ERA®
Endometrial Receptivity Analysis
Patented in 2009

igenomix
PIONEERS IN REPRODUCTIVE GENETICS
ERA® analyzes the expression of 248 genes using NGS to determine the personalized window of implantation for each patient.
The ERA® test is a molecular tool based on gene expression used to determine when the endometrium is receptive

- The test consists of:
  - NGS analysis of the expression of 248 genes involved in endometrial receptivity.
  - An informatic predictor that analyzes the gene expression data and classifies the endometrium as “Receptive” or “Non Receptive” with a sensitivity of 90% and a specificity of 97%.

Patented in 2009: PCT/ES 2009/000386
Scientific Support: IGENOMIX Publications

- The development of the ERA® diagnostic tool was published in the paper by Díaz-Gimeno et al., 2011 (Fertil Steril. 2011 Jan;95(1):50-60, 60.e1-15).

- ERA®'s effectiveness and consistency was demonstrated in the paper by Díaz-Gimeno et al., 2013 (Fertil Steril. 2013 Feb;99(2):508-17).

The Diagnostic Value of Endometrial Evaluation:

- The ERA test diagnoses the state of the endometrium during the Window of Implantation and determines the optimal timeframe for embryo transfer.

- In a blinded study, the ERA test classified endometrial receptivity better than histology using the Noyes criteria.

### KAPPA VALUE

<table>
<thead>
<tr>
<th>Pathologist 1 (P1)</th>
<th>Pathologist 2 (P2)</th>
<th>P1 vs P2</th>
<th>ERA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.618 (0.446-0.791)</td>
<td>0.685 (0.545-0.824)</td>
<td>0.622 (0.435-0.839)</td>
<td>0.922 (0.815-1.000)</td>
</tr>
</tbody>
</table>

0.61-0.80  
Good Concordance  
0.81-1.00  
Very Good Concordance

Clinical Support: IGENOMIX Publications

- ERA clinical applicability to patients with implantation failure was demonstrated in publications by Ruiz-Alonso et al., 2013 and 2014.

- A randomised study is currently in progress to assess its applicability to patients without any prior assisted reproduction treatment (ClinicalTrials.gov Identifier: NCT01954758).
Clinical Support: External Publications

A number of IVF clinics around the world have published their clinical experience using the ERA test in their patients to assess endometrial receptivity and adjust the timing of transfer based on ERA test results.

Canada
Tan et al, 2018
J Assist Reprod Genet, 2018 Jan; doi: 10.1007/s10815-017-1112-2

Japan
Hashimoto et al, 2017

India
Mahajan, 2015
ERA indications - Which patients may benefit?

- **Recurrent implantation failure patients:**
  - Two or more implantation failures with good quality autologous embryos or one failed implantation with good quality donor eggs.

- **Patients with morphologically normal endometrium:**
  - ERA® after intervention in the case of a congenital uterine abnormalities.

- **Patients with normal, atrophic or hypertrophic endometrium:**
  - ERA® can be used for patients with normal, atrophic or hypertrophic endometrium so long as the endometrial appearance is consistent for all cycles.
ERA biopsy cycle - When should the biopsy be taken?

- **Hormone replacement therapy**
  
  Patients start estradiol therapy from the 1st or 2nd day of the menstrual cycle. Ultrasound assessment is performed between 7-10 days after the start of estradiol administration. Start the progesterone (P4) intake when a trilaminar endometrium >6mm is reached with a serum progesterone <1ng/ml (within the 24 hours prior to starting exogenous progesterone). Progesterone is administered for five full days (120 hours).

- **Natural cycle**
  
  hCG (recombinant or urinary) is administered according to routine parameters in a natural cycle (follicle size >17mm). The biopsy will be taken 7 days (168 hours) after the hCG triggering.

- **Controlled ovarian stimulation**
  
  ERA® cannot be performed in a controlled ovarian stimulated cycle. Therefore it should be performed in a subsequent HRT or natural cycle as indicated above.
How to take the endometrial biopsy

Prior to biopsy, the ERA Cryotube should be prepared and labeled with the patient’s name and a second identifier (e.g. DOB or Medical Record Number/Unique Patient Identifier). The biopsied tissue should be introduced directly into the Cryotube and the Cryotube shaken vigorously for at least 10 seconds.

The amount of tissue should not exceed the white line on the Cryotube in order to ensure proper preservation of the RNA within the biopsy sample.

Storage

Immediately place the Cryotube in a refrigerator (4-8°C/39-46°F) and hold at this temperature for at least 4 hours. This preserved sample, in the original cryotube, can then be shipped at room temperature. Alternatively, the samples can be kept in the fridge for 3 weeks or frozen at -20°C/-4°F (recommended) until the time of shipment. The sample can then be shipped at room temperature (<35°C/95°F).

We recommend including an ice pack for shipments made during the summer months.
Shipment

- Samples are sent at room temperature (<35°C/95°F) accompanied with the patient’s informed consent and a completed requisition form. Samples shipped at room temperature should reach us in a maximum of 4 to 5 days.

