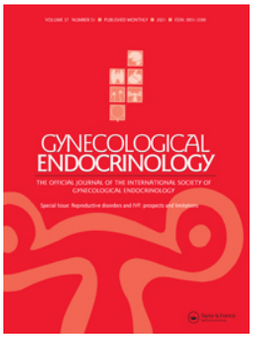

Gynecological Endocrinology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/igye20

Evaluation of embryo aneuploidy (PGT-A) and endometrial receptivity (ERA) testing in patients with recurrent implantation failure in ICSI cycles

Violeta Fodina, Alesja Dudorova & Juris Erenpreiss

To cite this article: Violeta Fodina, Alesja Dudorova & Juris Erenpreiss (2021) Evaluation of embryo aneuploidy (PGT-A) and endometrial receptivity (ERA) testing in patients with recurrent implantation failure in ICSI cycles, Gynecological Endocrinology, 37:sup1, 17-20, DOI: 10.1080/09513590.2021.2006466

To link to this article: https://doi.org/10.1080/09513590.2021.2006466

Published online: 23 Dec 2021.

GYNECOLOGICAL ENDOCRINOLOGY
2021, VOL. 37, NO. S1, 17–20
https://doi.org/10.1080/09513590.2021.2006466

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Evaluation of embryo aneuploidy (PGT-A) and endometrial receptivity (ERA) testing in patients with recurrent implantation failure in ICSI cycles

Violeta Fodina[a], Alesja Dudorova[a] and Juris Erenpreiss[a,b]

[a]Clinic 'IVF-Riga', Riga, Latvia; [b]Riga Stradins University, Riga, Latvia

ABSTRACT

Objective: The objective of a study was to assess the ability of the pre-implantation genetic testing of embryos for aneuploidy (PGT-A) and Endometrial receptivity array (ERA)—alone or in combination to improve the clinical outcomes in intracytoplasmic sperm injection (ICSI) cycles in patients with repeated implantation failure (RIF).

Methods: This was a retrospective study of the 253 cycles with a history of the previous RIF. They were divided into four groups: Group I - frozen embryo transfers without any additional tests or procedures (pure FET), $n = 72$ cycles; Group II - FET with PGT-A, $n = 87$; Group III - FET with PGT-A and ERA, $n = 72$; Group IV - FET with ERA, $n = 22$.

Results: Median age of the entire study group for the females was 35 years. Only Group II (FET + PGT-A) showed statistically significant higher chance in achieving both biochemical ($p = .01$, OR = 5.5) and clinical pregnancy ($p = .049$, OR = 2.3), as compared to the Group I (FET with no additional tests). Both Group III and Group IV failed to demonstrate better clinical outcomes as compared to the Group I.

Conclusions: Patients with RIF can benefit from testing for embryo aneuploidy using the PGT-A method, but the ability of the ERA test to improve the clinical outcome in ICSI cycles seems to be rather limited. Although the endometrium cycle is also weakened with age, the contribution of the embryo genetic quality is evidently more important for successful implantation, although in principle both factors reflect the reproductive health.

ARTICLE HISTORY
Received 31 May 2021
Accepted 14 July 2021
Published online 23 December 2021

KEYWORDS
Recurrent implantation failure; embryo aneuploidy; pregnancy loss; ICSI

Introduction

Recurrent implantation failure (RIF) is a common and serious problem in IVF and ICSI cycles. It is a repeated implantation failure after the embryo transfer (ET), although the ET numbers after which RIF is diagnosed may be different [1–3]. RIF can depend on several factors. Successful embryo implantation is an interactive process between the blastocyst and the uterus. Synchronized development of an embryo with uterine differentiation to a receptive state is a prerequisite to complete pregnancy. Implantation failure may occur even on early stages during the embryo attachment or migration, or an embryo can migrate through the luminal surface of the endometrium but the process could be disrupted before the formation of an intrauterine gestational sac [4]. Therefore, implantation failure is usually distinguished into two groups—the first group includes the women who never manifested the quantifiable marks of implantation, such as increased levels of hCG. The second group includes women who have an evidence of implantation (detectable hCG production), but it did not proceed beyond the formation of a gestational sac visible on ultrasonography two weeks later [1].

