Personalized Embryo Transfer Outcomes in Recurrent Implantation Failure Patients Following Endometrial Receptivity Array With Pre-Implantation Genetic Testing

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Abstract

Introduction
Implantation failure is a trending problem for pregnancy outcomes. Women’s reproduction rates can increase by in-vitro fertilization, which comes with frequent implantation failures. These failures can be mitigated by the personalization of embryo transfer depending on the patient’s implantation window. The study aimed to assess the importance of using an endometrial receptivity array (ERA) combined with pre-implantation genetic testing in patients with recurrent implantation failure (RIF) and the significant role of personalized embryo transfer (PET) after ERA in patients with a displaced window of implantation. The study also determined the efficacy of this approach in improving clinical outcomes.

Methods
We conducted this observational retrospective study following approval by the Ethics Committee of Wings In-Vitro Fertilization (IVF) Women’s Hospital, a unit of Reveba Infertility Clinics Pvt. Ltd., Ahmadabad (Approval No. 2019/002/31B). Two hundred ninety-one RIF patients were recruited and categorized into Group I (patients without ERA group) and Group II (ERA study group). Patients in the ERA study group were screened for ERA and subclassified into receptive and nonreceptive ERA groups. PET was performed for all subjects in the ERA study group according to their receptivity as assessed by ERA. We also screened some of the patients for ploidy (genetic) status of embryos by pre-implantation genetic testing for aneuploidy (PGT-A) before embryo transfer. The study had a power of 95% and an alpha of 0.05; therefore, 80 ± 2 subjects were required to conduct the study.

Results
The primary outcome was the clinical pregnancy rate followed by the implantation rate. We found an improved clinical pregnancy rate and implantation rate (73.5% and 78.6%) in the nonreceptive endometrial group after adjusting their embryo transfer schedule to their endometrial receptivity. The clinical pregnancy rate (64% and 65%) and implantation rate (65% and 74%) in receptive and nonreceptive ERA (respectively) were high in subjects with donor oocytes for IVF/intracytoplasmic sperm injection. In addition, patients who opted for PGT-A to eliminate the risk of transferring aneuploid embryos had significantly better implantation (88% and 95% receptive and nonreceptive, respectively) and clinical pregnancy rates (100% in both groups) compared to non-PGT-A screened patients (p<0.05; 34% and 37% clinical pregnancy rate, 96% and 57% implantation rate in receptive and nonreceptive groups, respectively).

Conclusion
Endometrial receptivity assessment is a highly beneficial method to assess the genetic expression of the endometrium and embryo transfer timing. In our study, in patients with recurrent implantation failure, this technology found receptivity issues and provided a chance to plan embryo transfer according to the window of implantation. The combination of PGT-A with ERA rules out the genetic issues related to embryos. In RIF patients, ERA results-guided PET improved the implantation rate and reproductive outcomes.

Introduction
In humans, implantation is the stage of reproduction at which healthy embryos adhere to the wall of the
receptive endometrium. The basic and frequent reason for implantation failure is aneuploidy embryos that lack developmental competency [1]. The implantation failure of euploid embryos determines a non-embryonic source with endometrial receptivity problems, representing another crucial cause of failure [1]. The window of implantation (WOI) is not universal for all women—it varies by individual. Studies observed displaced WOI in one of four recurrent implantation failures (RIFs) [2,3]. In assisted reproductive technology (ART) cycles, embryo transfer is synchronized with the WOI. The prognosis of the cycle should be monitored carefully from clinical and laboratory aspects.

In some cases, even though the patient has good endometrium and good-quality embryos, implantation is difficult to achieve. In such cases, two major issues must be assessed. The first is embryo ploidy status by pre-implantation genetic testing for aneuploidy (PGT-A), and the second is endometrial receptivity by endometrial receptivity array (ERA). PGT-A detects whole chromosome aneuploidy and potentially increases implantation and live birth rates, and decreases the rate of early pregnancy failure [4]. RIF is a significant indication for ERA, even after transferring euploid embryos. The determination of the WOI will improve implantation success in a subsequent cycle with personalized embryo transfer (PET). PET is performing an embryo transfer when the endometrium is in the optimal receptive stage [5]. This study aimed to assess the role of ERA and any beneficial effects of PET in improving clinical pregnancy rates (CPR) and implantation rates (IR) in RIF patients. The study also aimed to delineate the CPRs between receptive and nonreceptive ERA combined with PGT analysis in RIF patients.

