Original Article

Analysis of the Effect of Female Genital Tuberculosis on Ovarian Reserve Parameters

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Abstract

Background: Female genital tuberculosis (FGTB) is a known cause of female infertility. Worldwide incidence is 5%–10% and annual burden in India is around 4%–7%. It is known to cause tubal and endometrial damage. However, the effect on ovarian damage is poorly known. The availability of ovarian markers has contributed to an improved understanding of ovarian reserve in FGTB. **Aims:** The aim of this study was to assess ovarian reserve by measuring anti-Mullerian hormone (AMH) and antral follicle count (AFC) amongst infertile women and analyse the effect of GTB on ovarian reserve parameters. **Settings and Design:** This was a prospective study at a tertiary referral centre for infertility for 18 months. **Materials and Methods:** A total of 133 infertile women who underwent diagnostic hysterolaparoscopy and cartridge-based nucleic acid amplification test testing of an endometrial biopsy were included in the study. AMH and AFC of all the infertile women were assessed and compared between cases with and without FGTB. **Statistical Analysis Used:** Independent *t*-test was used to find the outcome differences in the distribution of values. $P < 0.05$ was considered statistically significant. **Results:** Fifty-eight (43.6%) cases were diagnosed with FGTB (Group I), and 75 (56.3%) cases were without FGTB (Group II). The mean AMH level 1.88 ng/ml (±1.52) and mean AFC 9.0 (±5.50) were significantly lower ($P < 0.001$) in Group I than in Group II with AMH 3.57 ng/ml (±2.93) and AFC 12.50 (±6.0). **Conclusion:** In women with prolonged infertility and low ovarian reserve, FGTB should be ruled out. Early diagnosis and treatment of GTB may prevent further decline of ovarian reserve and improve the reproductive outcome.

Keywords: *Anti-Mullerian hormone, antral follicle count, female genital tuberculosis, infertility, ovarian reserve*

Introduction

Female genital tuberculosis (FGTB) is a known cause of female infertility. Worldwide incidence is 5%–10% and annual burden in India is 4%–7%.[1] It mostly affects fallopian tubes (90%), endometrium (50%–80%) and ovaries (20%–30%) and rarely affects cervix, vagina and vulva. However, its effect on ovarian reserve is poorly understood and not well researched.[2,3] The ovarian reserve determines the functional age of the ovary and is an important predictor of a woman's reproductive capacity regardless of her chronological age. As direct bacteriological diagnosis of ovarian TB is difficult to make, to detect ovarian damage in people with FGTB, ovarian reserve evaluation has been used as a surrogate marker.[4]

Aims and objectives

The aims of this study were as follows:

- To assess the ovarian reserve by measuring anti-Mullerian hormone (AMH) and antral follicle count (AFC) amongst infertile women.

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- To analyse the effect of GTB on ovarian reserve parameters in infertile women detected with GTB.

Subjects and Methods

It is a hospital-based prospective study at a tertiary referral centre for infertility for 18 months, from January 2021 to July 2022. The institutional ethics committee approval was taken (CMCH/EC/2021/29-33). The study adhered to the principles of the Helsinki Declaration (2013). No sample size calculation was performed. Convenient sampling was done. All the patients of subfertility/infertility who were evaluated in the department and underwent diagnostic hysterolaparoscopy were registered after taking written informed consent. Ovarian reserve was assessed using hormonal assay (AMH) and ultrasonographic marker (AFC) and was correlated with hysterolaparoscopy findings and report of cartridge-based nucleic acid amplification test (CBNAAT).

Anti-Mullerian hormone

AMH was assessed irrespective of the day of the menstrual cycle. Serum AMH levels were determined using the MAGLUMI series fully automated chemiluminescent immunoassay analyser.

Antral follicle count

Transvaginal sonography was done in patients with empty bladder in the early follicular phase (day 2 or 3) of the menstrual cycle. It was performed using BPL (ECUBE-8) machine with an 8.8 MHz transvaginal probe in real-time B mode. It was done by a single operator according to the standardised protocol to avoid inter-observer variability. A number of follicles with a diameter of 2–9 mm were considered antral follicles and a number of follicles in both ovaries were added for the total AFC.[5,6]

Diagnosis of genital tuberculosis

Diagnosis of GTB was made out based on intraoperative findings of diagnostic hysterolaparoscopy, and endometrial sample was sent for CBNAAT testing in the same sitting. The presence of hydrosalpinx, pyosalpinx, tubo-ovarian masses and dense adhesions, tubercles on laparoscopy and a pale-looking endometrial cavity, tubercles, micropolyps, ulcers, periosteal adhesions, fibrosed ostia, intrauterine synechiae on hysteroscopy were considered features of GTB.

