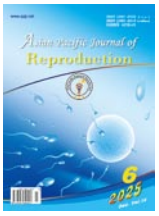

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A retrospective analysis of endometrial receptivity array-personalized embryo transfer and its outcome among women with implantation failure

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ABSTRACT

Objective: To assess whether personalized embryo transfer guided by endometrial receptivity array (ERA) improves implantation and pregnancy rates in women with implantation failure.

Methods: This retrospective case-control study was conducted on women with previous implantation failure. The women were divided into two groups, *i.e*, women who underwent ERA and those who underwent embryo transfer without ERA testing. ERA was performed using Igenomix. ERA results were interpreted as receptive or non-receptive. Women underwent frozen embryo transfer on the 6th day of progesterone (P+5). The primary outcomes were implantation rate, clinical pregnancy rate, abortion rate, and negative pregnancy rate.

Results: This study included 229 women with previous implantation failure, with 154 in the ERA group and 75 in the no ERA group. The mean age of the women of the ERA group was (32.2±4.1) years, and that of the no ERA group was (31.5±4.8) years. Women in the ERA group had a higher implantation rate (60.4%) and clinical pregnancy rate (57.1%) compared to those in the no ERA group (48.0% and 46.7%, respectively). In addition, implantation rate of the non-receptive ERA group was higher than the no ERA group (65% *vs.* 48%), and clinical pregnancy rate was also higher in the non-receptive ERA group than the no ERA group (65% *vs.* 47%). The abortion rate of the no ERA group was 9% and that of the non-receptive ERA group was 10%. 52% no ERA group women and 35% non-receptive ERA group women had negative pregnancy results.

Conclusions: Women who have undergone personalised embryo transfer guided by ERA have a higher clinical pregnancy rate than women who have not after previous implantation failure.

KEYWORDS: Endometrial receptivity array; Window of implantation; Personalized embryo transfer; Implantation failure; *In-vitro* fertilization

1. Introduction

In 2023, the World Health Organization (WHO) estimated that the lifetime prevalence of primary infertility was 9.6% and the period prevalence of primary infertility was 9.0%[1]. The advances in assisted reproductive technology (ART) have allowed the infertile

Key Points

Question: Does personalized embryo transfer guided by endometrial receptivity array (ERA) improve implantation and pregnancy outcomes in women with implantation failure?

Findings: In a retrospective study involving 229 women, those who underwent personalized embryo transfer based on ERA results had a higher clinical pregnancy rate (57.1%) and implantation rate (60.4%) compared to those who did not undergo ERA (46.7% and 48.0%, respectively). Notably, the non-receptive ERA group after personalized embryo transfer based on ERA had better outcomes than the no ERA group.

Meaning: ERA-guided personalized embryo transfer may improve clinical pregnancy and implantation rates, providing a more personalized approach to *in-vitro* fertilization treatment for women with implantation failure.

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couple to achieve a viable pregnancy.

Though there have been great advances in ART techniques leading to increased fertilization rates, pregnancy rates have remained fairly unchanged[2]. The likelihood of successful implantation after ART is determined by a multitude of factors, including female-related factors such as age, hormonal levels, endometrial and uterine status, embryo-related factors such as embryonic cleavage speed, euploidy, previous implantations of sibling embryos, male factors like genetic disorders, and external factors such as the performance of the laboratory and clinic and transfer policies[3].

Endometrial receptivity is defined as the period of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently invade the endometrial stroma and vasculature[4]. Following ovulation, progesterone further changes the endometrium to create an environment receptive to implantation and finally to maintain an early pregnancy[5].

Successful embryo implantation requires an appropriate embryonic development, coincident with a receptive endometrium. In humans, the uterus becomes receptive during the mid-luteal phase of the menstrual cycle (days 19–23), commonly known as the window of implantation[6]. Window of implantation refers to the period of maximal receptivity of the endometrium for the blastocyst and corresponds with the time of peak progesterone secretion. During this period, the endometrium shows a specific gene expression profile suitable for endometrial function evaluation[7].

