Clinical Evaluation of genetic screenings in overcoming Recurrent Implantation Failure patients

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Abstract

Objective: We evaluated the clinical usefulness of the endometrial receptivity array (ERA) and the preimplantation genetic test for aneuploidy (PGT-A) Genetic screenings in patients with severe or moderate recurrent implantation failure. Design: Retrospective multicenter cohort. Setting: University affiliate IVF centers. Population: Patients who failed to achieve implantation following transfer of [?]3 or [?]5 embryos at least in three single embryo transfers were evaluated as moderate or severe recurrent implantation failure, respectively. Methods: Patients with previous RIF were compared in PGT-A, ERA and PGT-A+ERA and control group. Multiple logistic regression analysis was performed and adjusted ORs were calculated with the aim to control possible bias. Main Outcomes Measures: Mean implantation rate and ongoing pregnancy rates per embryo transfer were considered as primary outcomes. Results: Of the 2,110 patients belonging to the moderate group, those who underwent transfer of euploid embryos after the preimplantation genetic test for aneuploidy had a higher implantation rate than those who did not. Additionally, the preimplantation genetic test for aneuploidy group had a significantly higher rate of ongoing pregnancy. The same outcomes measured for the 488 patients in the severe group did not reveal any statistically significant improvements. The use of the endometrial receptivity array did not significantly improve outcomes in either group. Conclusions: The preimplantation genetic test for aneuploidy may be beneficial for patients with moderate recurrent implantation failure. At its current level of development, the endometrial receptivity analysis by ERA does not appear to be clinically useful for patients with recurrent implantation failure.

Introduction

Infertility is a worldwide problem[1] contributing to rising demand for assisted reproductive techniques (ART)[2]. The European Society of Human Reproduction and Embryology (ESHRE) recently highlighted the expansion of ART treatments in Europe[3], and a similar trend was reported by the American Society for Reproductive Medicine (ASRM)[4]. ART outcomes for struggling couples have improved recently; yet, there are still significant numbers of unresolved cases [5]and frequently a significant number of embryos must be transferred for a successful pregnancy even with donated oocytes. Despite advances in ART, the implantation rate and several 'take-home' babies per initiated treatment or embryo transfer (ET) remain low[8]. Recurrent implantation failure (RIF) is one of the most common conditions affecting IVF outcomes and is diagnosed after the failure of varying numbers of ET[9,10]. Both the age of the mother and the type of embryo (cleavage stage or blastocyst) are also considered in the diagnosis[11].

The disparity in the definition of RIF likely stems from its multiple compounded etiologies[5]. Both the endometrium and the quality of embryos impact implantation[12]. However, objectively and uniformly defining good quality embryos is difficult and there is a lack of consensus on chromosome analysis following embryo biopsy. Regardless, endometrial receptivity and embryo quality may represent as high as ˜30% of factors influencing pregnancy success in IVF[8]. This contribution is unsurprising considering that uterine implantation is an intricate process requiring both a receptive endometrium and a competent embryo[13].

If conditions are appropriate, implantation is initiated by attachment of the blastocyst to the epithelial layer of the endometrium[14]. Attachment and invasion are optimal during an interval in the menstrual cycle termed the "window of implantation" (WOI)[15]. This window is classically diagnosed by endometrial histology, but this evaluation is subjective[16], histological dating through biopsy should not be part of the infertility workup without[17,18]. The endometrial receptivity array (ERA), based on the expression of 238 endometrial genes, may objectively diagnose receptivity[19]. The ERA test is superior to endometrial histology in its ability to detect temporal displacement of the WOI[20,21] and helped create personalized ET schedules that could result in better pregnancy rates[22,23], although no randomized controlled are currently unpublished. In contrast, RIF may result from the displacement of the WOI and/or its disruption by molecular pathologies independent of timing[24]. There is support for both mechanisms[25-27], highlighting the need for defining unique RIF etiologies[27].