In our setting, the Xpert® *Mycobacterium tuberculosis* complex/rifampin (MTB/RIF) ultra test was performed on the GeneXpert System, which is a qualitative, nested real-time polymerase chain reaction (PCR), *in vitro* diagnostic test for the detection of MTB complex DNA in tissue specimens. When MTB complex is detected, the Xpert MTB/RIF ultra test also detects RIF resistance-associated mutations of the rpoB gene.

Combination of tests and diagnostic algorithm

Ultimately, the diagnosis of FGTB is made by meticulous examination and suitable investigations such as endometrial sampling, radiology (in tubo-ovarian masses) and the use of hysteroscopy and laparoscopy. An algorithm has been developed for diagnosis of female genital tuberculosis in INDEX-TB Guidelines, as an initiative of Central TB Division, Ministry of Health & Family Welfare, Government of India. WHO in 2016 also issued guidelines for the diagnosis and management of FGTB. Every case which is diagnosed and treated is notified under NIKSHAY, a web-based notification system maintained by the Ministry of Health and Family Welfare, Government of India.[7]

Statistical analysis

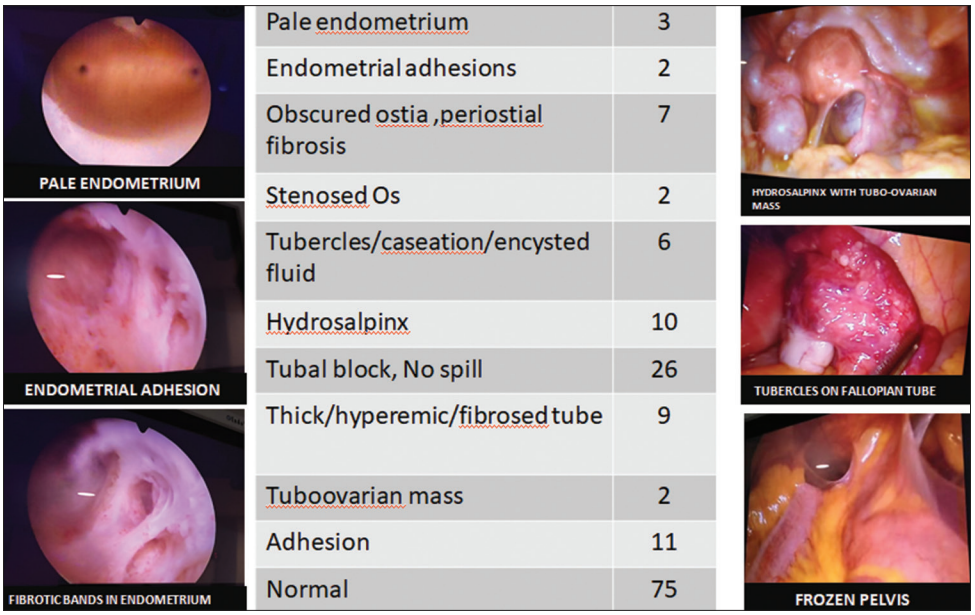
Patients were categorised into different age groups and baseline characteristics were calculated as means ± standard deviation, number percentage [*n*%]. Independent *t*-test was performed to compare age, hormone level (AMH) and ultrasonographic ovarian marker (AFC) and to find the outcome differences in the distribution of values across the different age groups. Statistical analysis including descriptive analysis was performed using the statistical package SPSS (version 23, IBM Corporation, City Armonk, State New York, United States). $P < 0.05$ was considered statistically significant.

Results

A total of 133 women were included in our study between the age groups of 21 and 45 years. All the females were evaluated and distributed amongst two groups. Fifty-eight (43.6%) cases were diagnosed with FGTB based on either Diagnostic Hysterolaparoscopy (DHL) findings and/or CBNAAT result and were included under Group I. Group II had 75 (56.3%) women who were not diagnosed with FGTB.