Implantation failure describes the situation in which a good-quality embryo has been transferred into the uterine cavity but has failed to establish a pregnancy. Implantation failure occurring more than once is termed recurrent implantation failure[3].

While the ART technique helps us overcome most infertility problems, implantation remains the rate-limiting step for the success of *in-vitro* fertilization (IVF)[8]. The endometrial receptivity array (ERA) was developed as a tool to pinpoint the window of implantation, thus maximizing endometrial receptivity and improving successful embryo implantation rates[9]. ERA is a molecular tool which can accurately predict endometrial receptivity status by transcriptomic analysis of 238 genes obtained by endometrial biopsy on progesterone+5 in hormonal replacement therapy cycle. Following analysis, a specific computational predictor classifies the samples according to their expression profile as receptive or non-receptive. The non-receptive endometrium is further classified as pre- or post-receptive, meaning that the endometrium has not reached the receptive phase yet or has already passed it[10].

ERA helps in better identification of each participant's personalized window of implantation and harnesses this knowledge to optimize IVF outcomes.

This study seeks to highlight the potential impact of ERA on clinical practice, participant outcomes, and the field of ART. So, our study aims to assess whether personalized embryo transfer (pET) after ERA improves implantation and pregnancy rates in women of IVF.

2. Methods

2.1. Study design and setting

The present research work was a retrospective case-control study conducted between August 2024 to March 2025 at the GarbhaGudi IVF centre.

An electronic-institutional database screening search of Medics software and also a manual file search were done at all the centres to find out the women who have undergone ERA and women with previous implantation failure.

2.2. Study participants

In our study, we included 229 women. We have calculated the sample size using the formula for comparing two independent proportions. Based on preliminary data from similar studies, we estimated a moderate effect size of 0.4 (Cohen's d) for the difference in clinical pregnancy rates between the ERA-guided pET group and the no ERA group[11]. Other parameters included a statistical power of 80% and a significance level of 0.05.

All women who underwent ERA testing were categorized as cases (n=154), and women without ERA testing were categorized as the control group (n=75). The samples collected from January 1, 2018, to December 31, 2023, were screened for inclusion and exclusion criteria. In our study, we included women of age between 25-40 years, body mass index (BMI) 17-35 kg/m^2, and endometrial thickness of >7 mm. All the women had undergone euploid embryo transfer after pre-implantation genetic testing (PGT), frozen embryo transfer (FET) with self and donor gametes, and had previously experienced 1/>1 implantation failure with morphologically good quality embryos. Women with infections like pelvic inflammatory disease and hydrosalpinx, reproductive-tract malformation, acquired uterine-cavity abnormalities like synechia, autoimmune factors, endometrial hyperplasia, and couples with karyotype abnormalities were excluded from the study.

The primary outcomes studied were implantation rate, clinical pregnancy rate and miscarriage rate following pET, and the secondary outcome studied was the proportion of displaced window of implantation.

2.3. Endometrial biopsy and ERA

Women who opted for ERA underwent an endometrial

biopsy in a programmed endometrial preparation cycle. The endometrium was prepared with micronized estradiol *via* oral and transdermal routes. A transvaginal ultrasound was performed to assess the endometrium after 7-10 days of estradiol, and progesterone was started once the endometrial lining was ≥7 mm and a trilaminar pattern was observed. Progesterone preparations used included vaginal micronized progesterone and/or intramuscular progesterone in oil and/or oral synthetic progesterone. Progesterone was initiated on the morning of day 1. Endometrial biopsy was performed with a suction pipelle on the 6th day of progesterone (P+5). The biopsy specimen was processed as per the manufacturer's protocol, and the ERA was performed using Igenomix. ERA results were interpreted as receptive or non-receptive (pre-receptive or post-receptive). Early-receptive (+12 hours) and late-receptive (-12 hours) results were classified as non-receptive.