Different techniques have been proposed to select the best embryos for transfer. Pre-implantation genetic screening (PGT-A) defines a normal embryo based on chromosomal status[28]. Chromosomal aneuploidies are the major cause of pregnancy loss and implantation failure[29]. Patients most likely to benefit from PGT-A are infertile women of advanced maternal age with a history of recurrent pregnancy loss or RIF[30,31]. Considering the multifactorial etiology of RIF, we used a large cohort to retrospectively evaluate the effectiveness of testing for endometrial (using ERA) and embryonic (using PGT-A) quality to improve clinical outcomes.

Material and Methods

Patients

This observational, retrospective, multicenter study evaluated ART results from couples with RIF between 2013 and 2018 using data from 17 IVIRMA clinics in Europe. Infertile patients between 18 to 45 years old at the first transfer who experienced RIF after repeated ART with their own or donated oocytes had a minimum of three embryos transferred in different single embryo transfers. Patients with lacked any evidence of prior implantation events, including previous births, voluntary interruptions of pregnancy, or clinical miscarriages were included in the study. Patients with an abnormal karyotype such as translocation or an inversion carrier and with thrombophilia, either congenital or acquired, were excluded. Patients presenting severe metabolic or endocrine disorders and patients with atrophic endometrium were not included in the study. Submucous myomas or polyps, previous ET with high difficulty, and/or bleeding without previous hysteroscopy correction were excluded[23].

Only embryos of good quality were transferred and day-5 embryos (blastocysts) were graded according to expansion and quality of the inner cell mass and trophoectoderm[32]. Patients who failed to achieve a pregnancy after transfer of three to five good quality embryos transferred in single embryo transfers were considered RIF. We have previously observed that 94.9% of patients with three embryos transferred achieve clinical pregnancy[33], so we compared patients who had at least three embryos transferred with patients who had a least five embryos transferred.

A moderate RIF (M-RIF) group consisted of patients who first received at least three transferred in single embryo transfer (SET) without achieving implantation and without having received PGT-A or ERA. Subsequent ETs were categorized depending on the treatment received (Figure 1). Severe RIF (S-RIF) patients undergone five transferred embryos summed across consecutive cycles without ERA or PGT-A testing. All subsequent ET were categorized after ERA, PGT-A, or both. Patients who underwent frozen ET had either natural or hormonal cycles.

Ethical Approval

This study was approved by the Ethics Committee of the Instituto Valenciano de Infertilidad (IVI), (identification code # 1801-FIVI-048-AP).

Data Collection

Age, BMI, years of infertility, mean number of previous fresh and frozen embryos transferred per patient,

mean number of oocytes retrieved or donated, mean number of MII oocytes inseminated, fertilization rate per inseminated oocyte, mean number of embryos available per oocyte pick-up, mean endometrial thickness, mean number of days for endometrial preparation, number of total ET, and number of frozen embryos transferred were recorded.

We determined the benefit of testing in terms of (1) mean implantation rate per transferred embryo defined as the number of gestational sacs divided by the number of embryos transferred, and (2) ongoing pregnancy rate per transferred embryo defined as positive pregnancy beyond 12 weeks gestation confirmed by ultrasound with fetal heart activity divided by the number of embryos transferred.

Endometrial Receptivity Analysis

The ERA (iGenomix, Valencia, Spain) is a transcriptomic analysis combined with artificial intelligence technology for dating the WOI[19]. The test assesses the expression of 238 genes that are biomarkers of endometrial dating. It has been hypothesized that the ERA can personalize the timing of ET, synchronizing embryonic development with the endometrial WOI of a given patient33.

The ERA was used to determine endometrial receptivity in a sample obtained seven days after the LH serum peak in a natural cycle or five days after progesterone administration in a hormone replacement cycle. Endometrial biopsies were collected from the uterine fundus, and samples were analyzed by iGenomix according to their protocol[19,20,23,33]. Endometria were classified by expression profile as receptive or pre- or post- receptive[34].

