Index:

1. Summary of the Endometrial Receptivity Analysis (ERA®) diagnostic process 02
2. The endometrial biopsy protocol 03
3. Standard procedure to send and receive the sample 06
4. Generic interpretation of the results 07

Annexes:

I. Scientific evidence 08
II. Questions and Answers about ERA® 10
Endometrial gene expression signature allows us to diagnose the woman’s endometrial receptivity status as another reproductive health parameter. This analysis is carried out with a tool that has been designed, developed and patented in 2009 (PCT/ES2009/000386) by IGENOMIX after more than 10 years of research.

The Endometrial Receptivity Analysis (ERA®) is a molecular tool that analyzes by NGS the transcriptome of 236 genes related to endometrial receptivity status.

The ERA® can be performed in natural or HRT cycles, according with the kind of cycle in which subsequently the embryo transfer is going to be performed. But not in controlled ovarian stimulation cycles. For that, an endometrial biopsy must be taken reproducing exactly the same conditions that will have the embryo transfer cycle (type of cycle, medication, way of administration...). This is very important in order to make the result reproducible.

The first biopsy will be performed always after five full days with progesterone administration (P+5) in HRT cycles (this is, 120 hours with progesterone administration), or 7 days after the hCG triggering (hCG+7) in natural cycles (this is, 168 hours after the hCG). In the case of transferring 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 that 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, this is, at P+3. When taking the endometrial biopsy it is very important to take enough quantity of tissue (30-50mg), being sure that there is all tissue and not blood or mucus. It is important too not to exceed the white line of the cryotube with the quantity of tissue, since it could lead to RNA degradation.

The ERA® result is based on analyzing the expression level of 236 genes with a computerized predictor designed and developed by IGENOMIX. After analyzing by NGS the genetic material (RNA) 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 endometrial cycle. In case that a patient is Non Receptive, a displacement of the window of implantation (WOI) could be validated by analyzing a second biopsy performed on the day specified by the first ERA® analysis.

The purpose of the ERA® test is to locate the optimum day of receptivity, even when there is a displaced WOI, in order to perform a personalized embryo transfer (pET).

For further details about the tool design, Annexe I provides the scientific evidence that supports this service.

The whole documentation regarding this service is available at www.igenomix.com/era-docs
Informed Consent

The patient must receive the necessary information about the complete process. The patient and the doctor will need to sign the ERA® informed consent 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 enough quantity of tissue, around 30-50mg, being sure that there is not only blood or mucus. It is important too not to exceed the white line of the cryotube with the quantity of tissue, since it could lead to 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 protocols at the clinic (or standard IGENOMIX HRT protocol can be provided).

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

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

In HRT cycle it is very important to be sure that there is no ovulation, and therefore it is recommended to always measure the endogenous progesterone level a day prior to the first day of progesterone intake. The level should be <1ng/ml, otherwise it is recommended to cancel the cycle and start again.
b) Natural Cycle with hCG: The day of the biopsy in a natural cycle after hCG injection is determined as follows:

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

In the case of transferring Day-3 embryos the biopsy should be done at P+5, LH+7 or hCG+7, since the ERA® checks the endometrium at the moment of implantation. In that 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, this is, at P+3.

Standard procedure to extract and collect the sample

- IGENOMIX will supply 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 MRN.

- After the biopsy has been performed, the sample will be transferred immediately to the supplied cryotube. The cryotube with the sample will be vigorously shaken for a few seconds. The image below is an example of an endometrial biopsy in the cryotube. Make sure that the cryotube actually contains endometrial tissue before sending it to our premises.

- The cryotube with the sample will be kept inside 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 padded envelope by a courier company. Samples may also be kept inside a refrigerator for up to 3 weeks or may be frozen at -20°C/-4°F (after the first 4 hours) if they are not to be sent immediately to IGENOMIX. In any case, deliveries should be sent at room temperature but should never take longer than 5 days.
Example of endometrial tissue sample in the stabilizing solution
3. Standard procedure to send and receive samples

Samples and documentation:

- Sample: Send the biopsy at room temperature (<35ºC/95ºF) in the ERA® cryotube. The tubes should be closed and sealed with film to prevent small leakage and sent following the instructions given by IGENOMIX in a padded envelope or similar.

- For shipment during the summer, when it can be reached temperatures >35ºC/95ºF, it is recommended to add an ice pack in the shipment.