Materials And Methods

Study design, location, and setup

We conducted this observational retrospective study of RIF patients who underwent ERA from December 2019 to August 2021 at the Wings In-Vitro Fertilization (IVF) Women’s Hospital, a unit of Reveba Infertility Clinics Pvt. Ltd., Ahmadabad. We retrieved patient data retrospectively from the clinic’s MediTEX software (MediTEX IVF, Regensburg, Germany). The study included patients with more than two unsuccessful fresh/frozen embryo transfers with two morphologically/PGT-A normal embryos. We included both self and donor cycles. The study excluded patients with confounding factors such as hydrosalpinx, endometrial polyp, submucous fibroid, immunological disorders, previous difficult embryo transfer, and thin endometrial thickness (i.e., < 6 mm) after hormone replacement therapy. Figure 1 presents the study design flowchart.

FIGURE 1: Study design flowchart

ERA: endometrial receptivity array; PGT-A: pre-implantation genetic testing for aneuploidy

Total patients recruited; N=291

Group I (Control group without ERA); n=72

Group II (ERA Group); n=219

ERA: (n=219)

PGT-A + ERA: (n=64)

Self cycle (n=122)

Donor cycle (n=97)

Receptive (n=178)

Nonreceptive (n=41)

Receptive + PGT (n=42)

Receptive Non-PGT (n=136)

Nonreceptive (n=74)

Nonreceptive + PGT (n=52)

Nonreceptive Non-PGT (n=19)
Participants were categorized into two groups: Group I consisted of RIF patients without ERA (n=72, control group), and Group II consisted of RIF patients with ERA (n=219; Study ERA groups). The study's primary outcome measures were IR, CPR, and abortion rate (AR). The clinical outcomes of the control group (without ERA) and ERA study group and subgroup's data were compared and statistically analyzed. The study was approved by the Ethical Committee of Wings IVF Women’s Hospital (Approval No. 2019/002/31B).

ERA protocol
All patients in the ERA study group started the ERA protocol on day two or day three of their menstrual cycle. Patients were advised to take one estradiol valerate tablet (2 mg) three times daily for four days, followed by twice daily for eight days. On the twelfth day of the regimen, the patient was called for endometrial assessment, hysteroscopy, and ERA. We added progesterone injections (100 mg, six injections total) on the 13th day of the regimen (considered as day zero). ERA was performed on the last day (P+5) of injection. After five days of progesterone injections, the first endometrial biopsy was collected from the uterus fundus and assessed for ERA transcriptomic analysis. According to Ruiz-Alonso et al., the ERA “consists of a customized array containing 238 genes expressed at the different stages of the endometrial cycle and is coupled to a computational predictor that can identify the receptivity status of an endometrial sample and diagnose the personalized WOI […] of a given patient regardless of the sample’s histological appearance” [5,6]. The endometrial gene expression profile was processed, and the ERA computational predictor diagnosed endometrial receptivity. The ERA test results were categorized into receptive ERA and nonreceptive ERA, conveyed in terms of hours based on the day of progesterone administration (i.e., 80 to 90 hrs [+3 hrs], 90 to 110 hrs [+3 hrs], 110 to 150 hrs [+3 hrs], 140 to 150 hrs [+3 hrs], and 150 to 160 hrs [+3 hrs] as P+3, P+4, P+5, P+6, and P+6 transfer, respectively).

Pre-implantation genetic testing and embryo screening
At Wings IVF Women’s Hospital, Ahmadabad, all embryo biopsy samples were processed and assessed by next-generation sequencing (NGS) at a single standard laboratory (Igenomix, New Delhi). PGT-A determined the status of the embryo into euploidy, aneuploidy, and mosaicism. The VeriSeq NGS (Illumina, San Diego, CA) platform was used to analyze the NGS result to detect abnormal chromosome segments as small as 1.5 million base pairs. According to the biopsy, NGS screening, and analysis (and according to copy variations), euploid embryos were a chromosome with two copies, monosomy is a chromosome with one copy, and trisomy is a chromosome with three copies. Any value between one and two or two and three was classified as a mosaic. More than 80% of mosaicism is categorized as aneuploidy, ~20% is euploid, and 20% to 80% are classified as a mosaic. After ERA testing, PGT-A was done, and the clinical pregnancy of respective patients was confirmed by assessment of β-subunit of human chorionic gonadotropin level after 15 days of embryo transfer.

Statistical analysis
To analyze the data, IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. was used. The number of participants should be at least 40 (80 in total) to assure 95% of the power of the study as the lower boundary of 95% one-sided confidence interval between the differences in the combination of Receptive ERA and PGT and PGT alone groups in the parameters of clinical pregnancy rate and implantation rate. Pregnancy outcomes, including IR and CPR, were statistically compared between receptive and nonreceptive patient groups and PGT-A and without PGT-A testing using chi-squared analysis. We considered p≤0.05 as statistically significant. We drew statistical interpretations in comparison by multivariate analysis between Group I and Group II and determined their significance.