Preimplantation Genetic Screening

Chromosomal analysis was performed by aCGH (array comparative genomic hybridization) or NGS (next-generation sequencing). Per iGenomix procedures and as specified by the manufacturer (Illumina), the 24sure aCGH platform has an effective 10-Mb resolution; therefore, only full chromosomal aneuploidies and segmental aneuploidies affecting chromosomal fragments larger than 10 Mb were identified[30,35]. Embryos were vitrified and transferred in subsequent natural or programmed cycles.

Statistical Analysis

Categorical and continuous variables are presented in the text and tables as percentages or means with standard deviations or 95% confidence intervals (95% CI). To compare means, ANOVA tests were used with Bonferroni post-hoc tests. For multivariate analyses to control possible bias and to calculate adjusted ORs, logistic regression analysis was performed considering the proportion of ET that used donated oocytes, the day of ET, the mean number of embryos transferred per procedure, age, and the mean of the prior number of embryos transferred per patient as clinically relevant variables. Because this study was conducted over a five year period and couples may have had consecutive ET with different diagnostic techniques (e.g., ERA and then ERA + PGT-A), meaning the groups lack independence and there is potential correlation between data from each group, generalized estimating equations were utilized to estimate the parameters of a generalized linear model with a possible unknown correlation between outcomes. Statistical significance was established at $P< 0.05$. Calculations were made with R version 3.5.0 (R core team)[36]. Estimation of statistical power was conducted to define the probability that a given test rejects a false null hypothesis to better interpret results, give context, and focus the discussion.

Results

Our analysis identified 2,110 patients with M-RIF and 488 with S-RIF. The general and clinical features of the cycles and patients included in the study are shown in Table 1. Table 2 summarizes the parameters of the test-guided IVF cycles among the different groups of patients. The retrospective nature of our study resulted in the differential distribution of some relevant variables, as shown in Tables 1 and 2. To avoid bias, we included those parameters with statistically significant differences and/or clinical relevance within a multivariate analysis model by using generalized estimating equations.

Some patients included in the M-RIF and S-RIF groups had infertility associated with uterine factors (Table S1). Patients who had the ERA had a higher percentage of uterine pathologies than control patients or those undergoing PGT-A (Table S1). Table S2 shows the number of patients scheduled for personalized ET after the ERA. The percentage of personalized ET (pET) was 25.7% and 39.3% in the S-RIF and M-RIF groups, respectively. Hence, a large percentage of patients had an asynchronous or displaced WOIs, particularly in the M-RIF group.

Univariate ANOVA of the M-RIF group revealed a statistically significant difference in the overall mean implantation rates of the subgroups ($P = 0.0053$). The use of PGT-A yielded a better implantation rate (45.9%) than standard IVF (35.89%) with an OR of 1.34, 95% CI: 1.17-1.55, $P < 0.001$. Implantation rates were not improved over standard rates by ERA. Significant differences were not detected between subgroups subjected to different tests (ERA vs PGT-A, ERA vs. PGT-A+ERA, PGT-A vs. PGT-A+ERA). Logistic regression models adjusted for control variables confirmed that within the M-RIF group, only the PGT-A test yielded significant improvement (AdjOR 1.22, 95% CI: 1.14-1.30, $P < 0.001$) over standard treatment. When comparing the other subgroups after adjusting for control variables, we found a statistically significant difference between the ERA and PGT-A subgroups (OR 0.84, 95% CI: 0.77-0.92, $P < 0.001$), but no other subgroup comparison reached significance.