- Documentation: A copy of the informed consent and a copy of the test requisition form must be completed for each sample and shipped along with the sample.

Shipping:

- Please inform us by email (www.igenomix.com/era-form) of each shipment of samples, indicating the number of samples and their clinical or reference record number.

- You may employ your usual courier company; if you wish, we can inform you about our pick up service.

IGENOMIX Contact details:

www.igenomix.com/era-form
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 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 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 do 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 do 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 such as poor embryo quality, genetic abnormalities, or previous pathologies.

Contact: [www.igenomix.com/era-form](http://www.igenomix.com/era-form)
Annexe I. Scientific evidence

The development of the molecular tool resulted from the translational research project published in Fertility & Sterility:


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


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


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

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ORIGINAL ARTICLE REPRODUCTIVE BIOLOGY

Objective: To compare the accuracy and reproducibility of the endometrial receptivity array (ERA) versus standard histological methods.


Setting: University-affiliated tertiary center.

Population: Women with varying age, gynecologic history, and cycle day on whom endometrial biopsies were performed (n = 113).

Intervention: ERA versus histology.

Main Outcome Measures: Accuracy and reproducibility of ERA versus histology.

Results: ERA was superior to histology for detecting endometrial receptivity.

Conclusion: ERA is a reliable and reproducible method for detecting endometrial receptivity.

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

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ORIGINAL ARTICLE INFERTILITY

Objective: To determine the clinical value of the endometrial receptivity array (ERA) in patients with repeated implantation failure (RIF), in order to personalize embryo transfer (ET) as a new therapeutic strategy.

GENETICS

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

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Background: To create a genomic tool composed of a characteristic transcriptome and a bioinformatic platform for endometrial evaluation, a new hypothesis of functional endometrial expression is proposed. A transcriptomic signature has been generated and validated using next-generation sequencing techniques.

Methods: A total of 150 endometrial biopsies were collected from women undergoing IVF or ET. The transcriptomic signature was validated using RNA-Seq in an independent cohort of 25 patients.

Results: The transcriptomic signature was validated as a predictive model with a sensitivity of 94% and a specificity of 96% for endometrial receptivity. The transcriptomic signature was applied to a clinical cohort of 60 patients, achieving a sensitivity of 93% and a specificity of 94% for endometrial receptivity.

Conclusion: The transcriptomic signature could be used as a diagnostic tool for predicting endometrial receptivity.

CASE REPORT INFERTILITY

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


Objective: To report a case of successful personalized embryo transfer (PET) after two failures of conventional transfer (CT) and to explore whether personalized embryo transfer (PET) could improve the success rate of IVF.

Methods: A 42-year-old infertile woman with poor ovarian response (B1) underwent PET after two failures of conventional transfer (CT). The PET strategy was based on the results of the ERA performed on day 17 of the cycle.

Results: The PET strategy resulted in a successful pregnancy, with a delivery of a healthy baby at 37 weeks of gestation.

Conclusion: Personalized embryo transfer (PET) could improve the success rate of IVF.

Key words: personalized embryo transfer / window of implantation / recurrent implantation failure / assisted reproduction
1) Clinical indication 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 ($\geq 6$ 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 have sufficient clinical data on uterus/endometrium with recognizable problems; the diagnosis of the ERA® test could reflect these conditions.

2) Type of cycle in which 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 ERA® endometrium expression profile has not been studied in cycles of controlled ovarian stimulation (COS).

3) Clinical management of 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 later 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 will 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 validate the personalized WOI (pWOI).

For this validation, 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 validated pWOI. The pWOI is also valid for subsequent embryo transfers in the case of failure of the first embryo transfer.
4) Performing 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 enough quantity of tissue, around 30-50mg, being sure that there is not only blood or mucus. It is important too not to exceed the white line of the cryotube with the quantity of tissue, since it could lead to RNA degradation. As far as possible avoid introducing other liquids or fluids, vaginal or uterine, or mucus or blood into the ERA® tube. This does not cause significant sample contamination but can affect the preservation of the sample and reduces the quality of the genetic material that can be obtained.

5) Submission of samples

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

Once in the ERA® tube, the sample can be stored in the refrigerator (4-8°C/39-46°F) for up to three weeks. The shipment to our facility, at room temperature, should not exceed 120 hours.