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## Research Article

# Altered endometrial receptivity causes failure of IVF/ICSI in cases with tubal factor infertility

## Abstract

**Objectives:** To study the prevalence of non-receptive endometrium in patients with tubal factor infertility and to compare that with patients with infertility due to other causes. Further, to analyse the association between endometrial non-receptivity in patients with tubal factor infertility (cases), and their previous history of diagnosis and treatment of genital tuberculosis.

**Materials and Methods:** We carried out an analytical cross sectional study with 63 patients of recurrent implantation failure, who took ERA test in a period between 01.05.15 to 30.04.17.

For patients aged 40 years or less, own egg IVF and for those aged 40 years ovum donor IVF was carried out.

All patients had HRT cycles. Endometrial Biopsy was taken when endometrial thickness was 7.5mm or more, after proper oestrogen priming and after 5 days of progesterone vaginal pessaries i.e. at P+5 days.

The sample was tested for ERA transcriptomic analyses

Observations were tabulated and analysed.

Patients in whom ERA result showed non-receptive report, were advised to undergo a repeat ERA test to exactly find the receptivity status. Most of the patients denied the repeat test due to time and financial constraints.

**Conclusion:** Tubal damage unilaterally or bilaterally has an impact on endometrial receptivity either due to damage causing causative agent or some unknown (yet to be discovered) chemical released from the fallopian tubes that acts on the endometrium at molecular level. This may be a reason for repeated implantation failures. Thus it is important to consider the cause of tubal damage and treat it before attempting ART.

## Introduction

The human endometrium is a dynamic, highly specialised and hormonally regulated tissue. It is a research ground for scientists. It displays a great range of cyclic complexities of growth and adaptation. Scientists have successfully unravelled the science of reproduction but for the dilemma faced at the endometrium which is the BLACK BOX in the fertility journey.

ART has replaced the other less effective treatments in infertility thus it is of clinical relevance to consider all prognostic factors available for each patient prior to attempting ART.

Tubal factor infertility and peritoneal pathology are common causes with incidence of 30–35% in ART group of

patients. Previous history of septic abortion, ruptured appendix, hydrosalpinx, tubal surgery for ectopic pregnancy suggest tubal damage. The obvious mechanism of infertility involves anatomic abnormalities which prevent the transmission of sperm, ovum and embryo.

Hence by IVF the mechanical disability created by blocked fallopian tubes is overcome in the embryology lab. In fact, IVF was first developed as a method to overcome infertility resulting from irreparable tubal disease, but the IR and PR did not correlate suggesting a more complex approach to treatment of tubal factor infertility.

For a successful implantation, a synchronous dialogue is required between the implanting embryo and the implantation site on the endometrium in a receptive host and at an optimal

time frame. Failed implantation may be caused by other factors such as poor embryo quality, genetic abnormalities or previous pathologies.

The endometrium during this small period acquires a functional and transient ovarian steroid hormone dependant maturity hence permitting blastocyst adhesion. This is called window of implantation and lasts from 12 hrs to 2 days and varies from patient to patient [1]. There are numerous structural and functional changes in the endometrium related to cytoskeletal reorganisation. These are induced by specific genomic signatures [2-4]. The test designed to support is ERA or Endometrial receptivity array. ERA is a customised array analyses the expression of 236 genes selected for endometrial receptivity profile. Analysis of expression of these genes utilizes next generation sequencing in conjunction with a bioinformatics tool which gives information about endometrial receptivity. This test is developed and patented by Igenomix, Spain. (PCT/ES2009/000386). Following ERA report recommendation, does not guarantee implantation but highlights the number of days of progesterone exposure of the oestrogen primed endometrium, to open the window of implantation.

This test enables the clinician to identify the receptivity status of the endometrium regardless of histopathology or embryo by means of bioinformatics [3]. Results are obtained by taking endometrial biopsy at LH+7 in a natural cycle and at P+5 days after proper oestrogen priming in an HRT cycle. It is superior to HPE and the results obtained are reproducible up to 29-40 months.

Recurrent implantation failure/RIF is an agonising condition which leads to failures of ART [5 6]. It is defined as more than or equal to three IVF failures in which one or two morphologically high grade embryos were transferred. The cases of RIF can be grouped into many categories as

- Pathological alteration of endometrial cavity as in hyperplasia, sub mucous myomas, polyps ,endometritis and synechiae-18%-27% [7].
- Hydrosalpinx [8]
- Increased incidence of chromosomal abnormalities [9]
- Lifestyle or hereditary causes or acquired thrombophilia. [10]

The pathological factors are correctable but the underlying implantation problem may persist.

## Aim

To study whether endometrial receptivity could be the cause of failure of IVF/ICSI in cases with infertility diagnosed with tubal factor(s).

## Objectives

- 1) To study the prevalence of non- receptive endometrium in patients with tubal factor infertility and to compare that with patients with infertility due to other causes.

