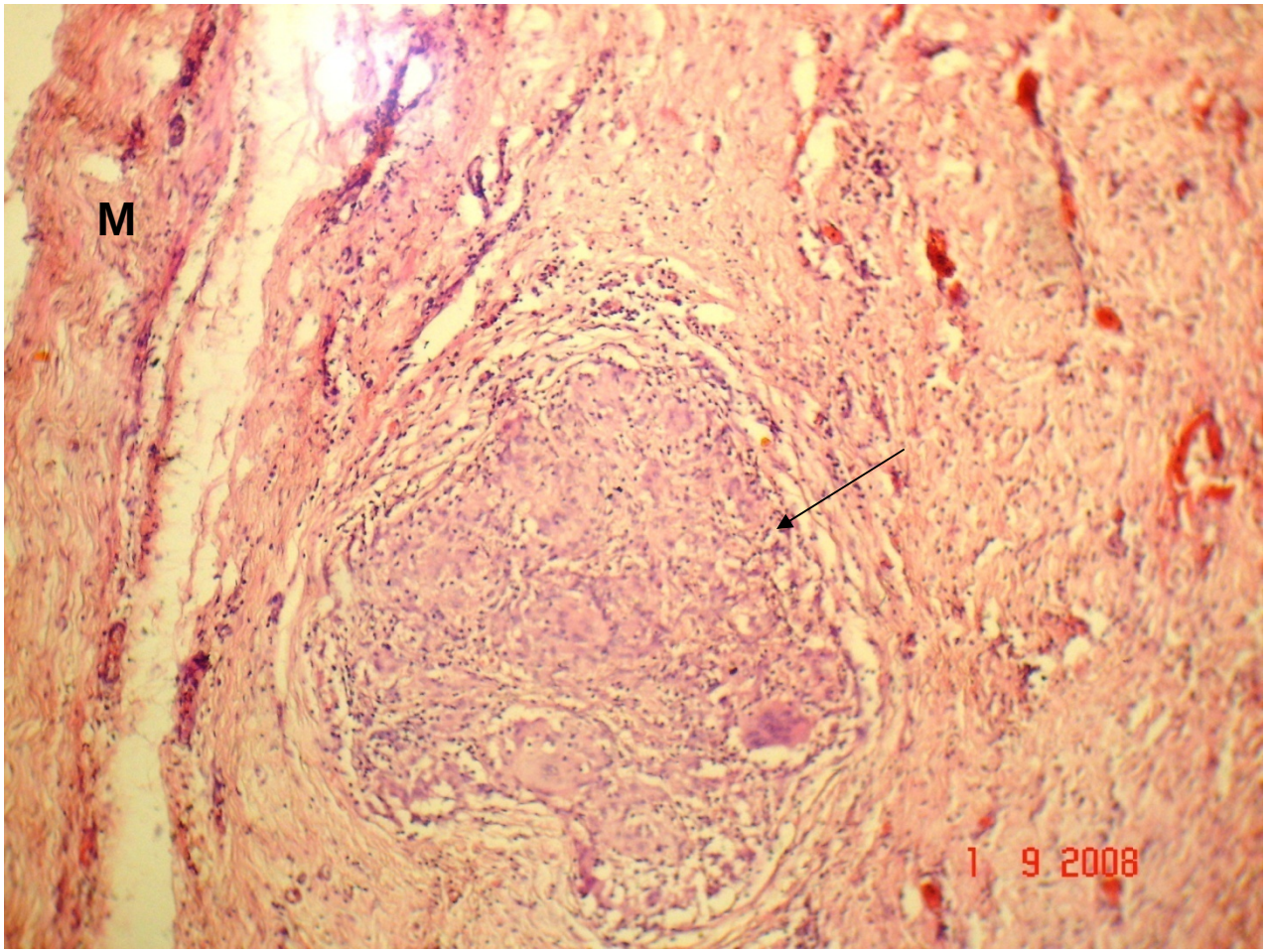



Arrow showing collection of lymphocytes in stroma close to a gland

G-Gland