2.4. FET

Women undergoing FET with standard timing had endometrial preparation as described in the mock cycles with FET on the 6th day of progesterone (P+5). In women who opted to have the ERA performed, endometrial preparation for FET involved the same estrogen and progesterone protocol as their ERA cycle. In women with non-receptive ERA results, the duration of progesterone was adjusted according to their ERA results; the duration of progesterone increased for a pre-receptive ERA and decreased for a post-receptive ERA according to specifications provided. If the ERA result was determined to be receptive, then the transfer was done with the same duration of progesterone as their mock cycle. All FETs were performed under abdominal ultrasound guidance as per the institutional norms.

2.5. Statistical analysis

Data were collected and analysed using coGuide REAP software version 2.0[12]. The dataset was first assessed for normality to determine the distribution of variables. Based on these distributional characteristics, appropriate statistical tests were applied. The *Chi*-square/Fisher's exact test was used to test the statistical significance of cross-tabulation between categorical variables. An independent *t*-test was used to compare the mean±standard deviation (mean±SD) of continuous variables between the two groups for normally distributed variables and the Mann-Whitney *U* test for non-normally distributed variables. Continuous variables were expressed as mean±SD for normally distributed data and median (IQR) for non-normally distributed data, while categorical variables were summarized as frequencies and percentages. A *P*-value < 0.05 was considered statistically significant.

2.6. Ethics statement

This study was approved by the GarbhaGudi Ethics Committee, affiliated with GarbhaGudi Institute of Reproductive Health & Research. Ethical clearance was obtained under approval number: GGIRHR/24/RI-2/18 dated 20-08-2024. All study procedures adhered to the principles outlined in the Declaration of Helsinki and the Indian Council of Medical Research (ICMR) ethical guidelines for biomedical research.

3. Results

3.1. Baseline characteristics

A total of 229 women were included in the study. The mean age and BMI of the women in the ERA group were (32.2±4.1) years and (25.8±4.8) kg/m^2, respectively, and in the no ERA group were (31.5±4.8) years and (27.1±4.8) kg/m^2, respectively. BMI between the ERA group and the no ERA group was statistically significant (*P*=0.04). Women of the ERA group were married for 6 (4, 9) years, and women of the no ERA group were married for 6.5 (4.5, 10.5) years. Menstrual length in days and number of previous failed embryo transfer for women of the ERA group were 30 (30, 32) and 1.7±0.7, respectively, and of the no ERA group were 30 (30, 35) and 1.3±0.5, respectively. The number of previous failed embryo transfer between the ERA group and the no ERA group was statistically significant (*P*<0.001) (Table 1).

3.2. Reproductive outcomes

The ERA group reported an implantation rate of 60.4% (*n*=93), a clinical pregnancy rate of 57.1% (*n*=88), and an abortion rate of 4.5% (*n*=7). The no ERA group had an implantation rate of 48.0% (*n*=36), a clinical pregnancy rate of 46.7% (*n*=35), and an abortion rate of 9.3% (*n*=7). Among 154 women of the ERA group, 12 (7.8%) opted for a PGT to check the ploidy status of the embryo. For the remaining 142 women (92.2%) of the ERA group, a morphological selection was done to select the best embryos and

Table 1. Baseline parameters of two groups.

Parameters	ERA (*n*=154)	No ERA (*n*=75)	*P*-value
Age, years	32.2±4.1	31.5±4.8	0.23
Married life, years,	6 (4, 9)	6.5 (4.5, 10.5)	0.20
BMI, kg/m^2	25.8±4.8	27.1±4.8	0.04
Menstrual length, days	30 (30, 32)	30 (30, 35)	0.23
Number of previous failed embryo transfer	1.7±0.7	1.3±0.5	<0.001

Data are expressed as mean±SD or median (IQR). ERA: endometrial receptivity array; BMI: body mass index.

Table 2. Comparison of reproductive outcomes of two groups.

