ORIGINAL ARTICLE

Role of endometrial receptivity array for implantation failure in *in-vitro* fertilization & intracytoplasmic sperm injection

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Biomedica - Official Journal of University of Health Sciences, Lahore, Pakistan
Volume 37(4):220-226
https://doi.org/10.51441/BioMedica/5-296

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ABSTRACT

Background and Objective: Assisted reproductive technique is an evolving field with many recent advances. The success rate is low in developing countries where financial concerns prevail predominantly. This prospective study was designed for the first time in any hospital in Pakistan to determine the role of Endometrial Receptivity Array (ERA) in patients with previous implantation failure to improve pregnancy outcome and to enhance the success rate of *in vitro* fertilization & intracytoplasmic sperm injection (IVF/ICSI).

Methods: This study was carried out at the Lahore Institute of Fertility & Endocrinology, Lahore-Pakistan from December, 2019 to October, 2020. A total of 16 patients were recruited after taking written informed consent. Only those patients were selected who had previous one or more implantation failures in IVF/ICSI cycles and had at least two or more good quality frozen embryos. RNA was obtained from the endometrial sample to check ERA through 238 genes expressed using RNA sequencing. Beta HCG level and scans were performed to confirm the clinical pregnancy.

Results: All enrolled patients had an ERA test and their embryos transferred according to personalized window of implantation (WOI). A total of 5 (31.3%) patients were stimulated with a long protocol while 11 (68.7%) underwent a short protocol. WOI was receptive in 12 (75%) patients, pre-receptive in 3 (18.2%) and post-receptive in 1 (6.2%), and most of patients showed receptivity at P5 (109-145 hours). Twelve patients (75%) had clinical pregnancy evident by positive beta human chorionic gonadotrophin (HCG) after embryo transfer. A significant association was found between WOI and receptivity ($P < 0.05$).

Conclusion: The results of ERA in our study seem promising, especially in patients with previous one or more implantation failures. Although we have limited number of patients keeping in mind its financial constraints, especially in the developing countries, still ERA is considered a way of hope, especially for those patients who have previous implantation failures.

Keywords: Endometrial Receptivity Array (ERA), window of implantation (WOI), trans-vaginal scan (TVS), frozen embryo transfer (FET), *in vitro* Fertilization (IVF), intracytoplasmic sperm injection (ICSI).

Received: 07 July 2021 **Revised date:** 10 August 2021 **Accepted:** 21 November 2021
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Introduction

Even though Pakistan is among the most crowded nations globally and has a populace development pace of around 2%, it likewise has a high infertility ratio (21.9%); 3.5% primary, and 18.4% secondary.[1] Furthermore, psychological impact associated with infertility and its treatment is still challenging.[2] Since the birth of first *in vitro* fertilization (IVF) baby, there were many hopes about the success of IVF, but the success rate of IVF and intracytoplasmic sperm injection (ICSI) is still not up to the desired level.[3] It needs to be improved further, notably in the developing countries, where finances are an impelling problem; patients pay for the treatment out of their pocket; hence, they avail a limited number of treatment attempts.[4]

Despite achieving good quality embryos, success rate of IVF & ICSI stagnates at a minimum standard. The major contributing factor of low success in such patients is implantation failure.[5] Successful IVF is a synchronized and timely transfer of a good quality embryo to the welcoming

endometrium.[6] The tight time frame during which the endometrium is ready to receive a healthy embryo is called the "window of implantation" (WOI).[7]

In normal IVF & ICSI cycle, it is assumed that every patient has a fixed WOI, transfer is usually timed around days 3 or 5.[8] However, few patients' windows are slightly away from mainstream and hence suffer from implantation failure. Hence, problem is that if good quality embryos fail to implant repeatedly, how endometrial receptivity (ER) can be checked to ascertain high chances of displaced WOI in such patients? The answer to the problem could be Endometrial Receptivity Array (ERA). The basis for ERA is that each woman has a unique WOI, and by knowing this factor, the chances of successful pregnancy can be enhanced. This is called "personalized embryo transfer" (PET) (Figure 1).