**Figure : 37**

# Picture Showing Tuberculous Salpingitis H&E (10X)

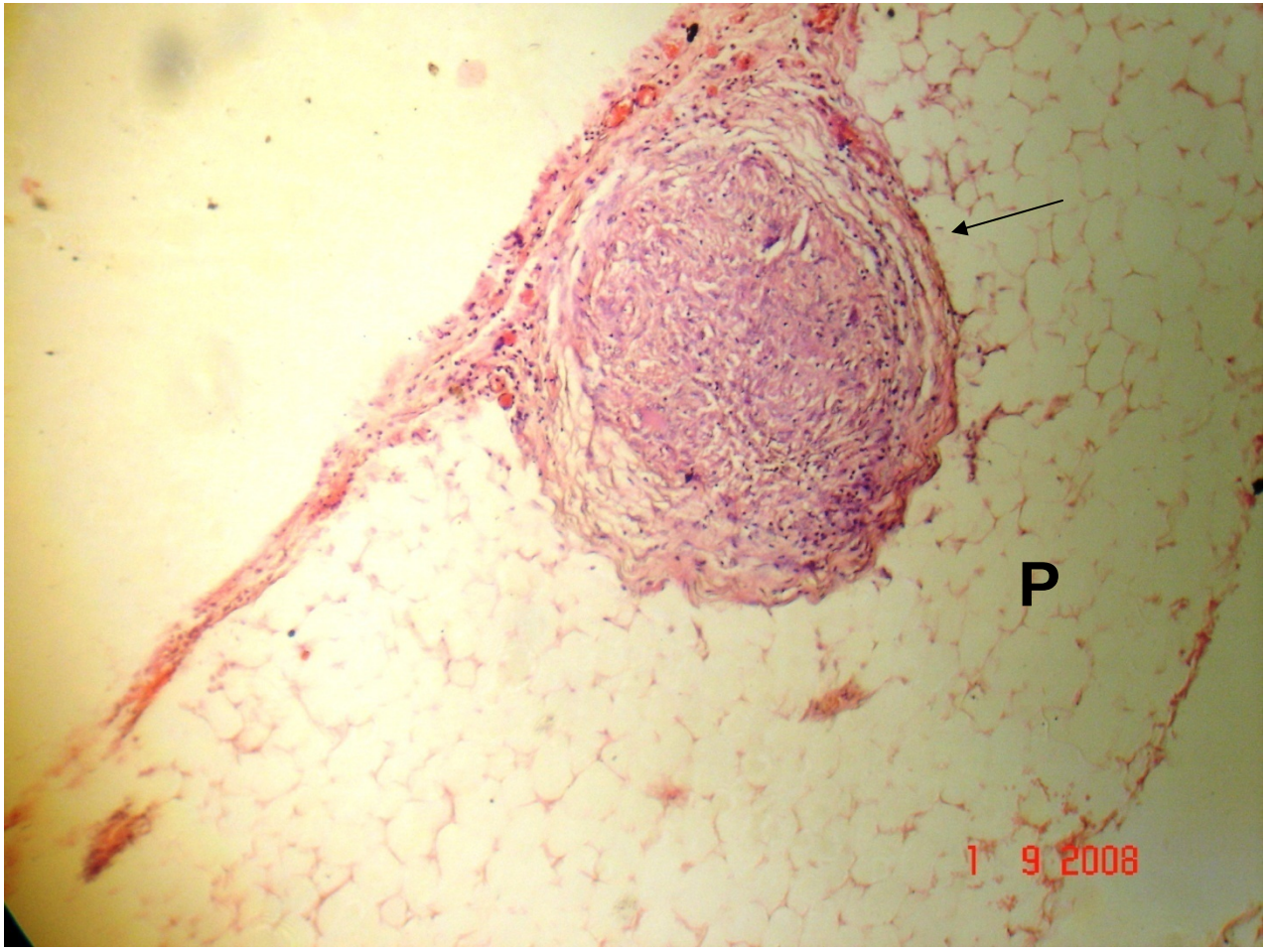



**Arrow pointing Granuloma**

