

ERA[®] Endometrial Receptivity Analysis
Operations Manual



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❖ 1. Summary of the Endometrial Receptivity Analysis (ERA®) diagnostic process

The Endometrial Receptivity Analysis (ERA®) is a patented, molecular tool that analyzes by Next Generation Sequencing (NGS) the transcriptome of 236 genes related to endometrial receptivity status thereby providing an additional reproductive health parameter. The purpose of the ERA® test is to identify the optimum day of receptivity, even when there is a displaced window of implantation (WOI), in order to perform a personalized embryo transfer (pET).

The ERA® can be performed in natural or Hormone Replacement Therapy (HRT) cycles, according to the kind of cycle in which the subsequent embryo transfer will be performed (not including 'fresh' controlled ovarian stimulation cycles). To ensure reproducible results, an endometrial biopsy must be taken reproducing exactly the same conditions as will be used in the embryo transfer cycle (e.g. type of cycle, medication, way of administration, etc.).

Ideally, the first biopsy will be performed always after five full days (i.e. 120 hours) of progesterone (P4) administration (designated as P+5) in HRT, or 7 days (i.e. 168 hours) after the human chorionic gonadotropin (hCG) trigger (designated as hCG+7) in natural cycles. Even if the intention is to transfer Day-3 embryos, the biopsy should be done at P+5 or hCG+7 since the ERA® checks the endometrium at the moment of implantation. In this way, if you have a receptive result at P+5 you will transfer a blastocyst at P+5 or a day-3 embryo two days earlier (i.e. P+3). When taking the endometrial biopsy, it is very important to take sufficient tissue (30-50mg), ensuring that it is all tissue and not blood or mucus. The tissue collected should not exceed the white line of the cryotube as this can result in RNA degradation which will affect the accuracy of test results.

The ERA® result is based on analyzing the expression level of 236 genes with a computerized predictor designed, developed and patented in 2009 (PCT/ES2009/000386) by IGENOMIX after more than 10 years of research. After analyzing by NGS the genetic material (RNA) extracted from the endometrial biopsy, it is possible to evaluate if the patient's endometrium is Receptive or Non-Receptive at a given moment of her menstrual cycle. If a patient has a Non-Receptive result after the first biopsy, a displacement of the window of implantation (WOI) can be confirmed by analyzing a second biopsy performed on the day specified by the first ERA® result.

See Appendix I for further details about the ERA® test and scientific evidence supporting its use.

Complete documentation regarding this service is available at www.igenomix.com/era-docs

2. The endometrial biopsy protocol

Informed Consent

The patient must be properly counselled and receive the necessary information about the complete ERA process. The patient and the clinician will both need to sign the ERA Test Requisition Form prior to the biopsy being taken.

Endometrial Biopsy

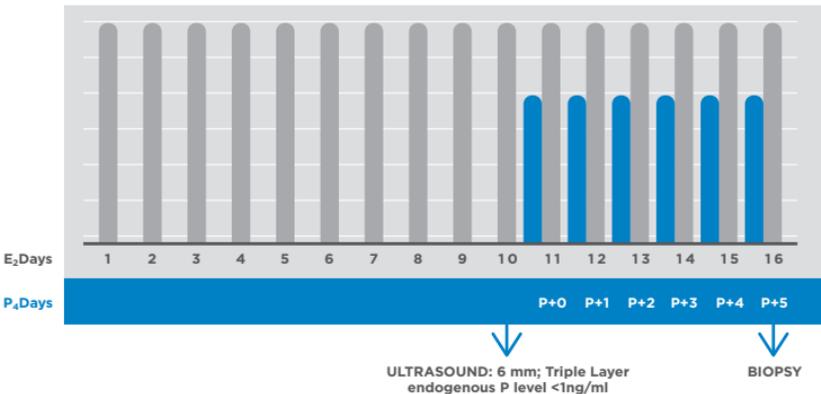
The endometrial biopsy can be taken in an HRT cycle or in a natural cycle. The endometrial biopsy will be taken from the uterine fundus using a Pipelle catheter (Genetics, Namont Achel, Belgium) or similar. When taking the endometrial biopsy it is very important to take sufficient tissue (30-50mg), ensuring that it is all tissue and not blood or mucus. The tissue collected should not exceed the white line of the cryotube as this can result in RNA degradation. The diagnosis of receptivity is valid for the type of cycle in which the test was performed, and therefore the embryo must be transferred in the same type of cycle and personalized window of implantation within which a 'Receptive' diagnosis was obtained.

a) Hormone Replacement Therapy Cycle: Involves treatment with oestrogen and progesterone to inhibit endogenous production of these hormones, following standard HRT protocols at the clinic (or the standard IGENOMIX HRT protocol can be provided).