- 2) To analyse whether previous history of diagnosis and treatment of genital tuberculosis is associated with endometrial non-receptivity in patients with tubal factor infertility (cases) in our study.

## Materials and Methods

We carried out an analytical cross sectional study with 63 patients of recurrent implantation failure, who took ERA test in a period between 01.05.15 to 30.04.17.

For patients aged 40 years or less, own egg IVF and for those aged 50years ovum donor IVF was carried out.

Inclusion criteria of patients in the study group

- All patients had an endometrial biopsy to detect genital TB / endometritis.
- All patients who had past history of genital TB, were treated with ATT and a repeat endometrial biopsy was negative for genital TB or endometritis.
- All the patients had undergone other tests to find any other cause of RIF as karyotyping both partners,3D USG to rule out uterine anomalies, tests to rule out antiphospholipid syndrome, tests for familial homocystenemia and thrombophilia
- All underwent ERA test
- All had 3 or more previous failed IVF cycles with about 4 morphologically high grade embryos transferred in total.

Criteria for labelling as Cases (Tubal Factors)

- All patients with RIF with tubal factor infertility i.e.1.tube(s) were blocked as diagnosed by HSG/Laparoscopy.
- Previous salpingectomy for ectopic pregnancy.
- Tubal clipping or occlusion for hydrosalpinx.

Criteria for labelling non-case

- patent fallopian tubes as documented by HSG/Laparoscopy, included POR, PCO, endometriosis, age related and unexplained infertility.

Exclusion criteria

- All cases with myomas, sub mucous polyps and septum, previous difficult ET and atrophic endometrium (5.5 mm).

All patients had HRT cycles. Biopsy was taken by pippelle catheter. Timing of biopsy was calculated after proper oestrogen priming of endometrium which measures more than or equal to 6.5 mm, then after 5 days of progesterone vaginal pessaries i.e. at P+5 days.

After biopsy, the endometrial tissue was transferred into a cryotube containing 1.5 ml endometrial tissue (RNA), vigorously

shaken for a few seconds and kept at 4 degree Celsius or in ice for minimum 4 hours. The sample were transported at room temperature for ERA transcriptomic analyses.

The observations were taken from our records at Southend fertility and IVF centres at Max Smart Saket, Max Gurgaon and Holy Angels hospital Vasant Vihar.

The presence and previous treatment taken of tuberculosis was noted down from the records.

Observations were tabulated and analysed.

Patients in whom ERA result showed non- receptive report, were advised to undergo a repeat ERA test to exactly find the receptivity status. Most of the patients denied the repeat test due to time and financial constraints.

**Interpretation of ERA Analysis result**

**Receptive**

Gene expression profile compatible with receptive endometrium.

**Non- receptive/Pre-receptive**

Endometrium displaced window of implantation showing endometrial lag or increased duration of progesterone.

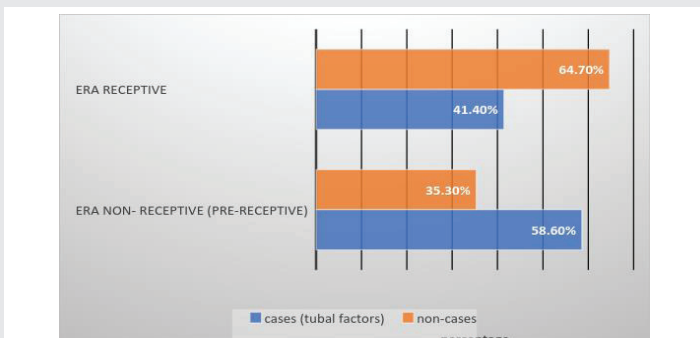
**Non- receptive/Post-receptive endometrium**

Displaced window of implantation due to progesterone excess.

**Observations (Table 1)**

**Table 1:**

Era results	Cases with tubal factor	Non -cases without tubal factor
Era non- receptive (Pre-Receptive)	17	12
Era receptive	12	22
Total	29	34



Prevalence of ERA NR (Pre- R) in tubal factor patients

17/29 (58.6%)

Prevalence of ERA NR in non- cases /without tubal factor

12/34 (35.3%)

**Prevalence ratio of endometrial receptivity problem**

NR (Pre-R) ERA in tubal factor patients Vs non- tubal factor patients

1.66

The prevalence of non-receptive /pre -receptive endometrial receptivity was 1.66 times more in patients with tubal factor infertility in our sample as compared to those with non- tubal causes.

95% CI for difference in two proportions

(-0.76%-47.36%)

p=0.06

p value of difference >0.05

**Interpretation**

Confidence interval is wide and includes zero. This shows that the sample size is probably not adequate and results are not statistically significant.

p value is weakly suggestive of a difference i.e patients with tubal factor have a higher proportion of ERA NON-RECEPTIVITY (PRE- RECEPTIVE) suggestive of an endometrial lag.