Diagnosis of FGTB was made based on definite findings or two or more findings suggestive of GTB on hysterolaparoscopy [Figure 1] in 40 (30%) cases. Positive CBNAAT on endometrial sampling was reported amongst 18 (13%) cases. However, both DHL findings and CBNAAT report were positive for FGTB only amongst 15 (11%) cases. Seventy-five cases were negative for FGTB as their DHL findings were normal and CBNAAT was tested negative.

Tubal pathologies and tubal block were seen in 35 (26.3%), hydrosalpinx in 10 (7.5%), tubercles, caseation and encysted fluid in 6 (4.5%) and pelvic adhesions in 11 (8.2%). Hysteroscopy revealed normal endometrium in 75 (56.3%) and pale oligaemic endometrium in 3 (2%) women.



Pale endometrium	3
Endometrial adhesions	2
Obscured ostia ,periostial fibrosis	7
Stenosed Os	2
Tubercles/caseation/encysted fluid	6
Hydrosalpinx	10
Tubal block, No spill	26
Thick/hyperemic/fibrosed tube	9
Tuboovarian mass	2
Adhesion	11
Normal	75

Figure 1: Hysterolaparoscopy findings suggestive of genital tuberculosis

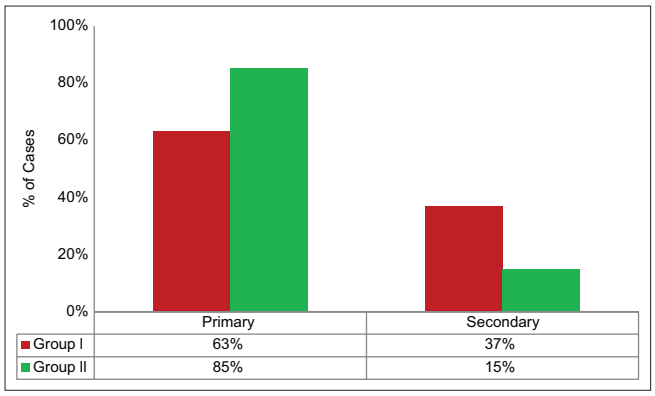


Figure 2: Distribution of cases in Group I and II according to type of infertility

Primary infertility was seen as more prevalent in both the groups. However, secondary infertility was more in Group I when compared to Group II [Figure 2].

Maximum cases with prolonged infertility of more than 10 years were seen in those affected with GTB (41% in Group I). In Group I, with increasing age and married life, the duration of infertility increased and the rate of secondary infertility increased [Figure 3].

Amongst cases with GTB, 24 (41%) were between 30 and 34 years which is the majority group in our study. Sixteen (28%) cases were between 25 and 29 years. Fifteen (25%) cases were above 35 years. Only 3 (5%) cases were between 20 and 24 years [Table 1].

The mean age was 31.71 years (±5.31) in Group I (with FGTB) and 29.89 years (±5.01) in Group II (without FGTB). The mean AMH level was 1.88 ng/ml (±1.52), which was significantly lower ($P < 0.001$) in Group I than in Group II with AMH 3.57 ng/ml (±2.93). The mean AFC was 9.0 (±5.50) in Group I and 12.50 (±6.0) in Group II, which clearly demonstrated that AFC is significantly low ($P < 0.001$) in Group I than Group II [Table 2].

AMH and AFC were found to be in significant positive correlation ($r = 0.68$, $P < 0.001$). We found a significant negative correlation between age and AMH ($r = -0.33$, $P < 0.01$) and AFC ($r = -0.29$, $P < 0.02$) in GTB. AMH and AFC were found to be decreasing with increasing age in different age subgroups. The difference in AMH and AFC in all age subgroups was significant between Group I and Group II [Figures 4 and 5].

Discussion

FGTB presents a diagnostic challenge due to its varied presentation, lack of sensitive and specific diagnostic techniques and its paucibacillary nature. The diagnosis of FGTB should be made based on any one of the following: laparoscopic appearance typical for GTB (caseous nodules, tubercles, pelvic adhesions, pale atrophic endometrium, fibrosed ostia and obscured ostia), any gynaecological specimen positive for AFBs on microscopy or positive for MTB on culture and any gynaecological specimen with findings consistent with genital TB on histopathological examination.[8] Due to the endometrium being shed during menstruation, typical caseative epithelioid granulomas, epithelioid cells and specialised Langhans giant cells may not appear on microscopy.[9]

Even with the availability of recent diagnostics, such as GeneXpert, with high diagnostic sensitivity and