Parameters	ERA (n=154)	No ERA (n=75)	P-value
Pre-implantation genetic testing (PGT)			
Number of morphologically screened transferred embryos	142 (92.2)	73 (97.3)	0.15
Number of PGT transferred embryos	12 (7.8)	2 (2.7)	
Implantation rate	93 (60.4)	36 (48.0)	0.08
Clinical pregnancy rate	88 (57.1)	35 (46.7)	0.14
Abortion rate	7 (4.5)	7 (9.3)	0.24
Negative pregnancy	61 (39.6)	39 (52.0)	0.08

Data are expressed as n(%). ERA: endometrial receptivity array.

Table 3. Comparison of reproductive outcomes between the no ERA group and the non-receptive ERA group.

Outcomes	No ERA (n=75)	Non-receptive ERA (n=51)	P-value
Implantation rate	36 (48)	33 (65)	0.06
Clinical pregnancy rate	35 (47)	33 (65)	<0.05
Abortion rate	7 (9)	5 (10)	>0.99
Negative pregnancy	39 (52)	18 (35)	0.06

Data are expressed as n(%). Out of 154 women who underwent personalized embryo transfer guided by endometrial receptivity array (ERA), 51 women had non-receptive ERA.

planned pET based on the window of implantation as per the ERA results. Reproductive outcomes were compared and found to be similar in both the ERA and no ERA groups women, as shown in Table 2.

3.3. Comparison of the no ERA group and the non-receptive ERA group after pET

Out of 154 women who underwent pET guided by ERA, 51 women had non-receptive ERA. Table 3 compares the reproductive outcome of the no ERA group and the non-receptive ERA group. According to it, the implantation rate of the non-receptive ERA group was higher than the no ERA group, *i.e*, 65% and 48%, respectively, and the clinical pregnancy rate was also higher in the non-receptive ERA group than the no ERA group, *i.e*, 65% and 47%, respectively. Clinical pregnancy rate between the the no ERA group and the non-receptive ERA group was statistically significant (P<0.05).

The abortion rate of both groups was almost the same, *i.e*, 9% for the no ERA group and 10% for the non-receptive ERA group. 52% no ERA group women and 35% non-receptive ERA group women have undergone a negative pregnancy.

4. Discussion

This was a retrospective study, in which we included 229 women, among whom 154 were in the ERA group, and 75 were in the no ERA group. PGT was performed in 12 women of the ERA group and 2 women of the no ERA group. Implantation rate and clinical pregnancy rate were higher in women with the ERA group, and abortion rate and negative pregnancy rate were lower in the ERA group than in the no ERA group. This signifies that ERA increases the chances of clinical pregnancy and live birth.

Our study also shows that the women with non-receptive ERA have a higher implantation rate and clinical pregnancy rate than the no ERA group. Women in the no ERA group have experienced more negative pregnancies than the non-receptive ERA group, and the abortion rate is the same in both groups. This signifies that the non-receptive ERA group also has better reproductive outcomes than the no ERA group.

There were a few retrospective studies that assessed the efficacy of ERA for repeated implantation failure. In a study by Bergin *et al*, the clinical pregnancy rate was 61.65% and 61.76%, live birth rate was 49.62% and 54.96% and miscarriage rate was 8.27% and 6.52% in the ERA and no ERA groups, respectively[13]. In a study by Amin *et al*, the clinical pregnancy rate was 23.0% and 48.4% in the no ERA and ERA groups, respectively. In women with the non-receptive ERA group, implantation rate and clinical pregnancy rate were higher in women who had undergone embryo transfer on progesterone (P)+6, *i.e*, 69.2% and 61.5%. The abortion rate was the same in non-receptive ERA, *i.e*, 11.1%[11]. In our study, clinical pregnancy rate was 57.1% and 46.7% in the ERA and no ERA groups. In women with the non-receptive ERA group, implantation rate was 65%, the clinical pregnancy rate was 65% and the abortion rate was 10%.