Univariate ANOVA of the implantation rate as calculated by the number of gestational sacs per number of embryos transferred revealed statistically significant differences between the M-RIF subgroups ($P = 0.005$). The highest implantation rate was in PGT-A (47.2%) versus the control group (35.8%), ERA (35.6%), and ERA+PGT-A (31.82%). For M-RIF patients, the implantation rate was higher after PGT-A testing than after standard IVF (OR 1.61, 95% CI: 1.24-2.11,$P = 0.002$). There were no statistically significant differences between other subgroups. When the ORs were adjusted by logistic regression models for control variables, the PGT-A subgroup was found to significantly differ from the control subgroup (AdjOR 2.69, 95% CI: 1.99-3.66, $P < 0.001$). In addition, the ERA subgroup was found to significantly differ from the PGT-A subgroup (AdjOR 0.40, 95% CI: 0.26-0.62, $P < 0.001$).

Table 3 shows the rates of ongoing pregnancy for all study groups based on the number of embryos transferred. Univariate ANOVA revealed statistically significant differences between M-RIF subgroups ($P = 0.05$). Post-hoc testing between M-RIF subgroups revealed that, again, only the PGT-A subgroup differed from the control group (1.51, 95% CI: 1.12-2.05, $P = 0.029$). There were no significant differences between other subgroups. When the multivariate analysis was applied to adjust for control variables, the PGT-A group was found to differ from the control group (AdjOR 2.19, 95% CI: 1.55-3.07, $P< 0.0001$). The ERA group was detrimental to the ongoing pregnancy rate compared to the PGT-A group (AdjOR 0.51, 95% CI: 0.31-0.83, $P < 0.0284$). No statistically significant differences emerged in the comparisons between other subgroups.

Univariate analysis of the mean implantation rate of the S-RIF subgroups revealed no statistically significant differences. The implantation rate per patient was 34.2% (95% CI: 30.68-37.81) for the control, 40% (95% CI: 25.40-54.60) for the ERA, 38.2% (95% CI: 28.02-48.37) for the PGT-A, and 33.3% (95% CI: 0-68.59) for the PGT-A+ERA groups. Logistic regression models with adjusted OR for control variables revealed no statistically significant differences between test and control groups or for multiple comparisons between subgroups.

Univariate analysis did not detect any statistically significant differences in the implantation rate calculated per S-RIF subgroup considering the number of gestational sacs and the number of embryos transferred. The implantation rates were 34.8% (95% CI: 31.63-37.99) for control, 37% (95% CI: 23.21-52.45) for ERA, 39.8% (95%CI 29.78-50.46) for PGT-A, and 33.3% (95%CI 4.33-77.72) for PGT-A+ERA patients. A logistic regression model with adjusted OR for any control variables revealed no statistically significant difference for any group or the multiple comparisons between all groups. No statistical significance was detected when comparing ERA vs PGT-A, ERA vs ERA+PGT-A, or PGT-A vs PGT-A+ERA.

Table 3 shows the rates of ongoing pregnancy for all study groups based on the number of embryos transferred. Univariate analysis was not statistically significant for contrasts between S-RIF subgroups. The

multivariate analysis did not detect statistically significant differences between treatments (PGT-A, ERA, or PGT-A+ERA) and the control group or in the multiple comparisons between the subgroups.

Comment

Main Findings

Our results confirm that PGT-A is a useful tool for assessing chromosomal viability and is essential for RIF patients to avoid the transfer of aneuploidy embryos. Confirming the euploid status of embryos significantly improves sustained implantation rates over rates achieved when selecting embryos based on morphology alone[39]. Patients with RIF have increased numbers of embryo anomalies[37], including translocations, mosaicism, inversions, and deletions, which can be resolved with the use of PGT-A[38].

Interpretation

In some cases, even euploid embryos are unable to implant[40], indicating an etiology independent of the embryo's genetics. Although PGT-A help to select embryos with a higher probability of implanting in these patients, any euploid embryos not implant suggesting that chromosomal status is not the only factor to consider.

