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ASSISTED REPRODUCTION TECHNOLOGIES

Does the endometrial receptivity array really provide personalized embryo transfer?

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Abstract

Purpose The aim of the present study was to determine the percentage of infertility patients who are diagnosed with a non-receptive endometrium according to the endometrial receptivity array (ERA) test and to examine whether adjusting the embryo transfer day according to the proposed shift in the window of implantation improves the pregnancy rate compared to non-ERA-tested patients.

Methods A single-center retrospective cohort study, including 53 consecutive good prognosis patients (0–2 previous frozen embryo transfers) admitted to our IVF unit for a mock cycle prior to their frozen day-5 embryo (blastocyst) transfer cycle. The mock cycle included an endometrial biopsy for both the ERA test and histological assessment by the Noyes criteria (study group). The next cycle frozen embryo transfer (FET) in the study group was adjusted according to the ERA results. The control group consisted of patients who underwent FET cycles at our clinic during the same period, without performing the endometrial biopsy and ERA testing.

Results During the study period, 503 patients (control group) underwent FET cycles without performing the ERA testing and 41 patients had FET following an ERA test. There were no between-group differences in patients' age, number of previous transfers, endometrial thickness, number of transferred embryos, and ongoing pregnancy rates (35.2 vs. 39%, respectively, p = NS). Out of the 53 patients who performed the ERA test before their first or second FET, five endometrial samples (9.4%) were found to be post-receptive, 29 (54.7%) pre-receptive, and only 19 samples (35.8%) were receptive. Women in the study group with pre- or post-receptive endometrium on ERA testing, the appropriate adjustment in timing of FET according to the ERA test resulted in a 33.3% pregnancy rate, which is comparable to the 35.2% background ongoing pregnancy rate of the control group.

Conclusions Performing the ERA test in a mock cycle prior to a FET does not seem to improve the ongoing pregnancy rate in good prognosis patients. Further large prospective studies are needed to elucidate the role of ERA testing in both good prognosis patients and in patients with recurrent implantation failure.

Keywords ERA · Endometrial receptivity

Introduction

With the recent trend toward single embryo transfer (ET) adopted in an attempt to reduce the risk of multiple pregnancy [1], the remaining extra embryos are cryopreserved, allowing further possibilities for conception following subsequent frozen-thawed embryo transfer (FET) cycles. Moreover, studies comparing fresh and frozen-thawed embryo transfer (FET) cycles in normal responders have recently demonstrated a significantly higher clinical pregnancy rate per transfer in the FET versus the fresh cycles [2–5], a difference that may be attributed to the high estrogen levels in the fresh stimulated cycles, negatively affecting the endometrium, with the consequent impaired endometrial receptivity [2–5].

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Successful embryo implantation requires an appropriate embryonic development, coincident with a receptive endometrium. In the human, the uterus becomes receptive during the mid-luteal phase of the menstrual cycle (days 19–23), commonly known as the window of implantation [6]. Implantation is an intricate process, which is further controlled by a number of complex molecules, e.g., hormones, growth factors, and cytokines. The initial steps in the implantation process are the apposition and attachment of the blastocyst to the epithelial layer of the endometrium, followed by the invasion of the trophoblast between its epithelial cells. While the ART technique helps us overcome most infertility problems, implantation still remains the rate limiting step for the success of IVF [7].

The observations described above led to the search for methods to assess the quality and the receptiveness of the endometrium for successful implantation. In the past, endometrial biopsy was the method of choice, used to evaluate endometrial development and dating. The endometrial histological appearance, as described by histological criteria described by Noyes et al. [8], suggested possible asynchrony between the embryonic and the endometrial development or out of phase (OOP) endometrium in patients with recurrent implantation failure.

Recently, a new technique using array molecular analysis and bioinformatics to create a customized array to identify markers of endometrial receptivity has been developed. The endometrial receptivity array (ERA) is based on analysis of expression of 238 genes that are thought to be involved in the receptivity of the endometrium to implantation. It has been suggested that the ERA test will enable the determination of a personalized window of implantation (WOI) [9]. This test is done by obtaining endometrial biopsy samples on day LH+ 7 in a natural cycle or on the 6th day of progesterone supplementation during an HRT cycle. Results are expressed as pre-receptive, receptive, or post-receptive. If the result is non-receptive, the embryo replacement timing may be adjusted in a subsequent cycle enabling personalized embryo transfer [10]. The ERA test is offered commercially but appears to be based on a relatively small number of samples.

The aim of the present study was to determine the percentage of good prognosis (0–2 prior FETs) infertility patients who were determined to have a non-receptive endometrium according to the ERA test and to examine whether adjusting the suggested day of transfer according to the ERA test increases the pregnancy rate compared to a similar group of women without ERA testing.

Materials and methods

This single-center retrospective cohort study included 53 consecutive patients admitted to our IVF unit, between April 2016 and March 2017 for a mock cycle prior their frozen day-5 embryo (blastocyst) transfer cycle that included an endometrial biopsy for the ERA test.