The day of the endometrial biopsy in an HRT cycle is determined as follows:

- Ultrasound assessment is performed after 7 to 10 days of estradiol priming. When a trilaminar endometrium ≥ 6 mm is obtained along with a serum progesterone level < 1 ng/ml (to be measured), progesterone treatment begins. The day on which the progesterone treatment begins is referred to as P+0, and the biopsy is taken on day P+5, after five full days (120 hours approx.) of progesterone administration. For example, if progesterone administration begins on Wednesday the patient will be referred for endometrial biopsy on the following Monday.

In HRT cycles it is very important to measure the endogenous progesterone level a day prior to the first day of progesterone administration to ensure that there is no ovulation. The level should be < 1 ng/ml, otherwise it is recommended to cancel the cycle and start again.



b) Natural Cycle with an hCG trigger: The day of the biopsy in a natural cycle after hCG injection is determined as follows:

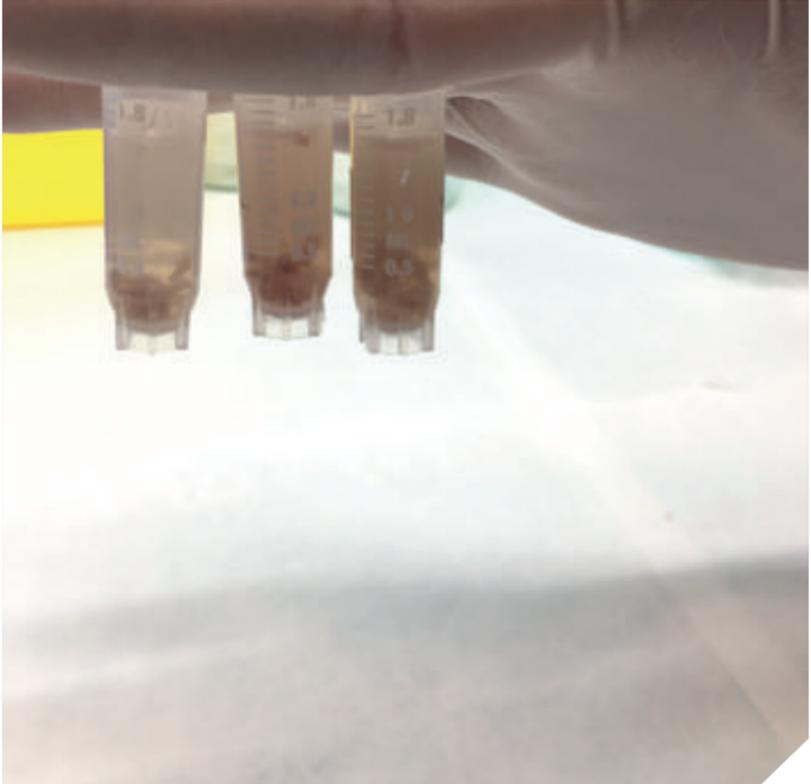
- hCG (either recombinant or urinary) is administered according to routine parameters in a natural cycle (follicle size > 17 mm). The day of the hCG administration is referred to as day hCG+0 and the endometrial biopsy is taken seven days (168 hours approx.) later (hCG+7). For example, if the hCG injection is on Monday the patient will be referred for endometrial biopsy on the following Monday.

If the intention is to transfer Day-3 embryos the biopsy should still be done at P+5, LH+7 or hCG+7, since the ERA® checks the endometrium at the moment of implantation. In this way, if you have a receptive result at P+5 you will transfer a blastocyst at P+5 or a day-3 embryo two days earlier (i.e. P+3).