In the case of S-RIF, the use of PGT-A was insufficient to improve IVF outcomes, suggesting that different tools may be needed to assess embryo quality and take endometrial factors into account. Even though PGT-A can mitigate maternal age effect in IVF patients, patients in the oldest group (> 42 years) had different implantation rates than those in younger groups (<35–42 years)[41].

The temporal window of endometrial receptivity to blastocysts is limited, and most of the histologic criteria using markers of endometrial receptivity are subjective and lack accuracy and predictive value[18]. The ERA was created for more accurate endometrial dating throughout the luteal phase[20]. In this study, we found that numerous M-RIF and S-RIF patients had displaced WOIs and qualified for pET. This is consistent with previous studies that report a 25–30% contribution of the endometrial factor to implantation failure[42,43]. The higher percentage of pET in the M-RIF group could be explained by the higher percentage of uterine pathologies, diagnosed by ultrasound, which could affect ERA results. Unfortunately, the ERA test did not improve implantation or ongoing pregnancy rates in this study and thus cannot be used as a predictor of a healthy WOI. This is consistent with a prior study in which a personalized adjustment of progesterone did not improve pregnancy outcomes in RIF patients receiving euploid embryos (as confirmed by PGT-A)[43].

Although PGT-A is considered an important tool for patients with advanced maternal age, the standard use of PGT-A is actively debated[44]. PGT-A may not improve overall pregnancy outcomes in all women, but there was a significant increase in the ongoing pregnancy rate per ET with the use of PGT-A in women aged 35-40 years with two or more embryos that could be biopsied[45]. The use of PGT-A should be addressed according to clinical history, as there is not sufficient evidence to recommend PGT-A for all infertile patients[46]. Moreover, the use of PGT-A in patients with RIF could improve live birth rates in ET compared to patients with no PGT-A at all[47].

Demonstrating a healthy embryo is necessary before considering whether the endometrium might contribute to implantation failure, but chromosomal status is not enough. Novel embryo assessment and selection procedures, such as time-lapse imaging and metabolomics, may help to better evaluate embryo quality and viability[48]. These should be evaluated for their usefulness in RIF as well. In addition, more data on PGT-A in S-RIF could be worth pursuing. After these results, fertility status seems not to be related to endometrial dating by ERA, deep characterization of endometrial pathology is needed to evaluate properly the endometrial factor in IVF cycles.

Strengths and Limitations

To our knowledge, this study is the largest to evaluate the clinical usefulness of ERA for RIF patients. However, its retrospective nature and strict inclusion criteria for defining subpopulations mean that some comparisons were carried out with moderate sample sizes and a limited number of transferred embryos. This

particularly affected the S-RIF PGT-A, ERA, and PGT-A+ERA subgroups. From a clinical perspective, this affects how the data should be interpreted. When comparing M-RIF subgroups, our study was powered to detect a 10% effect of using ERA results to guide ET. For the S-RIF subgroups, which had even lower numbers of patients, our study was powered to detect an approximate 20% effect. Our work is underpowered for confirming smaller differences. As more data is collected, some clinical benefit of the test might be detected, but our research indicates that any beneficial effect of PGT-A in S-RIF patients is limited.

Conclusions

The main discovery of this study is to distinguish into two types of implantation failure patients, the ones that could be benefited by PGT-A and the ones that are not benefited due to the implantation failure origin. There is no clinical evidence that ERA test benefits any patient, pET cannot be based on the morphological characteristics of the embryo and chromosomal screening should be considered for M-RIF patients. Additionally, ERA cannot identify the most appropriate time for embryo transfer and cannot detect uterine diseases making the endometrium unsuitable for implantation. New technologies may be necessary to assess the endometrial aspect of implantation. A more thorough investigation of the effect of pET on reproductive outcomes could also shed light on the role of the endometrium. Prospective studies with enough power are needed to evaluate whether ERA has a clinical benefit. Although S-RIF was uncommon in our population of IVF patients, it warrants further study because designing treatments for this condition will likely prove challenging.

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