Riestenberg *et al* had assessed the ERA in the women undergoing first embryo transfer. They observed clinical pregnancy rate was 65.4% and 67.4% and the live birth rate was 55.6% and 56.5% in the no ERA and ERA group, respectively. Clinical pregnancy rate was 69.00%, and live birth rate was 60.92% in the non-receptive ERA group, respectively[14]. A retrospective study by Bahgat *et al* in women with a displaced window of implantation and pET showed a clinical pregnancy rate of 57.0% and livebirth rate of 45.5%[15]. The results were quite similar to the studies having a history of implantation failure and to our study as well.

Another study by Mahajan *et al* divided women into two groups– Group 1 women with recurrent implantation failure undergoing ERA

followed by pET and group 2 women with 1 previous implantation failure followed by pET based on ERA report. The results showed ongoing pregnancy rates of 44.5% and 14.3% in groups 1 and 2, respectively, and concluded that pregnancy rates were better after pET in women of recurrent implantation failure[10]. A multicentre clinical trial by Ruiz-Alonso *et al* investigated the outcomes of clinical pregnancy rate and implantation rate among women with receptive ERA followed by pET. The results indicate 50% clinical pregnancy rates and 38.5% implantation rates[16]. Another study by Tan *et al* showed that women with at least one previously failed euploid embryo transfer found that implantation and ongoing pregnancy rates were higher, 73.7% *vs*. 54.2% and 63.2% *vs*. 41.7%, respectively, compared to women without pET[17]. A retrospective observational multicenter cohort study by Cozzolino *et al* showed no improvement in clinical pregnancy rate and live birth rate in the pET group following ERA[18].

Few systematic reviews and meta-analyses were performed to assess the efficacy of pET by ERA. In one systematic review by Liu *et al*, there was no difference in ongoing pregnancy rate or live birth rate between women undergoing routine ET without ERA test and those who followed pET with ERA in relatively good-prognosis infertile women. The meta-analysis revealed that ongoing pregnancy rate/live birth rate of women with recurrent implantation failure undergoing pET who had non-receptive ERA increased to the level of those undergoing standard embryo transfer with receptive ERA[19]. In another systematic review and meta-analysis byArian *et al*, the live birth or ongoing pregnancy rate for the ERA group was not significantly different compared with the no ERA group. The rates of implantation, biochemical pregnancy, clinical pregnancy, and miscarriage were also comparable between the ERA group and the no ERA group[20].

As implantation failure is a major challenge for women undergoing IVF, our study can act as evidence that proves the use of ERA to personalize embryo transfer based on endometrial receptivity may offer a more targeted approach, improving the chances of successful implantation and pregnancy. Additionally, personalized medicine is an emerging trend in fertility treatments, and the study could contribute to the growing body of evidence supporting individualized approaches.

Limitations of our study are the variability in participant populations, as implantation failure can result from various factors beyond endometrial receptivity, such as embryo quality, genetic factors, or immune issues, which might not be fully controlled. The other limitation is a nonrandomized study design. Because women elected for or against the ERA and were not randomized, it is possible that women who elected to undergo the ERA differed fundamentally from those who did not.

In conclusion, ERA can offer an individualized approach to the identification of the window of implantation and embryo transfer. Our study concludes that women undergoing ERA have a higher implantation rate, higher clinical pregnancy rate, and lesser abortion rate than the women who have not undergone ERA. As there are a smaller number of studies that reveal the significant role of ERA in achieving better clinical outcomes, there is a need for more such studies with a greater sample size in the Indian population.

Conflict of interest statement

The authors declare no conflicts of interest.

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Authors’ contributions

Dr Asha S Vijay made substantial contributions to the conceptualization of the study, critically reviewed the study and performed the final revision of the manuscript. Dr Raajam Murali SR contributed to the study design, supervised the data-collection process, and critically reviewed the study. Dr Pallavi R Gangatkar was responsible for data collection, drafted the manuscript, and performed the literature review. Dr Deepthi Venkatesh assisted in the literature review and contributed to the drafting and formatting of the manuscript. Dr Shweta Ashok Harnal supported the literature review and helped draft and format the manuscript. Dr Tanvi Khandekar conducted the statistical analysis and prepared the tables. All authors read and approved the final manuscript.

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