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ASSISTED REPRODUCTION TECHNOLOGIES

Clinical utility of the endometrial receptivity analysis in women with prior failed transfers

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Abstract

Purpose To determine the utility of the endometrial receptivity analysis (ERA) in women with prior failed embryo transfers (ET).

Methods This was a retrospective study of patients who underwent an ERA test with a subsequent frozen ET. Women were classified based on their indication for an ERA test: (1) ≥ 1 prior failed ET (cases), or (2) as a prophylactic measure (controls). A subset analysis of women with ≥ 3 prior failed transfers was performed. Pregnancy outcomes of the subsequent cycle were examined, including conception, clinical pregnancy, and ongoing pregnancy/live birth.

Results A total of 222 women were included, 131 (59%) women with ≥ 1 prior failed ET and 91 (41%) controls. Among the 131 women with ≥ 1 prior failed ET, 20 women (9%) had ≥ 3 prior failed ETs. The proportion of non-receptive ERA tests in the three groups were the following: 45% (≥ 1 prior failed ET), 40% (≥ 3 prior failed ETs), and 52% (controls). The results did not differ between cases and controls. The pregnancy outcomes did not differ between women with ≥ 1 prior failed ET and controls. In women with ≥ 3 prior failed ETs, there was a lower ongoing pregnancy/live birth rate (28% vs 54%, $P = 0.046$).

Conclusion Women with ≥ 1 prior failed ET and ≥ 3 prior failed ETs had a similar prevalence of non-receptive endometrium compared to controls. Women with ≥ 3 prior failed ETs had a lower ongoing pregnancy/live birth rate despite a personalized FET, suggesting that there are additional factors in implantation failure beyond an adjustment in progesterone exposure.

Keywords Endometrial receptivity · Endometrial receptivity analysis · Endometrium · Failed embryo transfer

Introduction

Endometrial receptivity is the ability of the uterine lining to permit attachment and invasion of the blastocyst [1]. The 4- to 5-day period of receptivity, or window of implantation (WOI), was presumed to be constant in all women. In an effort to improve live birth rates with the transfer of good quality embryos, attention has now turned to etiologies leading to implantation failure.

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While molecular, ultrasound, and histologic markers have been proposed for the detection of endometrial receptivity, none has proven reliable and able to predict fertility or clinical pregnancy [2–5]. More recently, endometrial phase-specific transcriptomic profiles have been identified in women with and without gynecologic disorders [6–11], in hopes of developing a tool to more reliably differentiate receptive and non-receptive endometrium [12–14]. In 2009, a diagnostic test was developed to detect the transcriptomic signature specific to receptive endometrium [15]. The endometrial receptivity analysis uses Next Generation Sequencing to measure the expression of 248 genes to classify the endometrium as receptive or non-receptive and to diagnose a personalized WOI, advising an adjusted progesterone duration in a subsequent cycle for those found to have non-receptive endometrium [16].

The ERA has gained traction as of late, especially for women with a history of failed embryo transfer (ET) or recurrent implantation failure. However, there remains limited and conflicting evidence of its reproducibility [17, 18] and effect on reproductive outcomes and live birth rates [19–27]. In this study, we sought to examine our experience with the ERA, analyzing outcomes after an ERA test for patients with and without prior failed ET.

Materials and methods

This retrospective cohort study included all patients who underwent an ERA test at two centers between January 2016 and February 2019 with a subsequent FET cycle using a single embryology laboratory. This study (Pro00056716) was approved by the Institutional Review Board at our institution.

Women were classified based on their indication for an ERA test: (1) ≥ 1 prior failed ET (cases), or (2) as a prophylactic measure due to having only a single euploid embryo or physician/patient preference (controls). Given the heterogeneity in defining recurrent implantation failure in the literature [19–21, 24–26, 28–30], we chose ≥ 1 prior failed ET to define the cases as the two centers primarily proceed with euploid embryo transfers. We also examined the subset of cases in which women had ≥ 3 prior failed ETs. Women who had the ERA test as a prophylactic measure did not have a history of implantation defects and were designated as the control group.