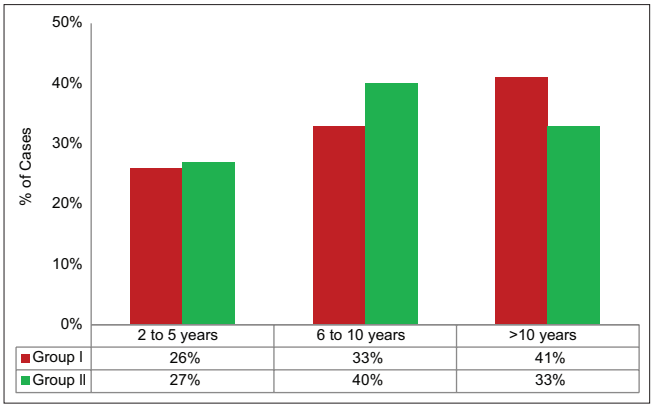


Figure 3: Distribution of cases in Group I and II according to duration of infertility (years)

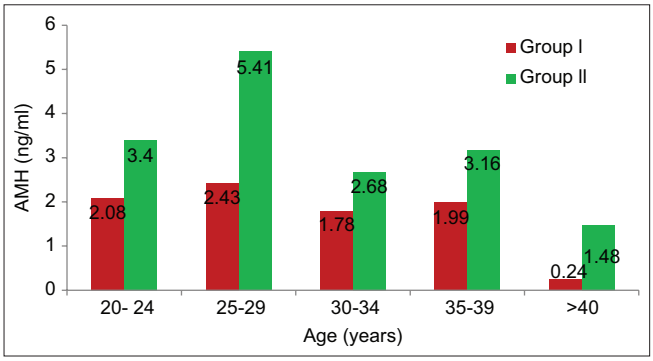


Figure 4: Mean values of anti-Mullerian hormone (ng/ml) in different age subgroups. AMH: Anti-Mullerian hormone

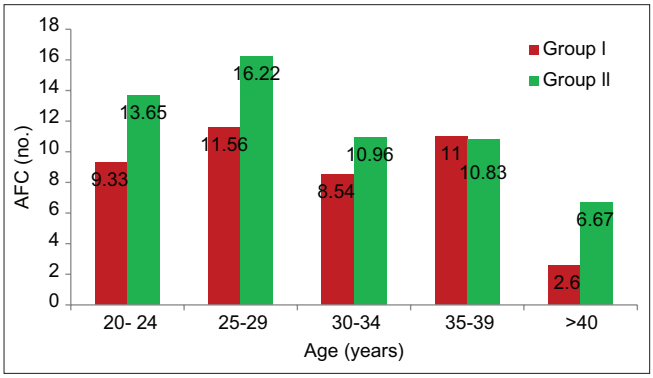


Figure 5: Mean values of antral follicle count in different age subgroups. AFC: Antral follicle count

specificity, diagnosis may still be lower because genital TB is a paucibacillary disease. As a result, most cases of genital TB would probably be identified based on a multimodal approach just like other extrapulmonary TBs. Importantly, there are no established standards for evaluation.[8]

The WHO-approved rapid diagnostic tests are (CBNAAT/GeneXpert MTB/RIF) for the detection of extrapulmonary tuberculosis. A positive CBNAAT result is a useful confirmatory test, but a negative test does not always rule out TB. Sharma *et al.*, 2014, observed GeneXpert to have a 33%–50% sensitivity and 100% specificity for the diagnosis of FGTB.[10]

As ovarian reserve declines with age, it is considered an important determinant for predicting the success of any

Table 1: Age distribution of female genital tuberculosis-positive cases (Group I, *n*=58)

Age group (years)	Genital TB positive, number of cases (%)
20–24	3 (5)
25–29	16 (28)
30–34	24 (41)
35–39	10 (17)
>40	5 (9)
Total	58 (100)

TB=Tuberculosis

Table 2: Comparison of various parameters between cases with female genital tuberculosis (Group I) and those without female genital tuberculosis (Group II)

	Independent sample *t*-test			
	Groups	*n*	Mean±SD	*P*
Age (years)	I	58	31.71±5.31	0.046
	II	75	29.89±5.01	Significant
AMH (ng/mL)	I	58	1.88±1.52	<0.001
	II	75	3.53±2.93	Significant
AFC	I	58	9.33±5.73	0.001
	II	75	12.64±6.12	Significant