Sample preparation

- IGENOMIX will supply an ERA® kit that includes a cryotube for each biopsy. The IGENOMIX cryotube contains 1.5 ml of a transparent stabilizing solution for the RNA in the tissue. Label the cryotube either with the Patient's initials, DOB and date of biopsy or with the Patient's initials and Unique Patient ID Number.
- After the biopsy has been performed, the sample is transferred immediately to the supplied cryotube. The cryotube with the sample should be vigorously shaken for a few seconds. The image below is an example of an endometrial biopsy in the cryotube. Please ensure that the cryotube actually contains endometrial tissue before sending it for testing.
- The cryotube with the sample must be stored in a refrigerator (4-8°C/39-46°F) immediately after being taken for at least 4 hours. After this time, samples may be sent to IGENOMIX at room temperature (<35°C/95°F) inside a plastic container (provided in the ERA kit) using a courier company (i.e DHL, TNT...). Samples may be kept inside a refrigerator for up to 3 weeks or may be frozen at -20°C/-4°F (following the first 4 hours in a fridge) if they are not to be sent immediately to IGENOMIX. In any case, shipping to IGENOMIX (conducted at room temperature) should never take longer than 5 days in transit. When the outside ambient temperature exceeds 35°C please include an ice pack along with the plastic container (containing the sample) or contact the laboratory for further instructions on how to send the sample.

Example of endometrial tissue sample in the stabilizing solution



❖ 3. Standard procedure for sending samples to the laboratory

Sample(s) and documentation:

- Sample: The prepared sample in the ERA® cryotube is transported at room temperature (<35°C/95°F) (check previous section for sample preparation). Please ensure that the cryotube is properly closed and ideally sealed with film to prevent leakage and placed in the plastic container (provided with the ERA kit).
- Documentation: The ERA Test Requisition Form must be completed and included with each sample shipment

Shipping:

- IGENOMIX will arrange the shipping. To do so please visit www.igenomix.com/era-form and completing the required fields for each sample shipment.
- When the outside ambient temperature exceeds 35°C please include an ice pack along with the plastic container (containing the sample) or contact the laboratory for further instructions on how to send the sample.

IGENOMIX Contact details:

www.igenomix.com/era-form

❖ 4. Generic interpretation of the results

Interpreting the results:

Once the sample has been analyzed, there are several possible results:

Receptive: This gene expression profile is concordant with a normal receptive endometrium.

Pre-receptive: This gene expression profile is concordant with an endometrium at a pre-receptive stage. It may be due to the displacement of the window of implantation, and to confirm this a second biopsy on the recommended day should be analyzed.

Post-receptive: This gene expression profile is concordant with an endometrium at a post-receptive stage. It may be due to the displacement of the window of implantation, and to confirm this a second biopsy on the indicated day should be analyzed.

Proliferative: This gene expression profile is concordant with an endometrium at a proliferative stage. It is recommended to contact the ERA® laboratory to discuss the type of cycle in which the biopsy was taken.

Non-informative: The profile analyzed does not match the control gene expression profiles present in the ERA® predictor. It is recommended to contact the ERA® laboratory to discuss the protocol and repeat the biopsy.

Insufficient RNA: It was not possible to determine the gene expression profile of the sample because there was not enough biopsy material. This occurs in approximately 2.5% of samples received. It is recommended to perform a second biopsy.

Invalid RNA : It was not possible to determine the gene expression profile of the sample due to the poor quality of genetic material obtained. This occurs in approximately 3% of samples received. It is recommended to perform a second biopsy following the sample stabilization instructions.

The aim of this test is to provide physicians with an objective molecular diagnosis of the patient's endometrial reproductive health. Depending on the result of this analysis, the physician may use it to guide personalized embryo transfer (pET).

Following ERA® report recommendations does not guarantee implantation. Failed implantation may be caused by other factors including, but not limited to, poor embryo quality, genetic abnormalities, or previous pathologies.

Contact: www.igenomix.com/era-form

❖ Appendix I. Evidence

The development of the molecular tool resulted from a translational research project:

- A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. Diaz-Gimeno, et al. 2011. *Fertil. Steril.*, 95:50-60, 60.e1-15

Accuracy and reproducibility of the ERA® test was proven in:

- The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. Diaz-Gimeno, et al. 2013. *Fertil Steril.* 99(2):508-17

Clinical applicability in patients with repeated implantation failures was demonstrated in:

- The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Ruiz-Alonso, et al. 2013. *Fertil Steril.* 100(3):818-24
- What a difference two days make: "personalized" embryo transfer (pET) paradigm: a case report and pilot study. Ruiz-Alonso, et al. 2014. *Hum Reprod.* 29(6):1244-7.