For most patients, the ERA test was performed with the standard programmed hormone replacement cycles used in our clinics. Generally, patients underwent treatment with escalating doses of oral estradiol to a maximum of 6–8 mg daily for 10–14 days. Transvaginal ultrasonography was used to assess the endometrial thickness. Then, intramuscular progesterone (P) injections were administered, and a biopsy was performed in a standard sterile fashion after five days of progesterone (P+5). A limited number of patients used vaginal progesterone instead of intramuscular progesterone for luteal phase support. There were also a limited number of patients who underwent a natural cycle and the biopsy was performed on day LH+7. The specific protocol for the ERA cycle was based on physician preference. ERA results were classified as receptive, early receptive, late receptive, pre-receptive, and post-receptive. Early, late, pre-, and post-receptive results were classified as non-receptive, as an adjusted progesterone duration was applied for a personalized FET in the next cycle.

In the subsequent FET cycle, the protocol for endometrium preparation was adjusted for all patients with a non-receptive ERA test. All ETs were frozen blastocyst-stage transfers and > 90% were known euploid embryos by PGT-A testing. Pregnancy outcomes included conception defined as a positive bhCG, clinical pregnancy defined as a gestational sac visualized on ultrasound, and ongoing pregnancy/live birth.

Descriptive statistics were performed to compare the groups in terms of demographics, ERA test results, and pregnancy outcomes. Continuous variables were expressed as mean and standard deviation using the student's *t* test or median (interquartile range) using the Wilcoxon rank-sum test as appropriate. Categorical variables were expressed as percentages and compared using the chi-square test. For statistical testing, cases with missing data were excluded. Women with ≥ 1 prior failed ET were compared to controls, and a subset analysis of women with ≥ 3 prior failed ETs were compared to controls as well. We also compared pregnancy outcomes in only women with a non-receptive ERA test. Lastly, we compared pregnancy outcomes for non-receptive ERA tests vs receptive ERA tests in the three separate groups. Based on the sample size of the entire cohort, the study had 80% power to detect a 20% difference in pregnancy outcome with a confidence level of 95%. Statistical analyses were performed using Stata IC 13.1 (StataCorp, College Station, TX).

Results

A total of 222 women were included in this study—131 (59%) cases (≥ 1 prior failed ET) and 91 (41%) controls (no prior failed transfer). Included in the 131 cases were 20 women with a history of ≥ 3 prior failed ETs.

Demographic characteristics and ERA test results are presented in Table 1. Maternal age and proportion of gestational carriers was similar between cases with ≥ 1 prior failed ET and controls. Prior gravidity and parity were higher in the control group (Table 1). A subset of cases with ≥ 3 prior failed ETs had similar demographics compared to controls (Table 1). Of women with ≥ 1 prior failed ET, 45% had non-receptive ERA tests. Of women with ≥ 3 prior failed ETs, 40% had non-receptive ERA tests. Of the controls, 52% had non-receptive ERA tests. The proportion of non-receptive ERA tests did not differ between cases and controls (Table 1).

Table 1 Demographic characteristics and ERA results

	≥ 1 prior failed ET $N = 131$	≥ 3 prior failed ETs $N = 20$	Controls $N = 91$	P value[c]	P value[d]
Age (at ERA)[a]	37, 5.3	38, 5.2	38, 5.5	0.7482	0.498
Gravida[b]	0 (0, 2)	1 (0, 2)	1 (0, 2)	0.0315	0.828
Para[b]	0 (0, 0)	0 (0, 0)	0 (0, 1)	0.0236	0.164
Gestational carrier (n (%))	16 (12)	1 (5)	10 (11)	0.780	0.417
Number of Prior failed transfers	1 (1, 2)	3 (3, 4.5)	--	--	--
ERA results (n (%))				0.350	0.638
Preceptive or early receptive	50 (38)	6 (30)	36 (40)		
Receptive	72 (55)	12 (60)	44 (48)		
Post-receptive or late receptive	9 (7)	2 (10)	11 (12)		

[a] Mean, standard deviation

[b] Median (interquartile range)

[c] ≥ 1 prior failed ET compared to controls

[d] ≥ 3 prior failed ET compared to controls

Pregnancy outcomes of subsequent FET cycles, of which 83% were single embryo transfers and 27% double embryo transfers, are shown in Table 2. There was no difference in conception, clinical pregnancy, or ongoing pregnancy/live birth between women with ≥ 1 prior failed ET and controls (Table 2). In a subset of women with ≥ 3 prior failed ETs, the ongoing pregnancy/live birth rate was significantly lower than controls, 28% vs 54% respectively ($P = 0.046$) (Table 2). These findings were duplicated when the analysis was limited to only women with a non-receptive ERA test (Table 3). Women with ≥ 3 prior failed ETs and an non-receptive ERA test had a significantly lower ongoing pregnancy/live birth rate compared to controls with a non-receptive ERA test, 13% vs 51% respectively ($P = 0.044$) (Table 3), even after an adjusted progesterone duration as recommended by the ERA test.