- For more instructions regarding sample packaging, please contact us via [www.igenomix.com/era-form](http://www.igenomix.com/era-form)

Confirm shipping by accessing the following link: [www.igenomix.com/era-form](http://www.igenomix.com/era-form)
ERA (ENDOMETRIAL RECEPTIVITY ANALYSIS)

Patient information
Unique pat id.: MUE-000
Sample type: Endometrial biopsy
Patient name:
Patient DOB:

Sample information
Date received: 20/12/2017
Report Date: 28/12/2017
Progesterone: 0.2 ng/mL

Clinic information
Clinic: IVF Clinic
Clinician: Dr.
No. biopsy: 1

Measure date:
First intake of P 14/12/2017 9:00 AM
Date of biopsy: 19/12/2017 9:00 AM
Cycle type: HRT P+5 (120 hours)

TEST RESULTS:

RECEPTIVE: EARLY RECEPTIVE

Recommendation: The personalized embryo transfer (pET) of a blastocyst/s should be performed with 132 ± 3 hours of progesterone administration (12 hours later than the time at which this endometrial biopsy was performed). A new endometrial biopsy is not required. **
ERA Results - Interpretation

**Receptive:**
This gene expression profile is compatible with a normal receptive endometrium. We recommend performing a blastocyst(s) transfer following the same protocol utilized during the Endometrial Receptivity Analysis biopsy cycle.

**Early receptive:**
The gene expression profile is concordant with an endometrium that is at the beginning of the receptive stage. We recommend progesterone administration (HRT cycle) or rest (natural cycle) for 12 hours more relative to when the biopsy was taken before performing a blastocyst(s) transfer.

**Late receptive:**
The gene expression profile is concordant with an endometrium that is at the end of the receptive stage. We recommend progesterone administration (HRT cycle) or rest (natural cycle) for 12 hours less relative to when the biopsy was taken before performing a blastocyst(s) transfer.
ERA Results - Interpretation

- **Example 1:**
  A patient with a delayed WOI shows a pre-receptive result at P+5.

- **Pre-receptive:**
  This gene expression profile is concordant with an endometrium at a pre-receptive stage. According to the specific profile obtained it could be directly recommended the blastocyst(s) transfer by adding 1 more day of progesterone exposure. In some cases (when 2 more days with progesterone exposure are needed) a new endometrial biopsy could be required.

**HRT CYCLE**

<table>
<thead>
<tr>
<th>Delayed WOI [ERA® P+5: PRE]</th>
</tr>
</thead>
</table>

**Endometrium’s state:**

- Pre-receptive
- Receptive
- Post-receptive
ERA Results - Interpretation

Example 2:
A patient with an advanced WOI shows a post-receptive result at P+5.

Post-receptive:
This gene expression profile is concordant with an endometrium at a post-receptive stage. To validate this result, the analysis of a second biopsy on the recommended day is needed.
Personalized embryo transfer (pET)

A blastocyst (day 5-7) should be transferred on the day in which the endometrium was found to be receptive. A day-3 embryo should be transferred two days earlier than the day in which the endometrium resulted receptive.
Samples with No Result

- **Invalid RNA (3%)**, possible causes:
  - High temperatures (>35°C) during the shipment
  - Sample size too large
  - Too much blood or mucus

- **Insufficient RNA (2.5%)**, possible causes:
  - Sample size too small
  - Too much blood or mucus

- **Non informative (1%)**

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**3% invalid RNA**

**2.5% insufficient RNA**

**1% non informative**
ERA® Decision Tree

- If the patient results receptive or pre-receptive (needing just 1 more day with progesterone exposure) at P+5, then pET is directly recommended by following the specific indications given in the report. This happens in >90% of received samples.

- A 2nd endometrial biopsy is required if the ERA result is post-receptive (needing 1 day less of progesterone administration) or pre-receptive (but needing 2 additional days of progesterone administration).
Clinical outcome

Updated results 31/01/2018
ERA® results

>32,000 patients

>60 countries

More than 1,000 clinics

SAMPLES AT P+5 ANALYZED BY NGS

1st ERA Biopsy

Receptive
Non-receptive

30.2
69.8
**ERA® results**

- In the case of post-receptive result or a pre-receptive result needing 2 more days of progesterone exposure, a 2nd endometrial biopsy is required.

**NON RECEPTIVE CASES**

- 0.5% Proliferative
- 10.5% Post-receptive
- 89% Pre-receptive

More than 95% of pre-receptive samples need just 1 more day of progesterone administration. In these cases pET can be confidently performed by following the report recommendations without a 2nd biopsy.
Receptivity and obesity

- Patients with body mass index (BMI) >= 30 show higher risk of having displaced WOI.