AMH=Anti-Mullerian hormone, AFC=Antral follicle count, SD=Standard deviation

Table 3: Mean values of anti-Mullerian hormone (ng/mL) in different age subgroups

	AMH (ng/mL)		
Age (years)	Group I	Group II	*P*
20–24	2.08±1.01	3.4±1.35	0.13
25–29	2.43±1.44	5.41±4.69	0.02
30–34	1.78±1.76	2.68±1.72	0.08
35–39	1.99±0.93	3.16±2.39	0.16
>40	0.24±0.18	1.48±1.19	0.05

AMH=Anti-Mullerian hormone

Table 4: Mean values of antral follicle count in different age subgroups

	AFC (*n*)		
Age (years)	Group I	Group II	*P*
20–24	9.33±6.03	13.65±3.97	0.12
25–29	11.56±4.13	16.22±7.59	0.04
30–34	8.54±5.85	10.96±4.78	0.12
35–39	11±6.63	10.83±6.83	0.95
>40	2.6±1.67	6.67±2.08	0.02

AFC=Antral follicle count

fertility treatment. AMH in the peripheral circulation is derived primarily from antral follicles and represents the ovarian reserve. This makes AMH along with AFC more clinically useful than other ovarian ageing markers currently available, such as inhibin B, estradiol (E2) and follicle-stimulating hormone, which are all menstrual cycle dependent and represent relatively late markers of the ongoing process of primordial follicle pool depletion.[11] Furthermore, AMH, like AFC, has a high sensitivity and specificity and little intra- and inter-cycle variation, making it the most commonly used marker to forecast ovarian response in *in vitro* fertilisation (IVF).[12,13]

It is known that women with low ovarian reserves do not respond well to ovarian stimulation. Patients who are infected by GTB are found to have insufficient AMH levels during ovarian reserve evaluation, especially before IVF. Laparoscopy and hysteroscopy can occasionally show tubal and endometrial involvement caused by tubercle bacilli, but little is known about ovarian involvement. The poor outcomes seen during the intrauterine insemination or IVF cycle, especially in response to ovulation induction, may be caused by MTB's toxic effects on ovarian reserve.[14] The assessment of ovarian reserve is considered one of the routine tests before starting IVF cycle to predict ovarian response.

We used the two most sensitive indicators of ovarian reserve AMH and AFC to assess ovarian reserve and found that infertile women with GTB had substantially lower AMH and AFC than the uninfected group, which suggests a compromised ovarian reserve [Tables 3 and 4].

In our study a clear evidence of declining ovarian reserve is seen with increasing age in women affected with FGTB in different age groups [Figures 6 and 7] similarly as reported in a study done by Gupta and Rai, 2018.[15] They found the median AMH level 1.23 ng/ml which was significantly lower in cases with GTB than controls (AMH: 2.50 ng/ml). Moreover, the median AFC 6.0 (4.0–8.0) was significantly lower in genital TB than control 11.0 (8.25–12.0).[15]

In a study done by Jirge *et al*., 2018, the women in Group I (women with latent GTB diagnosed by DNA PCR testing of an endometrial biopsy) had significantly lower AMH (median: 1.6 [0.7, 2.6] ng/ml vs. 2.3 [1.2, 4.0] ng/ml; $P < 0.001$) and AFC (median: 6 [4, 9] vs. 7 [5, 11]; $P < 0.001$) than those in Group II (women negative for latent GTB by PCR).[4]

In our study, the mean AMH level was 1.88 ng/ml (±1.52), which was significantly lower ($P < 0.001$) in Group I, than in Group II with AMH 3.57 ng/ml (±2.93). The mean AFC was also significantly lower 9.0 (±5.50) in Group I than 12.50 (±6.0) in Group II.