ERA is an effort to reveal the personalized WOI, particularly, in patients with previous implantation failure. Recurrent implantation failure is defined as "failure to achieve pregnancy after embryo transfer".[10] A total of 73% failed cycles occur due to failed embryo implantation either due to aneuploid embryo or implantation failure. Three in every 10 women have a displaced WOI failure.[9]

Another thought is that implantation failure is not pathology, but it is the inability to develop accurate time-based harmony between the developing embryo and the endometrium.[11] ERA is a technique to see genomic expression of endometrium to time embryo transfer. Data derived suggests that embryo transfer timing is decided by expression of genes from endometrium. Total RNA is obtained from endometrial biopsy and used for genomic expression to check receptivity.[12] ERA evaluates 238 genes expressed during WOI using RNA sequencing.[13] Following analysis, endometrium is classified as receptive or non-receptive (NR). The NR 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, respectively.[14] Recurrent implantation failures may be due to a previously deserted WOI. These are the patients who are candidate for PET decided by ERA.[14]

Methods

It was a prospective study on couples coming for the IVF/ICSI at the Lahore Institute of Fertility & Endocrinology, Lahore-Pakistan from December, 2019 to October, 2020. The institutional ethical review committee approved this study. We selected 16 patients for ERA, and written informed consent was obtained. We selected only those patients with previous one or more implantation failures in IVF/ICSI cycles and have at least two or more good quality frozen embryos. All cases of hydrosalpinx, submucous fibroid or with previous difficult ET were excluded.

For ERA, there were two cycles. First was Biopsy Cycle, which was initiated by prescribing tablet Progynova 2 mg three times a day from first day of the cycle followed by trans-vaginal scan on cycle day-8 for endometrial thickness that should be >6 mm. In addition, serum progesterone (P4) levels were checked and ensured that they were <1 ng. After that, P4 pessary was inserted from CD-9. Endometrial biopsy was performed on P + 5 or 120th hour after starting P4. Biopsy results usually took 2-3 weeks. Endometrial sample was sent to Igenomix lab (Dubai) with all precautionary measures. RNA was obtained from the endometrium sample, and it

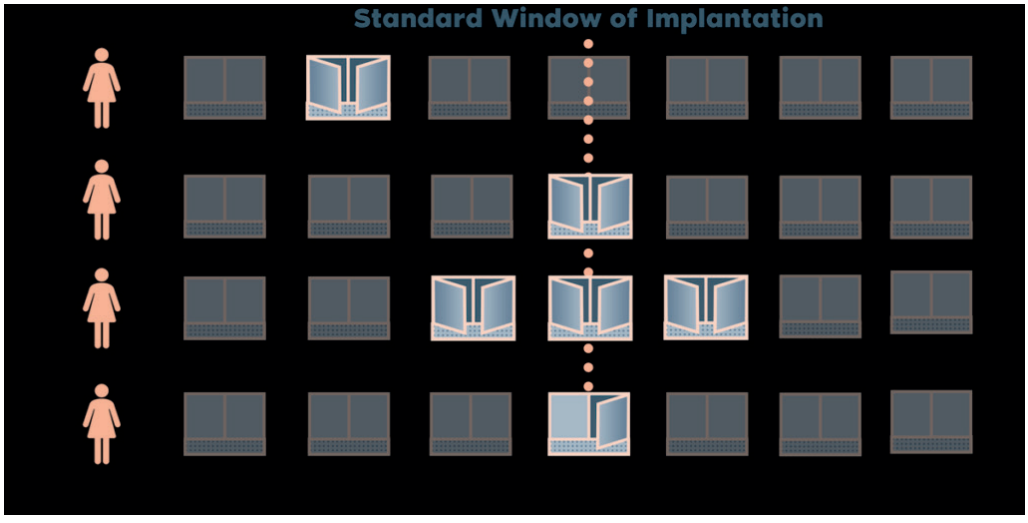

Figure 1. *Window of implantation (courtesy IGENOMICS).*[9]

was used for genomic expression to check its receptivity. The result was either receptive or NR endometrium. A receptive endometrium showed that the WOI was located on taking the sample. The recommendation was to proceed with embryo transfer under the same condition as for biopsy. A NR result showed a displaced WOI. In that case, with the ERA computational predictor, we estimated WOI by another biopsy. The NR endometrium was further classified as pre or post receptive. A biopsy cycle was repeated according to displaced WOI until we got the receptive endometrium.

The second cycle is transfer cycle, similar to biopsy cycle for embryo transfer as calculated by ERA WOI.

Statistical analysis

The data were analyzed using SPSS-25.0. For categorical variables descriptive analysis and for numerical variables mean and standard deviation was calculated. Pearson Chi-Square test was used to find the association between different variables with the 0.05% or 5% level of significance.

Results

The mean age of the patients was 32.25 ± 4.38 and body mass index (BMI) was 25.31 ± 3.51. The mean baseline hormonal parameters of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol (E2), and anti-Mullerian hormone (AMH) was 6.02 ± 1.69, 6.34 ± 4.78, 16.28 ± 10.77, 45.61 ± 30.69, and 4.76 ± 3.54, respectively. Similarly, the mean thyroid stimulating hormone (TSH) level was 2.62 ± 2.36, triiodothyronine (T3) was 8.89 ± 2.90, and thyroxin (T4) was 15.10 ± 30.52. In these selective patients, Rubella antibodies IgG mean was 88.80 ± 93.68, and IgM was 0.47 ± 0.26, therefore considered negative.