A prospective, randomized study on the effectiveness of the ERA® test in patients who have not received previous assisted reproduction treatments is presently underway (ClinicalTrials.gov Identifier:NCT01954758). Interim results from this study were presented at the American Society of Reproductive Medicine (ASRM) 2016 Scientific Congress (*Fertil Steril.* 2016 Sep;106(3):e46-e47). This abstract was awarded Prize Paper by the Society of Reproduction, Endocrinology and Infertility (SREI).

ARTICLE IN PRESS

ORIGINAL ARTICLE REPRODUCTIVE BIOLOGY

The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity

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Objective: To compare the accuracy and reproducibility of the endometrial receptivity array (ERA) versus standard histology method.
Design: Comparative prospective study (May 2008–May 2012).
Setting: University-affiliated fertility clinic.
Patients: 100 fertile women, regular cycles, aged 30–40 years with a body mass index (BMI) of 16–30 kg/m².
Interventions: Each received histology and ERA on the same day. For the ERA, a custom study, 17 targeted microarray probes were the testing set (n = 16) for ERA and a standard testing set including, and a dating set (n = 1) for comparison.

(ORIGINAL ARTICLE: WFSR107)

The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

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Objective: To evaluate the clinical value of the endometrial receptivity array (ERA) in patients with repeated implantation failure (RIF) for getting their personalized embryo transfer (pET) as a novel therapeutic strategy.

GENETICS

A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature

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Objective: To create a genomic tool composed of a customized microarray and a bioinformatic pipeline for human endometrial arrays and to detect pathological endometrial stages. To define the transcriptomic signature of human endometrial receptivity.
Design: Two cohorts of endometrial samples along the menstrual cycle were used: one to assess the genes to be included in the customized microarray endometrial receptivity array (ERA), and the other to be analyzed by ERA to assess the predictive for endometrial timing and to define the transcriptomic signature. A third cohort included only pathological endometrial samples was used to test the predictive for pathological endometrialization.
Setting: Fertility clinic, private and public.
Patients: Healthy fertile women (N) and women with implantation failure (P) in hydroalpinic (H).
Interventions: Custom endometrial arrays.
Main Outcome/Measure: The gene expression of endometrial biopsies.
Results: The ERA included 134 selected genes. The transcriptomic signature was defined by 130 genes. The predictive showed a specificity of 0.887 and sensitivity of 0.870 for endometrial timing, and a specificity of 0.977 and a sensitivity of 0.897 for the pathological classification.
Conclusions: The diagnostic tool for endometrial receptivity endometrial timing and genealogy. The transcriptomic signature is a potential endometrial receptivity biomarker disease (short list) (P) (101, 88, 128) by American Society for Reproductive Medicine.
Key Words: Endometrial receptivity, endometrial dating, recurrent miscarriage, transcriptomic signature, predictive

Human Reproduction, Vol 39, No 4, April 2014, pp 1244–1249, 2014
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Human reproduction

CASE REPORT Infertility

What a difference two days make: “personalized” embryo transfer (pET) paradigm: A case report and pilot study

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ABSTRACT: Embryo implantation requires at the blastocyst and attach during the receptive stage of the endometrium. Issues in timing of implantation (DPO), receptivity, if it has been assumed that the WVO is always correct in all women. However, results also analysis of endometrial receptivity (ERA) and personalized embryo transfer (pET) (pET) that is displayed in one out of four patients suffering from recurrent implantation failure (RIF) of endometrial stage and duration of the cycle. In order to improve the diagnostic approach, here, we report a retrospective analysis of a successful personalized embryo transfer (pET) after four IVF and three miscarriage duration failed attempts in which different embryo transfer strategies were attempted. This case report is complemented by a pilot study of 17 patients undergoing accurate donation and who suffered failed implantations with routine embryo transfer (ET) but were then treated with pET after the personalized diagnosis of their WVO.

Key words: personalized embryo transfer / timing of implantation / recurrent implantation failure / assisted reproduction

❖ Appendix II. ERA® Frequently asked questions (FAQ)

1) What are the clinical indications for the ERA® test?