In another study by Malhotra *et al*., 2012, significantly lower values of AFC were found in patients with GTB 10.3 (±2.47) than 11.8 (±2.76) in controls ($P < 0.001$).[16]

Datta *et al*., 2019, also observed that AMH concentration was much lower in PCR positive group than PCR negative with statistical significance.[17]

Malhotra *et al*., 2014 observed a trend of poor ovarian blood flow in patients with genital TB when compared to those without the disease, which was statistically significant. However, the mean value of AMH was found 3.1 (0.63–12.2) ng/ml in women without FGTB (Group I, $n = 185$) and 4.8 (2.6–7.7) ng/ml in women with FGTB (Group II, $n = 69$), which was not significant ($P = 0.21$). There was no significant difference in AFC amongst the two groups 6.5 (3–24), $P = 0.85$. Hence, the huge difference in number of patients

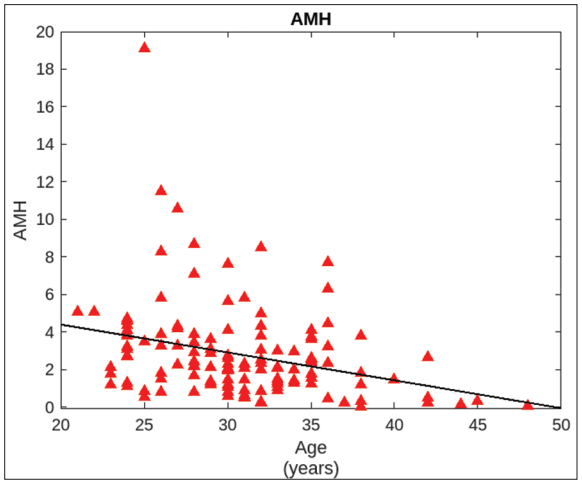


Figure 6: AMH level in different age groups

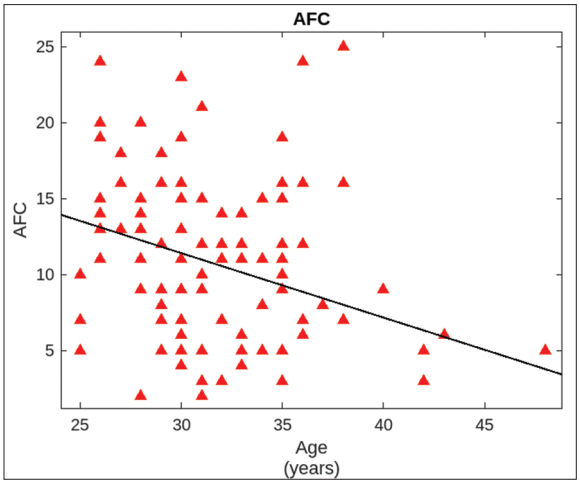


Figure 7: AFC level in different age groups

amongst Group I (n = 185) and Group II (n = 69) could be the cause of non-significant findings.[18]

The results of our study are in concordance with the previously mentioned studies. In some studies, it is seen that AMH, AFC and Doppler parameters have improved when assessed before and after antitubercular treatment. Similar improvements in ovarian response and embryo quality have been observed in women with recurrent implantation failure who have been identified with and treated for latent TB infection, suggesting that after antitubercular treatment, the ovarian environment has improved qualitatively. These results also give us the idea that antitubercular therapy may be able to prevent or even rectify the negative effects of FGTB on ovarian reserve.[19]

Even in a study done by Sharma *et al.,* 2016, it was found that laparoscopic findings were improved. Post-antitubercular treatment findings such as tubercles (in pelvic peritoneum, fallopian tubes and ovaries) and caseous nodules were resolved. However, advanced fibrotic lesions (e.g. pelvic and perihepatic adhesions and bilateral blocked tubes) did not improve with Antitubercular Treatment (ATT).[20]

A timely diagnosis and the implementation of effective antitubercular therapy are, therefore, essential for a better outcome. In addition, awareness amongst clinicians regarding the need for evaluation of ovarian reserve in women with suspected or confirmed FGTB is important.

Limitations

Since this was a time-limited study, we could not assess whether genital TB was the sole cause of infertility. The reassessment of ovarian reserve after antitubercular treatment was not done. Other causes for poor ovarian reserve, such as body mass index, genital infections, drugs and medical disorders, were not taken into consideration.

Conclusion

In women presenting with prolonged duration of infertility and low ovarian reserve, FGTB should also be considered a cause of infertility. These women may experience an accelerated decline in ovarian reserve while being asymptomatic, thus having reduced chances of conceiving naturally.

A high index of suspicion should be kept in cases of prolonged infertility and low ovarian reserve to rule GTB as an aetiology based on either clinical or highly sensitive CBNAAT testing.

Few studies have proven that antitubercular treatment can prevent further decline and even improve ovarian reserve and infertility outcome. Prompt initiation and adherence to ATT may improve further fertility outcome.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data will be made available from the corresponding author reasonable request.

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