**M-Muscle**

**Figure : 38**

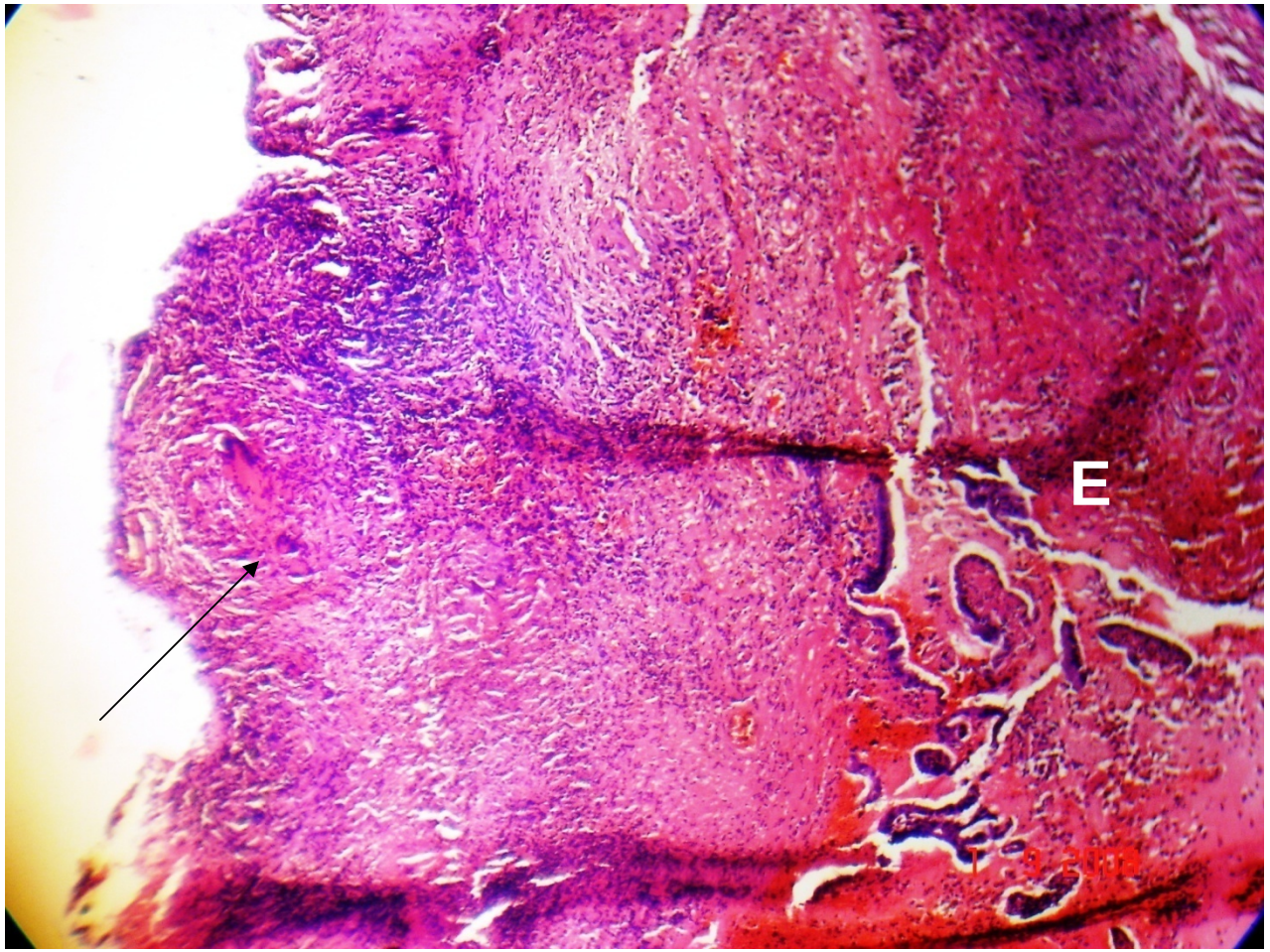
# Tuberculous Granuloma In The Peritoneum H&E (10X)