The ERA® has been tested in patients who have had implantation failure with embryos of good morphological quality.

This test is recommended for patients with a morphologically normal uterus and normal endometrial thickness (≥ 6 mm), where the uterus and endometrium are unlikely to be the problem.

Its use for other indications should be decided by the gynecologist. We do not yet have sufficient clinical data on uterus/endometrium with recognizable problems; the diagnosis of the ERA® test could reflect these conditions.

2) In which type of cycle can the test can be performed?

The ERA® test should be performed in an HRT cycle or a natural cycle. The diagnosis of receptivity is valid for the type of cycle in which the test was performed, therefore embryo transfer must be performed in the same type of cycle (and implantation window in the case of personalization) in which a 'Receptive' diagnosis was obtained.

The ERA® test diagnoses the endometrial receptivity of a woman placed under a defined hormonal cycle, HRT or natural. We have observed that some patients may be sensitive to these hormonal differences and the time of receptivity varies according to the type of cycle.

The embryo transfer can be performed in the cycle following the completion of the ERA® test or in any subsequent cycle deemed suitable by the patient and her doctor. The consistency of the test has been verified up to two years later.

The ERA® test is not performed in ovarian stimulation cycles because it is known that the process of stimulation affects the endometrium. The endometrial expression profile has not been studied using ERA® in cycles of controlled ovarian stimulation (COS).

3) What is the clinical management of an embryo transfer following the ERA® test?

The endometrial biopsy must be taken in an HRT cycle or a natural cycle for receptivity diagnosis by the ERA® test.

Sample processing to diagnosis requires several days. If you want to perform embryo transfer based on the ERA® result you must wait for a subsequent cycle.

Result ‘Receptive’

If the patient has frozen eggs or embryos, or has fresh eggs or embryos from ovum donation: transfer the embryos in the same type of cycle (HRT or natural) in which a ‘Receptive’ ERA® test outcome was obtained.

If the patient does NOT have eggs or embryos frozen and wants to use her own eggs: an ovarian stimulation cycle should be performed for egg or embryo cryopreservation. Transfer the embryos in a subsequent cycle, in the same type of cycle (HRT or natural) in which a ‘Receptive’ ERA® test outcome was obtained.

You can transfer embryos in the COS cycle, however the result of the ERA® test DOES NOT apply to COS cycles, as mentioned above in the point: ‘Type of cycle in which the test can be performed’.

Result ‘Non-receptive’ with the recommendation of a new window of implantation (WOI)

If the result of a first ERA® test is ‘Non-receptive’ and the expression profile analysis suggests that the window of implantation may be displaced, it is necessary to confirm the personalized WOI (pWOI).

For this confirmation, take an endometrial biopsy in the suggested pWOI. If the result of the second ERA® is “Receptive” egg or embryo thawing and the subsequent transfer must be scheduled to coincide with the day of the patient’s confirmed pWOI. The pWOI is also valid for subsequent embryo transfers in the case of failure of the first embryo transfer.

4) How do I perform an endometrial biopsy?

The biopsy of the uterine fundus is performed according to standard procedures with a Pipelle catheter or similar.

When taking the endometrial biopsy it is very important to take sufficient tissue (30-50mg), ensuring that it is all tissue and not blood or mucus. The tissue collected should not exceed the white line of the cryotube as this can result in RNA degradation. As far as possible avoid introducing other liquids or fluids (vaginal or uterine/mucus or blood) into the ERA® cryotube. This does not cause significant sample contamination but can affect the preservation of the sample and reduces the quality of the genetic material and thus the accuracy of the result that can be obtained.

5) What are the handling, storage and shipping requirements for the biopsy samples?

After taking a biopsy the tissue must be transferred to the ERA® cryotube, provided by IGENOMIX, that contains a transparent RNA stabilizing solution. The ERA® cryotube containing the sample should be stored immediately in the fridge (4-8°C/39-46°F) for at least four hours. After this refrigeration period the sample can be sent to our facilities at room temperature with the customer courier service of choice.

For convenience, once in the ERA® cryotube and following the mandatory four hour refrigeration period, the sample can be stored in the refrigerator (4-8°C/39-46°F) for up to three weeks or frozen at -20°C. The shipment to our facility, at room temperature, should not exceed 5 days (120 hours).