Lastly, we compared pregnancy outcomes for women with a non-receptive ERA test vs a receptive ERA test (Table 4). There was no difference in conception, clinical pregnancy, or ongoing pregnancy/live birth for women with ≥ 1 prior failed ET (Table 4A), women with ≥ 3 prior failed ETs (Table 4B), or controls (Table 4C).

Discussion

In our study, the ERA test did not differentiate between those with and without a history of implantation failure. The proportion of non-receptive ERA results were similar when comparing (1) women with ≥ 1 prior failed ET to controls and (2) women with ≥ 3 prior failed ETs and controls. In the subset of women with ≥ 3 prior failed ETs, there was a lower ongoing pregnancy/live birth rate compared to controls. This finding remained significant when the analysis was limited to women with a non-receptive ERA test who underwent an adjusted progesterone duration for a personalized FET. This suggests there are additional factors, such as the underlying etiology of infertility or differences in ovarian function, involved in implantation failure beyond an adjustment in progesterone exposure.

The pregnancy outcomes in the subsequent FET cycle did not differ between women with ≥ 1 prior failed ET and controls; however, the sample size of the cohort ($N = 222$) was powered only to detect statistically significant differences of 20%. An interesting finding of our study is that there may be no added benefit to doing a prophylactic ERA test for women

Table 2 Pregnancy outcomes in the subsequent FET cycle after ERA test: cases vs. controls

	≥ 1 prior failed ET $N = 131$	≥ 3 prior failed ETs $N = 20$	Controls $N = 91$	P value[a]	P value[b]
Conception (n/N (%))	92/131 (70)	12/20 (60)	70/90 (78)	0.213	0.099
Clinical pregnancy, (n/N (%))	78/130 (60)	10/20 (50)	60/90 (67)	0.315	0.161
Ongoing pregnancy/ live birth (n/N (%))	57/121 (47)	5/18 (28)	43/80 (54)	0.357	0.046

[a] ≥ 1 prior failed ET compared to controls

[b] ≥ 3 prior failed ETs compared to controls

Table 3 Pregnancy outcomes in the FET cycle after a non-receptive ERA test: cases vs. controls

	≥ 1 prior failed ET $N = 59$	≥ 3 prior failed ETs $N = 8$	Controls $N = 47$	*P* value[a]	*P* value[b]
Conception (*n*/*N* (%))	40/59 (68)	5/8 (63)	38/47 (81)	0.130	0.245
Clinical pregnancy (*n*/*N* (%))	33/58 (57)	3/8 (38)	31/47 (66)	0.344	0.126
Ongoing pregnancy/live birth (*n*/*N* (%))[c]	21/55 (38)	1/8 (13)	22/43 (51)	0.199	0.044

[a] ≥ 1 prior failed ET compared to controls

[b] ≥ 3 prior failed ETs compared to controls

with no prior failed ETs, including gestational carriers or women with a limited number of embryos. Based on Table 4, controls had comparable pregnancy outcomes regardless of whether they had a receptive ERA test or a non-receptive ERA test leading to a personalized FET. Larger studies are needed to confirm this.

The literature reports a wide range of prevalence of non-receptive ERA results, 12–66%, owing to the heterogeneity in the patient population and the varying definition of non-receptive. Our study classified early and late receptive ERA results as non-receptive, because the results led to an adjusted progesterone duration for a personalized FET in the next cycle. Previous studies have corroborated our results in which the proportion of non-receptive ERA tests are similar between those with implantation failure and controls. In a study by Ruiz *et al*, cases were defined as ≥ 3 failed cycles with ≤ 4 morphologically high grade embryos and controls were defined as ≤ 1 prior failed cycle. The cases did not have a statistically higher proportion of non-receptive tests compared to controls (26% vs 12%, $P = 0.182$) [19]. Likewise, Tan *et al* showed a similar proportion of non-receptive tests in cases (≥ 2 prior failed transfers, 46%) compared to controls (1 failed transfer, 37%; 0 failed transfers, 43%) [21].