**P-Peritoneal fat**

**Figure : 39**

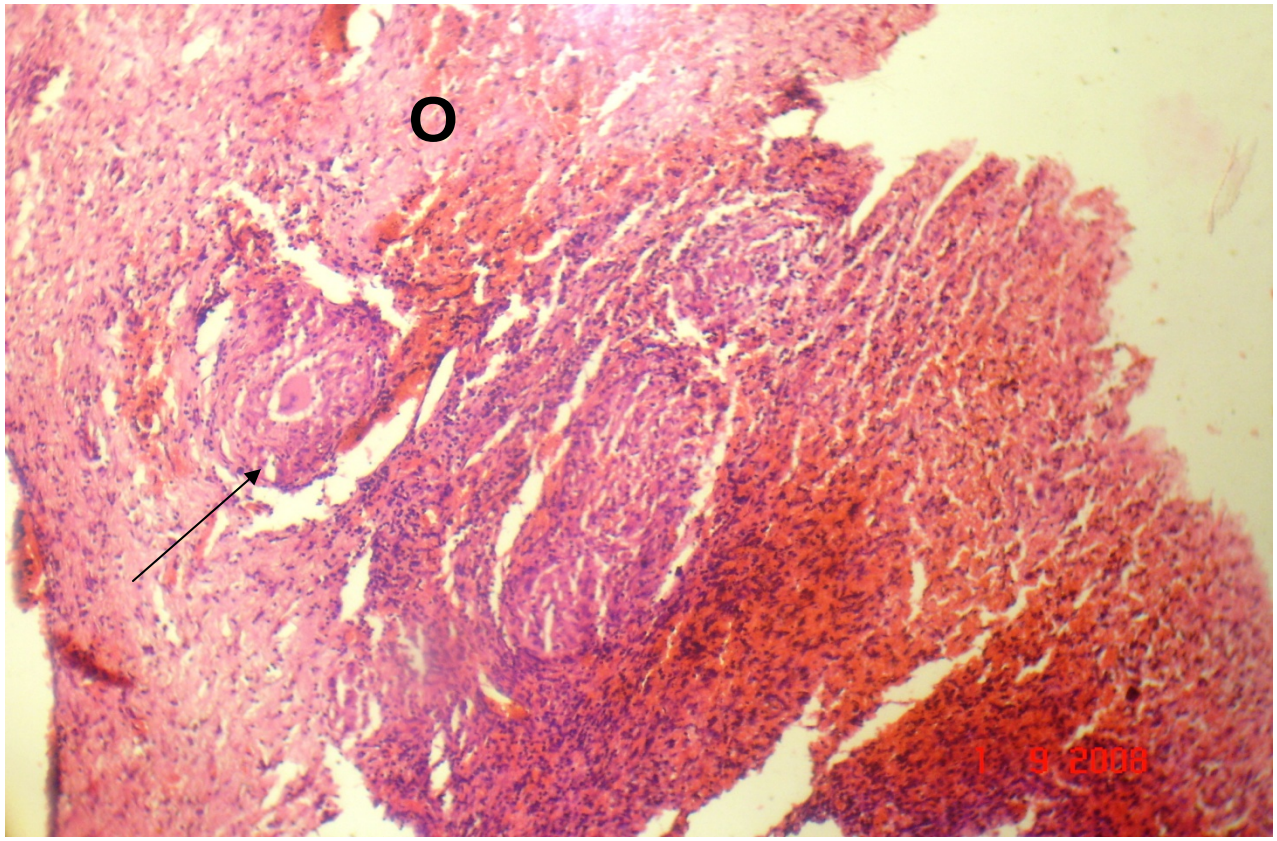
## Tuberculosis Of The Endocervix  H&E (10X)




## E-Endocervix

**Figure : 40**

# Tuberculous Granuloma In The Ovary H&E (10X)




**Arrow showing granuloma in the ovary**

**O-Ovarian parenchyma**