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Normal (19-24.9) (n=163)</th>
<th>Overweight (25-29.9) (n=47)</th>
<th>Obese (≥30) (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>38.4</td>
<td>38.5</td>
<td>37.7</td>
</tr>
<tr>
<td>BMI (mean)</td>
<td>21.9</td>
<td>26.8</td>
<td>32.8</td>
</tr>
<tr>
<td>% non receptive patients by ERA®</td>
<td>25.2%</td>
<td>25.5%</td>
<td>36.4%</td>
</tr>
</tbody>
</table>

IGENOMIX Publication. Lathi R B et al. 2014, PCRS.
**Receptivity and endometrial thickness**

- Patients with atrophic endometrium (<6mm) show higher risk of having displaced WOI

<table>
<thead>
<tr>
<th>Endometrial thickness (mm)</th>
<th>Receptive</th>
<th>Non receptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>6/4 (42.85%)*</td>
<td>8/14 (57.14%)*</td>
</tr>
<tr>
<td>6-12</td>
<td>333/431 (77.26%)*</td>
<td>98/431 (22.73%)*</td>
</tr>
<tr>
<td>&gt;12</td>
<td>24/37 (64.86%)</td>
<td>13/37 (35.13%)</td>
</tr>
</tbody>
</table>

P: 0.0003 by Chi-square test

*IGENOMIX Publication: Valbuena D et al 2016 ESHRE.*
Clinical Outcome

Published Case Report regarding clinical applicability of the ERA® test in a RIF patient

- **Previous ART treatments**
  1. IVF with fresh day-3 ET
  2. IVF with fresh day-3 ET
  3. IVF with fresh day-5 ET
  4. IVF with frozen day-5 ET in a natural cycle
  5. OD with day-3 ET in an HRT cycle (P+2)
  6. OD with day-3 ET in a natural cycle
  7. OD with day-5 ET in an HRT cycle (P+5)

- **INTERVENTION**
  Finding Personalized WOI (ERA® Test)

- 8. OD with day-5 ET in an HRT cycle (P+7)
  Successful twin pregnancy

**Abbreviations**
- ET: Embryo Transfer
- HRT: Hormonal Replacement Therapy
- IVF: In-Vitro Fertilization
- OD: Ovum Donation
- WOI: Window of Implantation

**IGENOMIX Publication**
## pET - personalised embryo transfer

### pET Clinical Outcome

<table>
<thead>
<tr>
<th></th>
<th>pET after 1st ERA</th>
<th>pET after 2nd ERA</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of pET performed</td>
<td>175</td>
<td>86</td>
<td>261</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>53.4% (126/236)</td>
<td>60.5% (66/109)</td>
<td>55.7% (192/345)</td>
</tr>
<tr>
<td>Pregnancy rate (bhCG +)</td>
<td>69.7% (122/175)</td>
<td>73.3% (63/86)</td>
<td>70.9% (185/261)</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>9.8% (12/122)</td>
<td>4.8% (3/63)</td>
<td>8.1% (15/185)</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>9% (11/122)</td>
<td>3.2% (2/63)</td>
<td>7% (13/185)</td>
</tr>
<tr>
<td>Ectopic</td>
<td>0.8% (1/122)</td>
<td>1.6% (1/63)</td>
<td>1.1% (2/185)</td>
</tr>
<tr>
<td>Ongoing / Pregnancy</td>
<td>80.3% (98/122)</td>
<td>90.5% (57/63)</td>
<td>83.8% (155/185)</td>
</tr>
<tr>
<td>Ongoing / pET</td>
<td>56% (98/175)</td>
<td>66.3% (57/86)</td>
<td>59.4% (155/261)</td>
</tr>
</tbody>
</table>
ERA® Randomized Controlled Study

Interim results from a prospective randomized controlled study presented at ASRM show the improvement on Pregnancy Rate and Ongoing Pregnancy Rate.

<table>
<thead>
<tr>
<th></th>
<th>Fresh ET</th>
<th>Deferred ET</th>
<th>Personalized ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>117</td>
<td>122</td>
<td>117</td>
</tr>
<tr>
<td>Transfers (n)</td>
<td>60</td>
<td>74</td>
<td>49</td>
</tr>
<tr>
<td>Preg. Rate/ET (%)</td>
<td>61.7%</td>
<td>60.8%</td>
<td>85.7%*</td>
</tr>
<tr>
<td>Implantation Rate (%)</td>
<td>35.3%</td>
<td>41.4%</td>
<td>47.8%</td>
</tr>
<tr>
<td>Ongoing PR/ET (%)</td>
<td>43.3%</td>
<td>44.6%</td>
<td>55.1%</td>
</tr>
</tbody>
</table>

* p=0.003 by Chi-Square test

Award Winner
Society for Reproductive Endocrinology and Infertility (SREI)

Our paper **PROSPECTIVE, RANDOMIZED STUDY OF THE ENDOMETRIAL RECEPTIVITY ANALYSIS (ERA®) TEST IN THE INFERTILITY WORKUP TO GUIDE PERSONALIZED EMBRYO TRANSFER VERSUS FRESH TRANSFER OR DEFERRED EMBRYO TRANSFER** has been awarded as Prize Paper by the Society for Reproductive Endocrinology and Infertility, in this year’s ASRM Annual Meeting.