Results
Demographics of the study group
Two hundred ninety-one patients were included in the study (Table 1). Group I (the control group) contained 72 participants with a mean age of 35 ± 4.5 years, mean body mass index of 26.4 ± 1.3 kg/m2, and mean active marriage life of 5 ± 1.6 years. The control group and Group II subjects had a mean implantation failure of 2.2 ± 0.9 and 2.4 ± 0.8, respectively.
**Patient Demographics**

|                  | Group I/Control Group; n=72 (Mean ± SD) | Group II/ERA Group; n=219 (Mean ± SD) |
|------------------|-----------------------------------------|---------------------------------------|
| Average female age (years) | 35 ± 4.5                                | 36 ± 4.2                              |
| BMI              | 26.4 ± 1.3                               | 27.5 ± 1.1                            |
| Active marriage life (years) | 5 ± 1.6                                 | 5 ± 1.2                               |
| Average no. of failures | 2.2 ± 0.9                               | 2.4 ± 0.8                             |

**TABLE 1: Study population demographics**

BMI: body mass index; ERA: endometrial receptivity array; SD: standard deviation.

**Reproductive outcomes of Group I and Group II**

Patients in Group I reported a CPR of 23% (n=17), an IR of 4.1% (n=3), and an AR of 17% (n=13). Patients in Group II had a CPR of 48.4% (n=106); an IR of 50.6% (n=111), and an AR of 2.2% (n=5). Among 219 patients, 64 (29.2%) opted for a PGT-A with ERA to check the ploidy status of the embryo. For the remaining 155 patients (70.7%), a morphological selection was done to select the best embryos and planned PET based on the WOI as per the ERA results. Reproductive outcomes were significantly better in Group II patients than in Group I patients (p=0.05; Table 2).

**TABLE 2: Reproductive outcomes comparisons**

PGT: pre-implantation genetic testing; ERA: endometrial receptivity array

**CPR and IRs in receptive and nonreceptive ERA groups**

Among 219 patients assessed for ERA, 178 were receptive, and 41 were nonreceptive as per ERA results. Of the 178 receptive ERA patients, 83 reported positive pregnancy 15 to 18 days after embryo transfer, and all had confirmed implantation (47%). However, in the nonreceptive ERA group, patients were regrouped under three headings according to their PET timing. The only patient with P+3 turned positive and showed successful implantation. The second group was P+4 (n=27), where 18 (66.6%) had implantation. The third group was P+6 (n=13), where nine (69.2%) had implantation. For all ERA nonreceptive patients, the total IR was 28 of 41 (68%; Table 3), higher than the ERA receptive group but not significantly higher. This trend is the same, with a CPR of 45.5% compared to 60.9% in the ERA receptive and nonreceptive patients. We found no significant difference in AR between the ERA receptive and nonreceptive groups (Table 3).
TABLE 3: Clinical outcomes in ERA receptive and ERA nonreceptive patients

| Group                  | PET | IR, n (%) | CPR, n (%) | AR, n (%) | Negative Pregnancy, n (%) |
|------------------------|-----|-----------|------------|-----------|---------------------------|
| Receptive (n=178)      | P+5 (n=178) | 83 (47%) | 81 (45.5%) | 2 (2.4%) | 95 (53.3%)                |
|                        | P+3 (n=1)     | 1 (100%) | 1 (100%)   | 0 (0%)    | 0 (0%)                    |
| Nonreceptive (n=41)    | P+4 (n=27)    | 18 (66.6%) | 16 (59.2%) | 2 (11.1%) | 9 (33.3%)                 |
|                        | P+6 (n=13)    | 9 (69.2%)  | 8 (61.5%)  | 1 (11.1%) | 4 (30.7%)                 |

ERA outcome in donor and self-oocyte cycles

We also analyzed data by respective type of gametes (self-gametes, n=122; egg donor cycles, n=97) transferred into the patient and their respective IRs. The IRs are high in the donor gametes group (64.8% in the ERA receptive group, 73.9% in the ERA nonreceptive group) compared to the self-gametes (33.6% in the ERA receptive, 61.1% in the ERA nonreceptive group) regardless of endometrial receptivity (Table 4). Likewise, the CPRs were 64% in the donor group and 33% in the self-gamete groups among the ERA receptive participants and 65% in the donor versus 56% in the self-gamete groups in the ERA nonreceptive participants.