Primary and secondary infertility were reported by 9 (56.3%) and 7 (43.7%) patients, respectively. Most women had regular menstrual cycle 14 (87.5%), while 2 (12.5%) had irregular cycle.

Regarding etiology of infertility, 4 (25.0%) had tubal factor, 3 (18.3%) had unexplained infertility, 5 (31.3%) had male factor, and 4 (25%) had both male and female factor

Out of 16 patients who were stimulated, 5 (31.3%) underwent long protocol and 11 (68.7%) had a short protocol. Furthermore, regarding antral follicle count (AFC), 3 (18.7%) had 8-15, and 13 (81.3%) had more than 15. The mean value of serum E2 & P4 levels and endometrial thickness on the day of decision were 3,468.36 ± 113.9 iu, 5.74 ± 2.39, 10.09 ± 1.43 mm, respectively (Table 1).

Mean of total oocyte (both mature and immature) count was 17.50 ± 6.67, 14.88 ± 5.59, and 2.63 ± 2.60, respectively. Single and double embryo were transferred in 7 (43.75%), more than two embryos were transferred in 2 (12.5%) patients. Out of 16, 2 (12.5%) were early blastocysts, 7 (43.8%) were expanded blastocysts, 5 (31.3%) were hatched, and 2 (12.4%) were morula (Table 3).

WOI was receptive in 12 (75%) patients, pre-receptive in 3 (18.2%), and post-receptive in 1 (6.2%), and most of patients showed receptivity at P5 (109-145 hours) (Table 2).

The significant association was found between WOI and receptivity ($P < 0.05$). Beta HCG was positive in 12 (75%) patients, and out of these, 8 (67%) patients had fetal cardiac activity (FCA) (Table 4).

Discussion

ART in Pakistan is evolving but at a very slow pace and with limited available data. ERA is an attempt to introduce new techniques in IVF/ICSI to enhance its success rate. It is a ray of hope particularly for those patients who have previous implantation failure in Pakistan as this test is introduced for the first time. Life Center Lahore is the first and the only center in Pakistan carrying out this test. Finances and resources both are the limiting factor for any new advancement particularly in developing countries. Hence, ERA in Pakistan with only 16 patients is justifiable as it opens the door for future innovation in IVF with limited resources.

The success of human implantation is a complex process depending upon a good quality embryo and endometrium ready to receive this embryo and a synchronous harmony amongst them.[15,16] Apart from the good quality embryo; ER is a big challenge now a days with limited available tests. Furthermore, the functional tests for endometrium are less accurate and have low predictive value. Microarray innovation (ERA) has permitted recognizable proof of the transcriptomic mark of the WOI. Subsequently, it leads to the improvement of an ER measure (time) for the finding of adjusted ER.[17] Therefore, genomic expression of endometrium is an attempt to enhance the implantation rate in IVF & ICSI.[13,18]

The personalized way of embryo transfer enhances the success rate of ART, and it also enhances the clinical pregnancy rate. A total of 75% of our patient's ERA results showed that endometrium was found to be receptive which is comparable with the study of Patel et al.[7] ERA helps finding out a personalized WOI for majority of these patients with previous implantation failure. In addition, the results of positive beta HCG in our 12 patients, as a marker of receptivity, are comparable to the study of Simon et al.[12] and Mahajan et al.[16]

ERA test is accurate and sensitive in identifying genetic expressions of the endometrium to pinpoint embryo transfer timing.[19,20] PET guided by ERA significantly improves

Table 1. *Demographic parameters of ERA patients.*

Parameters	Mean ± SD
Mean Age (years)	32.25 ± 4.38
Mean BMI (kg/m^2)	25.31 ± 3.51
Infertility diagnosis	
Mean duration of infertility (years)	6.25 ± 4.15
Type of infertility *n* (%)	
Primary	9 (56.3)
Secondary	7 (43.7)
No. of attempts	2.25 ± 1.12
Menstrual cycle *n* (%)	
Regular	14 (87.5)
Irregular	2 (12.5)
Baseline hormonal parameters (mean ± SD)	
FSH mIU/ml	6.02 ± 1.69
LH	6.34 ± 4.78
Prolactin ng/ml	16.28 ± 10.77
E2 pg/ml	45.61 ± 30.69
AMH ng/ml	4.76 ± 3.54
TSH µIU/ml	2.62 ± 2.36
Infertility etiology *n* (%)	
Tubal	4 (25.0)
Unexplained infertility factor	3 (18.3)
Male factor	5 (31.3)
Both Male & Female factor	4 (25.0)
Stimulation parameters *n* (%)	
Protocol	
Long	5 (31.3)
Short	11 (68.7)
Ultrasonography parameters *n* (%) AFC	
<8	-
8-15	3 (18.7)
>15	13 (81.3)
Serum endocrine levels on decision day (mean ± SD)	
E2	3,468.36 ± 113.9
P4	5.74 ± 2.39
Endometrium thickness	10.09 ± 1.43

Data are presented as mean and standard deviation for continuous variables and *n* (%) for categorical variable.

pregnancy rates in patients with unexplained repeated implantation failure.[7] Although we have limited available data but results of ERA are promising. This test is still in an evolution phase and the authors are looking forwards to establish a local lab in Pakistan so that the financial issues and time delays can be overcome to get the maximum benefit for the public in Pakistan.