All the patients in the clinic, during this period, were offered to undergo ERA during a mock cycle, but only 53 patients decided to perform the ERA test.

We also sent part of the endometrial biopsy sample for histological endometrial dating in a routine pathology laboratory. We included women younger than 42 years old, undergoing their first to third embryo transfer attempt. A control group included all other women undergoing their first or second frozen embryo transfer at our clinic, younger than 42, during the same period of time, without performing the ERA testing. We did not include women with recurrent implantation failure so that the study group and control group would be comparable and to estimate the percentage of out of phase endometria in a relatively normal infertility population. The study was approved by the Research Ethics Board at Mount Sinai Hospital in Toronto. The sample size was sufficient to detect a difference of 20% in the ongoing pregnancy rate taking into account a power of 80% and a confidence level of 95%.

Artificial hormone replacement Patients started on day 2–3 of the cycle with oral administration of 2 mg of estradiol (Estrace) twice daily for endometrial preparation. The dose of estradiol was increased to 8 mg/day after 5 days. An ultrasound endometrial assessment was performed about cycle day 13. When the endometrial thickness was ≥ 7 mm with a triple line pattern, luteal support was begun using vaginal administration of progesterone suppositories (200 mg three times daily) with embryo transfer or endometrial biopsy performed on day 6 of progesterone.

Modified natural cycles Following spontaneous menstruation, patients were monitored by serial ultrasound for endometrial thickness, follicular development, and blood sampling for serum LH and progesterone levels, until the start of the LH surge was observed (LH level exceeded 180% of the baseline value), corresponding to the day prior to ovulation. On the following day, progesterone suppositories 200 mg three times daily were started. The endometrial biopsy or embryo transfer was performed on day 7 after the initiation of the LH surge.

The endometrial tissue was divided into two samples. One of the samples was sent for the ERA test and the other sample was sent to a commercial pathology laboratory for histological assessment using the criteria of Noyes et al. [8]. One to 4 months after the mock cycle, the same protocol was utilized (artificial hormone replacement or natural cycle) but the timing of the frozen embryo transfer was adjusted according to the ERA test results.

Table 1 Characteristics of patients performing ERA prior to the FET compared with patients undergoing FET without ERA (all the patients < 3 previous embryo transfers)

	ERA testing	Control group	*p* value
N	53	503	NS
Age (mean)	36.3 ± 0.4	35.6 ± 4	NS
Number of eggs retrieved (mean)	13.2 ± 7.1	12.8 ± 6.2	NS
Number of previous embryo transfers (mean)	1 ± 0.4	1.1 ± 0.6	NS
Endometrial thickness (mm) (mean)	8.95 ± 0.4	9.1 ± 0.2	NS
PGS	14/41 (34.1%)	74/503 (14.7%)	0.003
Number of freeze all cycles (%)	13 (24.5%)	119 (23.6%)	NS
Number of embryos transferred (mean)	1.1 ± 0.4	1.1 ± 0.3	NS
Implantation window			
Receptive (%)	19 (35.8)	–	–
Pre-receptive (%)	29 (54.7)	–	–
Post-receptive (%)	5 (9.4)	–	–
Ongoing pregnancy rate (%) (after correcting by the ERA results)	16/41 (39%)	177/503 (35.2%)	NS

Comparison of continuous variables between the two groups was conducted using Student's *t* test. Chi-square test was used for comparison of categorical variables.

Results

Fifty-three patients underwent the ERA testing during the period of the study. Forty-nine of the patients were prepared with artificial hormone replacement and four patients were biopsied during spontaneous natural cycles. Twelve patients did not undergo FET, 11 of these were for non- medical reasons, and one, because the embryo did not survive thawing. During the study period, 503 patients (control group) underwent FET cycles (456 with ART cycles and 47 with natural cycle FET) without performing the ERA testing.

The 503 control patients and 41 patients following an ERA test were similar in age (35.6 vs. 36.3 p = NS), number of oocytes retrieved during the fresh retrieval cycle (13.2 vs. 12.8 p = NS), number of previous transfers (1.1 vs. 1.0 p = NS), endometrial thickness (9.1 vs. 8.9 mm p = NS), and number of transferred embryos (1.1 vs. 1.1, p = NS). While more patients underwent preimplantation genetic screening (PGS) in the ERA group compared to the control group (34.1 vs. 14.7% p = 0.003), the ongoing pregnancy rates were comparable between the two groups (35.2 vs. 39% p = NS) (Table 1). When including only patients ≤ 40 of age, the ongoing pregnancy rates were still comparable (37.5 vs. 36% p = NS).

Out of the 53 infertile patients who performed the ERA test before their first or second FET, five endometrial samples (9.4%) were found to be post-receptive, 29 (54.7%) pre-receptive, and only 19 samples (35.8%) were receptive. When comparing the results of the ERA test with the Noyes (histologic assessment) criteria, only 47% of the results were comparable between the two groups and the strength of agreement between the two methods was found to be poor (Kappa = 0.041), and out of the 19 receptive samples, according to the ERA test, six (31.6%) were receptive according to the Noyes criteria (Table 2).