Some researchers attempted classify the reasons of RIF. Timeva *et al.* [5] have divided RIF causes in three main groups: (1) multifactorial RIF with the subgroups of maternal or paternal factors, hormonal or metabolic disorders, infections and thrombophilias; (2) endometrial RIF that is associated with thin (≤6 mm) endometrium, with or without variations in vascularity; (3) idiopathic RIF, which is unexplained failure to achieve pregnancy after transfer of good quality embryos, without any anatomical and histological changes in uterine cavity and endometrium, without any other disturbances in patient, patient-partner and embryos. Some other authors, in turn, have distinguished etiologic groups such as decreased endometrial receptivity, defective embryonic development, and also multifactorial RIF [6]. However, the two main causes of implantation failure are always present in the majority of classifications: uterine (endometrial) and embryo factors.

Endometrial environment plays a crucial role in embryo implantation and early placental development. There is a certain period of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently proceed to invade the endometrial stroma, the process is called endometrial receptivity [7]. This complex process provides the embryo with the opportunity to normally attach, implant and develop. There is a short period of time during the menstrual cycle, when the endometrial receptivity is optimal and embryo implantation is possible. This period is called 'a window of implantation' (WOI). In 2009, an Endometrial receptivity array (ERA) was developed based on the expression analysis of 248 genes [8]. The idea is to detect a specific point in time of endometrial cycle in which the WOI starts, allowing physicians to perform personalized embryo transfer. The accuracy and consistency of the ERA test had been demonstrated by its reliability and reproducibility for determination of the exact time of the WOI that can be used with better results in comparison to histological dating of endometrial receptivity [9]. Studies

CONTACT Juris Erenpreiss jerenpreiss@gmail.com

Table 1. Description statistics of the study groups.

Variable	Group I	Group II	Group III	Group IV	*p*
Age (median, IQR)	34.0 (37.0–32.0)	36.0 (38.0–34.0)	35.0 (37.0–33.0)	34.0 (38.0–32.5)	.21
Acquired oocytes (median, IQR)	13.0 (16.0–10.0)	13.0 (17.0–9.0)	11.5 (18.0–8.0)	11.5 (17.5–6.8)	.83
Acquired blastocysts (median, IQR)	5.0 (7.0–3.0)	7.0 (9.0–3.0)	5.0 (7.0–3.0)	4.0 (5.5–3.0)	**.02**

Group I – cycles with FET; Group II – cycles with FET + PGT-A; Group III – cycles with FET + PGT-A + ERA test; Group IV – cycles with FET + ERA test.

demonstrated that embryo-endometrial synchronization within an optimal time frame increases the chances of success in an assisted reproductive treatment [10]. It has been shown that WOI is displaced in 25% of RIF patients [11], and application of the ERA test is able to improve the outcomes of IVF in RIF patients [12–14]. However, a recent study including very large numbers of patients failed to demonstrate a beneficial effect of the ERA in RIF patients [15].

A lot of attention in search to improve the outcomes in RIF patients has been driven also to the embryo factors, implementing the pre-implantation genetic testing for embryo aneuploidy (PGT-A). PGT-A allows avoiding transferring an embryo with chromosomal aneuploidies that would lead to the implantation failure, pregnancy loss or embryo malformation. It has been shown that sorting by PGT-A is especially effective in improving live birth rates in patients with advanced maternal age, where embryo aneuploidies are more frequent, due to meiosis errors [16]. However, the data on the ability of the PGT-A to improve the implantation and live birth rates in RIF patients is also still a controversial issue. Several studies failed to demonstrate such a beneficial effect for the RIF patients [16–18], while other large-cohort studies have shown a beneficial effect, at least in patients with moderate RIF [15].

Therefore, we aimed to evaluate the ability of these two tests—ERA test and PGT-A test—to improve the outcomes of the ICSI treatment in our group of RIF patients.

Materials and methods

In this observational and retrospective study, we evaluated the ICSI results in our clinic from couples with RIF between 2017 and 2020. The patients who failed to achieve a clinical pregnancy after transfers of at least three good-quality embryos in different single fresh or frozen embryo transfers were considered RIF. Patients with an abnormal karyotype (translocations, inversions), with severe endocrine disorders, with atrophic endometrium were not included in the study. Only embryos of good quality which were defined as possessing the correct number of cells corresponding to the day of development—as the day-5 embryos (blastocysts) were transferred.