Table 2. *Window of implantation of ERA patients.*

Age	No. of attempts	WOI	Hours	Receptivity	No. of ET	Clinical pregnancy	FCA
34	3.00	P + 5	110.00	R	2.00	Positive	Negative
32	3.00	P + 5	115.00	R	2.00	Positive	Positive
28	1.00	P + 5	120.00	R	2.00	Positive	Positive
30	1.00	P + 5	120.00	R	1.00	Positive	Negative
29	3.00	P + 5	109.00	R	1.00	Positive	positive
39	2.00	P + 5	121.00	R	1.00	Positive	Negative
30	1.00	P + 5	120.00	R	1.00	Negative	Negative
26	1.00	P + 5	145.00	pre	3.00	Positive	Positive
39	4.00	P + 5	108.00	R	2.00	Positive	Positive
37	3.00	P + 5	145.00	pre	1.00	Positive	Negative
24	1.00	P + 4	96.00	post	3.00	Negative	Negative
32	1.00	P + 5	110.00	R	2.00	Negative	Negative
36	2.00	P + 5	108.00	R	1.00	Positive	Positive
34	3.00	P + 4	108.00	pre	2.00	Positive	Positive
31	4.00	P + 5	125.00	R	1.00	Positive	Positive
35	3.00	P + 5	145.00	R	2.00	Negative	Negative

Table 3. *Outcome parameters of ART treatment of ERA patients.*

Parameters	Mean ± SD
Total oocytes	17.50 ± 6.67
Mature	14.88 ± 5.59
Immature	2.63 ± 2.60
No of embryos [*n* (%)]	
1	7 (43.7)
2	7 (43.7)
3	2 (12.5)
Embryo stage [*n* (%)]	
Early blastocysts	2 (12.5)
Expanded blastocysts	7 (43.8)
Hatched blastocysts	5 (31.3)
Morula	2 (12.4)

Table 4. *Pregnancy outcomes in ERA patients.*

Variables		*n* (%)
No. of failed attempts	0	1 (6.3)
	1	2 (12.5)
	2	8 (50.0)
	3	4 (25.0)
	4	1 (6.3)
WOI	Receptive	12 (75.0)
	Pre-receptive	3 (18.2)
	Post-receptive	1 (6.2)
Hours of WOI	P4 (96-108)	2 (12.5)
	P5 (109-145)	14 (87.5)
Clinical pregnancy	Beta HCG positive	12 (75.0)
	Beta HCG negative	4 (25.0)
FCA	Present	8 (67.0)
	Absent	4 (33.0)

Conclusion

The ERA results in this study seem promising, especially in patients with previous one or more implantation failures. Although the study recruited limited number of patients keeping in mind the financial constraints, especially in developing countries like Pakistan, ERA may still be considered a way of hope, especially for patients with previous implantation failures.

Limitations of the study

The major limiting factor for the study is the small number of patients for ERA because of high procedure cost. ERA is an expensive test and labs are not currently available in Pakistan so we had to send the samples abroad for results which led to delays.

Acknowledgement

The authors are thankful to Dr. Rashid Latif Khan for motivating and guiding for the research. A special thanks to Dr. Shahzad Bhatti & the whole team of Lahore Institute of Fertility & Endocrinology particularly Dr. Nighat Mehmood & Miss Shezae for their help and support.

List of Abbreviations

E2 Estradiol
ERA Endometrial Receptivity Array
ICSI Intracytoplasmic sperm injection
IVF *In vitro* fertilization
P4 Progesterone

PET personalized embryo transfer
WOI Window of implantation

Conflict of interest
None to declare.

Grant support and financial disclosure
None to disclose.

Ethical approval
Ethical approval was granted by the Ethics Committee, Lahore Institute of Fertility & Endocrinology, Lahore, Pakistan vide letter No IRB/2019/012 dated 06-10-2019.

Authors' contribution
RN: Study design, acquisition and analysis of data
HLK: Conception of study and drafting of manuscript
YLK: Study design and drafting of manuscript
AA, AF: Data collection and drafting of manuscript
RN: Acquisition and analysis of data
ALL AUTHORS: Approval of the final version of the manuscript to be published.

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