Our study also confirms the findings of several studies which did not demonstrate improved pregnancy outcomes following the ERA test. Bassil et al. compared pregnancy outcomes in cases (0–2 previous frozen embryo transfers) who underwent FET after an ERA test and controls who underwent FET without an ERA test, and they did not show a difference in the ongoing pregnancy rate [22]. Neves et al. compared pregnancy outcomes in patients with ERA after ≥ 1 previous failed euploid ET to those without ERA after ≥ 1 previous failed euploid ET, and those with ERA after ≥ 2 previous failed donor ET to those without ERA after ≥ 2 previous failed donor ET. They did not find differences in pregnancy rate in the euploid-ET ERA and euploid-ET control group, and there was a significantly lower PR in the donor-ET ERA group compared to the donor-ET control group. The sample sizes were small, with $n = 24$ and $n = 23$ in their ERA groups, and the outcome of ongoing pregnancy/live birth was not included [23].

This is the largest cohort of patients with an ERA test after a failed embryo transfer to be compared to a control group without failed embryo transfer. Another strength of our study is that > 90% of transfers in the subsequent FET cycle after the ERA test were known euploid embryos, which eliminates

Table 4 Pregnancy outcomes in the subsequent FET cycle after ERA test: non-receptive ERA test vs Receptive ERA test. A) ≥ 1 prior failed ET, B) ≥ 3 prior failed ETs, C) controls

A	**Non-receptive ERA $N = 59$**	**Receptive ERA $N = 72$**	***P* value**
Conception (*n*/*N* (%))	40/59 (68)	52/72 (72)	0.582
Clinical pregnancy (*n*/*N* (%))	33/58 (57)	45/72 (63)	0.517
Ongoing pregnancy/live birth (*n*/*N* (%))	21/55 (38)	36/66 (55)	0.073
B	**Non-receptive ERA $N = 8$**	**Receptive ERA $N = 12$**	***P* value**
Conception (*n*/*N* (%))	5/8 (63)	7/12 (58)	0.852
Clinical pregnancy (*n*/*N* (%))	3/8 (38)	7/12 (58)	0.361
Ongoing pregnancy/live birth (*n*/*N* (%))	1/8 (13)	4/10 (40)	0.196
C	**Non-receptive ERA $N = 47$**	**Receptive ERA $N = 44$**	***P* value**
Conception (*n*/*N* (%))	38/47 (81)	32/43 (74)	0.463
Clinical pregnancy (*n*/*N* (%))	31/47 (66)	29/43 (67)	0.881
Ongoing pregnancy/live birth (*n*/*N* (%))	21/37 (57)	22/43 (51)	0.617

aneuploidy as a cause of failed transfer. The main limitation of our study is inherent to the control group. The control group was defined as women who do not have a history of failed ET, but 1/3 of the controls did not have a clinical pregnancy in the subsequent FET cycle and ultimately would be reclassified as cases. The ideal control group would be women with a non-receptive ERA test, who do not undergo an adjusted progesterone duration for a personalized FET in the next cycle. However, it is difficult to recruit women to be in this ideal control group. The underlying etiology for infertility may play a role in implantation failure and pregnancy outcomes. Although we reviewed this data, the data was heterogeneous and difficult to validate, thus it was not included.

In conclusion, our study contributes to the growing body of literature on the ERA test. The clinical utility of the ERA test may be limited as the prevalence of non-receptivity was similar between women with and without a history implantation failure as defined in this study. Furthermore, in women with ≥ 3 prior failed ETs, there was a lower ongoing pregnancy/live birth rate even after adjusting for progesterone duration in the next cycle, which points to additional factors involved in implantation failure. Large, prospective studies that include women with a non-receptive ERA test who do not undergo a personalized FET in the next cycle are needed to provide more definitive conclusions.

Compliance with ethical standards

Conflict of interest Dr. Jessica Chan is a scientific advisor for the women's health start up BINTO. All other authors declare that they have no conflict of interest.

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