Table 3 represents ongoing pregnancy rates according to the ERA test versus histological dating criteria. Of the patients with concordant results in the ERA and the Noyes criteria and who underwent adjustment of the timing of FET according to the ERA test (or no adjustment if the test was receptive), 39% conceived (16 out of 41). Fifty percent of patients with receptive results according to the ERA test conceived in their subsequent FET cycle. Of those with pre- and post-receptive endometrium who underwent the appropriate adjustment according to the ERA test results in their subsequent cycle, 33.3% conceived. This percentage is comparable to the

Table 2 Comparison of the Noyes histologic dating and ERA results

ERA (n = 53)	Results by NOYES and % of agreement with ERA
Receptive, n = 19 (35.8%)	6 patients (31.6%) receptive
	11 patients (57.9%) pre-receptive
	2 patients (10.5%) post-receptive
Pre-receptive, n = 29 (54.7%)	18 patients (62.1%) pre-receptive
	1 patient (3.4%) post-receptive
	10 patients (34.5%) receptive
Post-receptive, n = 5 (9.4%)	1 patient (20%) post-receptive
	2 patients (40%) pre-receptive
	2 patients (40%) receptive

Table 3 Ongoing pregnancy rates according to the Noyes histologic dating and ERA results

ERA ($n = 41$)	Ongoing pregnancy rate	Ongoing pregnancy rate (NOYES)	Results by NOYES and % of agreement with ERA		Control group (503)	p value
Receptive (14)	7/14 (50%)	2/4 (50%)	4	Receptive	Pregnancy rate: 177/503 (35.2%)	NS
		5/10 (50%)	10	Non-receptive		
Non-receptive before the adjustment (27)	9/27 (33.3%)	5/12 (41.6%)	12	Receptive		
		4/15 (26.6%)	15	Non-receptive		

*53 patients went through ERA biopsy but twelve patients did not undergo FET, therefore only 41 patients are included in the pregnancy outcome analysis

35.2% background ongoing pregnancy rate of the control group without any testing.

Discussion

In the present study of patients with 0–2 previous failed embryo transfers undergoing endometrial biopsy for ERA testing and histologic assessment by the Noyes criteria, we were not able to demonstrate an improvement in ongoing pregnancy rate by the adjustment of the timing of subsequent FET according to the ERA results. Moreover, even though that more patients in the ERA group utilized PGS with the transfer of euploid blastocyst, we were not able to demonstrate any advantage or improvement in ongoing pregnancy rate by the adjustment of the timing of subsequent FET according to the ERA results.

Furthermore, we did not find any concordance between the ERA and the Noyes histologic criteria, nor any correlation between the ERA and the Noyes results and ongoing pregnancy rates. Of the 53 study patients, only 35.8% were found to have a "receptive," implantation window according to the ERA test. This figure is in contrast to the report by Ruiz-Alonso et al. [11], who demonstrated that 88% of patients without RIF, who underwent an ERA test, were found to have a normal implantation window. In the present study, the patients undergoing endometrial biopsy were good prognosis patients and therefore this figure was very surprising.

Díaz-Gimeno et al. [10] compared the accuracy and reproducibility of the ERA test versus standard histologic methods and found that 16 of 49 (32.6%) were properly dated when using both methods (histology and the ERA). In our study, we found that 47% had similar results on both the ERA and histologic assessment. We therefore cannot conclude which method is more accurate with regard to the implantation window dating since both tests determined that only 34–35% of the samples had a normally timed implantation window. This percentage seems low for women without recurrent implantation failure.

The new transcriptomic and bioinformatics technologies applied to the development of the ERA are aimed to provide a test with a higher precision for endometrial dating making this molecular diagnostic tool more accurate and robust than the histological approach for endometrial dating that has been used since the 1950s [10]. In the present study of good prognosis patients, those with pre- and post-receptive endometrium by ERA testing and who underwent the appropriate adjustment in timing of FET according to the ERA test conceived only 33% of the time. This pregnancy rate is comparable to the 35.2% background ongoing pregnancy rate of the control group.

There are few limitations in our study. There are many factors that can influence the pregnancy rate such as the physician or the embryologist performing the transfer, difficulties in inserting the transfer catheter, endometrial thickness and pattern, and subendometrial contractions. Those factors were not controlled for in the study. Moreover, the study was a retrospective study and in order to confirm the findings a prospective randomized controlled study is recommended.

In conclusion, the results of the present study do not support the use of the ERA test in a mock cycle prior to FET as a way to improve pregnancy outcomes in good prognosis patients. Further large prospective studies are needed to elucidate the role of ERA testing or histological endometrial dating in good prognosis patients. Additional large studies are required for patients with suspected recurrent implantation failure since the ERA test may be helpful in those women. These studies will aid in patient counseling and in tailoring the appropriate timing of embryo transfer in women undergoing infertility treatment.

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