In total, 253 cycles of the assisted reproduction by means of ICSI were included into the study, and further divided into four groups: Group I - frozen embryo transfers without any additional tests or procedures (FET), $n = 72$ cycles; Group II - FET with PGT-A, $n = 87$; Group III - FET with PGT-A and ERA, $n = 72$; Group IV - FET with ERA, $n = 22$.

Whole-genome amplification (WGA) and chromosomal analysis - preimplantation genetic testing for aneuploidies

PGT-A was performed as also described elsewhere [16]. Sureplex reagent kit (Illumina) was used for WGA of trophectodermal cells. Electrophoresis and DNA quantification with Qubit was followed to evaluate the success of amplification. PGT-A was done either by aCGH (array Comparative Genomic Hybridization) with Illumina 24Sure arrays from 2015 to 2017 or Illumina VeriSeq PGS library preparation kit from 2018 to 2019 due to methodology change. Both methods allow to detect imbalanced aberrations of autosomes 1-22 and X, Y sex chromosomes in preimplantation embryos, but due to technical differences patients were divided in groups after methods used – aCGH or NGS. Low-level mosaicism, uniparental disomy, 69XXX variant and aberrations under 20 Mb cannot be determined. Reaction conditions are available under request. CNV analysis in both cases was performed with Bluefuse software. At least two molecular geneticists and at least one clinical geneticist interpreted the results. Chromosomal aberrations were classified according to guidelines: euploid, low-level mosaic (20–50%), high-level mosaic (50–80%), aneuploid. Decision regarding transfer was made according to chromosomes involved and type of aberration (whole chromosome vs. partial). Embryos with aneuploidy were not transferred.

Endometrial receptivity array

Endometrial biopsies were collected from the uterine fundus following the manufacturer's protocol [8], and samples were sent for the analysis by iGenomix. Endometria were classified by expression profile as receptive, pre- or post-receptive based on transcriptome signature [8].

Statistics

Descriptive statistics are presented using medians (interquartile ranges) and percentages. Differences between groups were tested by the Chi-square test. To adjust the results for the covariates, multivariate regression analysis was used. The age of the female, count of acquired oocytes after the pick-up procedure, and count of acquired blastocysts were evaluated as the potential covariates. The main end-points were divided into: (a) no pregnancy; (b) biochemical pregnancy (not proceeding further to the clinical pregnancy); (c) clinical pregnancy; (d) pregnancy loss (loss of the clinical pregnancy after the gestation week 7). In multivariate regression analysis, the results of Groups II-IV were compared with the 'reference' Group I (FET without any additional tests). To compare the results of the ICSI cycles, the end-points (b), (c) and (d) were compared with an end-point (a)—no pregnancy: in order to assess the ability of each approach to achieve better clinical outcomes as compared to 'control' FET approach, and as compared with the negative outcome (no pregnancy).

All tests were two-tailed, $p < .05$ was considered significant. Statistical analyses were performed using SPSS 20.0 [SPSS Inc., Chicago, IL, USA].

Ethical approval

The local ethical committee of the Latvian University approved the study.

Table 2. Comparative analysis (p values) for counts of acquired blastocysts between the Groups I-IV.

	Group I	Group II	Group III	Group IV
Group I		**0.03**	0.52	0.13
Group II			0.11	**0.004**
Group III				0.07
Group IV				

Group I – cycles with FET; Group II – cycles with FET + PGT-A; Group III – cycles with FET + PGT-A + ERA test; Group IV – cycles with FET + ERA test.

Results

Table 1 shows the descriptive statistics of a study cohort. Median age of the entire study group for the females was 35 years. An age of a female and the counts of acquired oocytes showed no differences between the Groups I–IV. In turn, the counts of acquired blastocysts were higher in Group II, compared both to the Group I ($p = .03$) and Group IV ($p = .004$) (Table 2). All these factors were used as covariates in multivariate regression analysis.