TABLE 4: Clinical outcomes between self and donor cycles in ERA receptive and ERA nonreceptive patients

| Study Group             | IR, n (%) | CPR, n (%) | AR, n (%) |
|-------------------------|-----------|------------|-----------|
| Receptive donor (n=74)  | 48 (65%)  | 47 (64%)   | 1 (1.3%)  |
| Receptive self (n=104)  | 35 (34%)  | 34 (33%)   | 1 (0.9%)  |
| Nonreceptive donor (n=23)| 17 (74%) | 15 (65%)   | 1 (4.3%)  |
| Nonreceptive self (n=18)| 11 (61%) | 10 (56%)   | 2 (11.1%) |

CPRs in patients of ERA combined with PGT-A

Prenatal genetic screening was advocated for all the subjects; however, a minority of patients (29.2%) opted for PGT-A, whereas most (70.7%) opted for embryo selection based on the morphological criterion of embryos for transfer (Table 5). Interestingly, embryos screened by PGT-A and optimally transferred into the recipient resulted in high IR in both the groups, regardless of endometrial receptivity. The primary clinical outcomes, including IR and CPR for receptivity and nonreceptivity with PGT-A, were more significant and showed better results in positive pregnancy rates than without PGT-A (p<0.05; Table 5).

TABLE 5: Pregnancy outcome in PGT-A patients and Non-PGT-A patients

| Content | Receptive + PGT-A (n=42) | Receptive –PGT-A (n=136) | P-value | Nonreceptive +PGT-A (n=22) | Nonreceptive - PGT-A (n=19) | P-value |
|---------|--------------------------|---------------------------|---------|-----------------------------|----------------------------|---------|
| CPR     | 37 (88%)                 | 46 (34%)                  | 0.008   | 21 (95%)                    | 7 (37%)                    | 0.08    |
| IR      | 37 (88%)                 | 44 (96%)                  | 0.005   | 21 (100%)                   | 4 (57%)                    | 0.01    |
Index
under the nonreceptive category were screened using embryo morphology, and none opted for PGT-A screening to select embryos for transfer. This further supports the importance of embryo screening by PGT-A in RIF patients.

We anticipated that asynchrony between the euploid embryo and uterine receptivity might be the primary cause of the failure. Implantation is a complex mechanism of immunological, anatomical, endometrial, embryogenic, and many more unknown factors. For patients with implantation failure, despite good endometrial receptivity, euploid embryos, and no other known contributing factors, assessment is challenging and should be studied further to identify the causes.

The study determined the importance of PGT-A and ERA, which offer significant benefits in clinical outcomes during the cycle with positive pregnancy rates. Testing the embryo's genetic quality and endometrium receptivity significantly contributed to successful implantation.

Our study had several important limitations. The study design was retrospective and conducted in a single center. The sample size is small, making interpretations from the ERA nonreceptive group inconclusive compared to the ERA receptive group. Larger prospective studies would offer more statistical power to support our findings. As a small number of patients opted for PGT-A along with ERA testing in the present study, it is recommended for all participants to do both PGT-A and ERA in future prospective studies.

Conclusions
The data set from the present study reveals the significant role of ERA along with PGT in achieving better clinical outcomes for the first time in Indian patients. An ERA offered a personalized approach to identifying the WOI, adjusting the progesterone exposure, and then performing embryo transfer accordingly. There is an improvement in IR and CPR in RIF patients with a displaced WOI by transferring euploid embryos in a PET cycle. An ERA is a promising technique for RIF patients even after euploid embryo transfer. Further, PGT-A benefits RIF significantly, particularly in cases with optimal endometrial receptivity.

Additional Information
Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Wings IVF Women’s Hospital, Unit of Reveba Infertility Clinics Pvt. Ltd., Ahmadabad - Ethics Committee. issued approval No. 2018/002/31B/Memo Date: 08/05/2018 Memorandum This is in connection with the decision of the medical board to form a Ethics Committee to review all retrospective/prospective studies to be conducted on the patient’s data at Wings IVF Hospital, a unit of Reveba Infertility Clinics Pvt. Ltd. Following members have been notified through additional communication about their involvement in the Ethics Committee: 1. Dr. Girish Patel - Chairman of the Ethics Committee 2. Dr. Jayesh Amin - IVF Specialist 3. Dr. Ripal Patel - Associate IVF Specialist 4. Mr. Shriraj Rawal - Administrative officer/Member Secretary 5. Dr. G.Jaya Prakash - Subject Expert - Embryology 6. Dr. K.Murali Krishna - Subject Expert 7. Dr. Darpi Patel-Layman This committee shall meet every month to evaluate and see abstracts for studies to be conducted at WINGS IVF. Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue.

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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