So although the higher percentage of ERA Non Receptivity (Pre- Receptive) in tubal cases, suggestive of an endometrial lag, is not statistically significant, it is suggested by the analysis in our sample that NR (Pre R) endometrial receptivity may be as much associated with patients with tubal factor infertility (if not more) as compared to other patients in the study (Table 2).

**Table 2:**

Era results	Pts with GJB &Tubal factor	Pts with Tubal Factor without GTB
Non- Receptive (Pre -Receptive)	7	10
Receptive	10	2

Fischer exact test showed a p value ff 0.05

p value of 0.05 suggests that the higher proportion of patients with tubal factor infertility without genital TB showed an endometrial lag hence delayed endometrial receptivity Vs the patients of tubal factor infertility who had previously been diagnosed and treated for endometrial TB.

This was statistically significant.

**Discussion**

Fallopian Tubes are two, 7-14 cm long passages between ovaries and uterine cavity. In addition to being patent conduits for passage of sperms, oocytes and embryo. They also play various other important functions as ovum pick up by the congested pulsatile fimbriae and providing the essential environment for fertilisation and embryo nourishment by their secretions.

But is that all?

This study found an association between non-receptive endometrium (suggestive of progesterone deficiency) and tubal blockage in patients of RIF. This leads us to speculate that there may be a role of some chemical mediator released from the fallopian tube(s) which signals the arrival of the embryo to the endometrium and helps in opening the window of implantation. This may be in addition to the embryonic signals.

While there is hardly any data available in the literature which highlights the possible role of fallopian tubes in signalling the implantation cascade, some studies can be extrapolated as an indirect support to our hypothesis.

In a study on patients who had undergone tubal sterilisation, age was the only factor that influenced delivery rates significantly. The cumulative delivery rate for patients aged less than 37 years was 52.4% after IVF and 72.2% after reversal ( $P = 0.012$ ), while cumulative delivery rates for patients aged 37 years or older were, respectively, 51.4 and 36.6%, a difference that did not reach statistical significance. Surgical reversal is recommended for patients younger than 37; older patients are advised to opt for IVF [11].

This finding points to the importance of the fallopian tube, suggesting that in young sterilized women, tubal recanalisation may be more successful than IVF/ICSI in achieving pregnancy.

Another study found that high tubal damage grade is associated with low pregnancy rate in women undergoing in-vitro fertilization treatment [12]. This finding could also be suggestive of tubal signalling factor or residual endometritis leading to IVF failure and hence lower pregnancy outcome. Hence, tubal damage grade per se seems to be of importance for treatment outcome.

Our study also found that a higher number of RPL patients of receptive endometrium in RPL patients with tubal factor and treated genital TB.

Our results also speak to the importance of treatment of genital TB. A significantly higher proportion of RPL patients with tubal factor whose genital TB was detected and treated had a receptive endometrium. In our study, we detected GTB/endometritis by subjecting all our patients who to EB. Our results could imply that if the GTB is diagnosed and treated before attempting ART, the endometrial lag would be corrected in majority of the patients.

It could further imply that other probable causes of tubal blockage like chlamydia, *N.gonorrhoeae*, *Mycoplasma hominis*, *E.coli*, *Mobiluncus* and *Bacteroides ureolyticus* should be detected and treated before attempting ART. A study by Lyons et al., 2006 [13] supports this by showing that *Escherichia coli* in the Fallopian tubes of rabbits resulted in a dose-dependent deciliation.

### Limitations

It is a cross sectional study with limited number of patients, so no causality can be inferred although the study does provide

an evidence of some association and possibly generates a hypothesis.

The patients in study population were those who had undergone ERA. As this is an expensive and time consuming test, not all patients of RPL opted for it. This could be a source of potential bias in our study.

There was significantly more TB in tubal factor cases as compared to non-tubal cases ( $p=0.001$ ), 72.4% as against 23.5%. This could have acted as a confounder in our study.

Despite these limitations, our study finds a novel association between these factors which have not yet been researched extensively. Hence, our study forms the basis of further, more robust research to study this association between tubal factor and distorted endometrial receptivity.

### Conclusions

Tubal damage unilaterally or bilaterally has an impact on endometrial receptivity either due to damage causing causative agent or some unknown (yet to be discovered) chemical released from the fallopian tubes that acts on the endometrium at molecular level. This may be a reason for repeated implantation failures. Thus it is important to consider the cause of tubal damage and treat it before attempting ART.

### Recommendations

A bigger multi centric study could be carried out to generate better evidence of association.

We recommend a further case control study to take care of confounders and potential bias. Ideally cases (with tubal factor) and matching controls (without tubal factors), should be selected and all of them should be subjected to ERA test to confirm this hypothesis.

Also, the treating clinician should carry out extensive counselling of the patient with tubal factor infertility undergoing ART highlighting the probability of shift of endometrial receptivity.

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