Table 3 shows the clinical outcomes (no pregnancy, biochemical pregnancy, clinical pregnancy, and pregnancy loss) in Groups I–IV. When clinical outcomes of biochemical pregnancy, clinical pregnancy and pregnancy loss were compared to the end-point of 'no pregnancy', only Group II (FET + PGT-A) showed statistically significant higher chance in achieving both biochemical ($p = .01$, OR = 5.5) and clinical pregnancy ($p = .049$, OR = 2.3), as compared to the Group I (FET with no additional tests). Both Group III and Group IV failed to demonstrate better clinical outcomes as compared to the Group I. No differences were observed in terms of pregnancy loss between the groups.

Table 3. Clinical outcomes in Groups I-IV.

	Group				
	Group I	Group II	Group III	Group IV	Total
No pregnancy					
Count	35	25	30	11	95
%	48.6%	28.4%	41.7%	50.0%	40.8%
Biochemical pregnancy					
Count	4	15	1	1	18
%	5.6%	17.9%	1.4%	4.5%	7.7%
Clinical pregnancy					
Count	32	43	40	8	113
%	44.4%	49.3%	55.6%	36.4%	48.5%
Pregnancy loss					
Count	1	4	1	2	7
%	1.4%	4.5%	1.4%	9.1%	3.0%
Total					
Count	72	87	72	22	253

Group I – cycles with FET; Group II – cycles with FET + PGT-A; Group III – cycles with FET + PGT-A + ERA test; Group IV – cycles with FET + ERA test.

Discussion

We have demonstrated that implementing the pre-implantation genetic testing for aneuploidies is able to improve the rates of both biochemical and clinical pregnancies in RIF patients, as compared to the cycles where FET without PGT-A were carried out. It is well established that maternal age is associated with a rapid decline in the production of healthy and high-quality oocytes and euploid embryos resulting in reduced fertility in women older than 35 years of age [19], and also the results of our study are reflecting this phenomenon. In turn, we could not see the beneficial effect of the ERA in RIF patients. In addition, no beneficial effect was observed not only in FET + ERA group, but also in FET + PGT-A + ERA group. This can be explained by the presence of uterine pathologies in this group (which is the reason why the ERA test was performed), and the absence of uterine pathologies in the PGT-A group. Our results are in concordance with the study by Cozzolino *et al.* [15] where the authors also observed the improvement of the implantation rates only in the PGT-A group, and failed to demonstrate the beneficial effect in either pure ERA group or combined PGT-A + ERA group. It seems that uterine pathologies play one of the central roles in RIF, and in this group of patients, ERA test even in combination with the testing for embryo aneuploidies is not able to overcome the problem. On the other hand, PGT-A seems to be effective only in RIF patients without uterine pathologies.

In addition, pure PGT-A group also had the highest rates of biochemical pregnancies that did not proceed further to the clinical pregnancies (17.9%, as compared to 1.4–5.6% in other groups)—which could not be explained by the aneuploidy of an embryo. These data again confirm that many other factors apart from embryo quality (both morphological and genetic) and endometrial receptivity (WOI) play a crucial role in RIF. It has been suggested that uterine microbiome [20,21], different immunological factors like increase of the pro-inflammation interleukins [22], overproduction of the TNF-a [23], abnormal function of natural killers (NK) and specifically uterine NK cells [24], and other immunological and non-immunological factors might play a role in RIF. Also, male factor (sperm DNA integrity) might play a role in RIF [25], although some studies failed to confirm that [26].

The limitation of our study is a lack of the live-birth data from our patients, however, since we have the data for the clinical pregnancies and early pregnancy loss, we believe these data are representative enough for the clinical outcomes, and the proportion of the late pregnancy loss must be negligible.

In conclusion, our study is in accordance with the recent data [15] that patients with RIF without uterine pathologies can benefit from the embryo aneuploidy using the PGT-A method. The ability of the ERA test to improve the clinical outcome in RIF patients seems to be rather limited. However, this is still a poorly investigated and controversial field, and further studies are necessary to elucidate other factors that play a role in RIF that would help to improve the ability to assist these couples in achieving successful pregnancies.

Disclosure statement

There is no conflict of interest.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

[1] Coughlan C, Ledger W, Wang Q, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online. 2014;28(1):14–38.

[2] Polanski LT, Baumgarten MN, Quenby S, et al. What exactly do we mean by "'recurrent implantation failure'? A systematic review and opinion"? Reprod Biomed Online. 2014;28(4):409–423.

[3] Thornhill AR, deDie-Smulders CE, Geraedts JP, ESHRE PGD Consortium, et al. ESHRE PGD Consortium 'Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. Hum Reprod. 2005;20(1):35–48.

[4] Maesawa Y, Yamada H, Deguchi M, et al. History of biochemical pregnancy was associated with the subsequent reproductive failure among women with recurrent spontaneous abortion. Gynecol Endocrinol. 2015;31(4):306–308.
[5] Timeva T, Shterev A, Kyurkchiev S. Recurrent implantation failure: the role of the endometrium. J Reprod Infertil. 2014;15(4):173–183.
[6] Margalioth EJ, Ben-Chetrit A, Gal M, et al. Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod. 2006;21(12):3036–3043.
[7] Lessey BA, Young SL. Structure, Function, and Evaluation of the Female Reproductive Tract [Internet]. Eighth Edi. Yen & Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management: Eighth Edition. Elsevier Inc.; 2019. 206-247.e13 p.
[8] Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Fertil Steril. 2011;95(1):50–60.
[9] Díaz-Gimeno P, Ruiz-Alonso M, Blesa D, et al. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. Fertil Steril. 2013;99(2):508–517.
[10] Ruiz-Alonso M, Galindo N, Pellicer A, et al. What a difference two days make: "personalized" embryo transfer (pET) paradigm: a case report and pilot study. Hum Reprod. 2014;29(6):1244–1247. Jun
[11] Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 2013;100(3):818–824.
[12] Mahajan N. Endometrial receptivity array: clinical application. J Hum Reprod Sci. 2015;8(3):121–129.
[13] Tan J, Kan & A, Hitkari J, et al. The role of the endometrial receptivity array (ERA) in patients who have failed euploid embryo transfers. J Assist Reprod Genet. 2018;35(4):683–692.
[14] Hashimoto T, Koizumi M, Doshida M, et al. Efficacy of the endometrial receptivity array for repeated implantation failure in Japan: a retrospective, two-centers study. Reprod Med Biol. 2017;16(3): 290–296.
[15] Cozzolino M, Diaz-Gimeno P, Pellicer A, et al. Evaluation of the endometrial receptivity assay and the preimplantation genetic test for aneuploidy in overcoming recurrent implantation failure. J Assist Reprod Genet. 2020;37(12):2989–2997.
[16] Rubio C, Bellver J, Rodrigo L, et al. Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: Two randomized trials. Fertil Steril. 2013;99(5):1400–1407.
[17] Sato T, Sugiura-Ogasawara M, Ozawa F, et al. Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. Hum Reprod. 2019;34(12):255.
[18] Hatirnaz S, Ozer A, Hatirnaz E, et al. Pre-implantation genetic screening among women experiencing recurrent failure of in vitro fertilization. Int J Gynecol Obstet. 2017;137(3):314–318.
[19] Mikwar M, MacFarlane AJ, Marchetti F. Mechanisms of oocyte aneuploidy associated with advanced maternal age. Mutat Res Rev Mutat Res. 2020;785:108320.
[20] Fanchin R, Harmas A, Benaoudia F, et al. Microbial flora of the cervix assessed at the time of embryo transfer adversely affects in vitro fertilization outcome. Fertil Steril. 1998;70(5):866–870.
[21] Selman H, Mariani M, Barnocchi N, et al. Examination of bacterial contamination at the time of embryo transfer, and its impact on the IVF/pregnancy outcome. J Assist Reprod Genet. 2007;24(9):395–399.
[22] Liang PY, Diao LH, Huang CY, et al. The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. Reprod Biomed Online. 2015; 31(6): 823–826.
[23] Boomsma CM, Kavelaars A, Eijkemans MJC, et al. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum Reprod. 2009;24(6):1427–1435.
[24] Lédée N, Petitbarat M, Rahmati M, et al. New pre-conception immune biomarkers for clinical practice: Interleukin-18, interleukin-15 and TWEAK on the endometrial side, G-CSF on the follicular side. J Reprod Immunol. 2011;88(2): 118–123.
[25] Zini A, Boman JM, Belzile E, et al. Sperm DNA damage is associated with and increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2008;23(12):2663–2668.
[26] Coughlan C, Clarke H, Cutting R, et al. Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. Asian J Androl. 2015;17(4):681–685.