1. What is the ERA test?
   The ERA test is an endometrial evaluation test designed, developed and patented (PCT/ES2009/000386) by IGENOMIX. This molecular diagnostic tool allows to analyze the level of expression of 238 genes related with the status of endometrial receptivity. It consists of a personalized microarray with probes for these 238 genes onto which an RNA sample obtained from endometrial tissue is hybridized. After the hybridization, the intensities of the signals are scanned and the ERA predictor classifies the samples into receptive or non-receptive depending on the expression profile.

2. What is the purpose of the ERA test?
   The ERA test is used to evaluate the stage of an endometrium and to determine if it presented a receptive or non-receptive genetic profile when the biopsy was taken. In the case that the stage is non-receptive, the test allows us to find a personalized window of implantation for each patient.

3. Who should use the ERA test and why?
   The ERA test has been proven in patients who have experienced implantation failures with good quality embryos (at least 3 implantation failures in young women or 2 in patients 37 years-old or more). A displaced window of implantation is detected in approximately 25 % of these patients.

   This test is indicated for patients with an apparently normal uterus and with an endometrium with a normal thickness (6 mm or more).

4. Advantages of the ERA test
   The classical method of endometrial dating is based on histological criteria. However it has been proven that these criteria do not discriminate between fertile and infertile patients and are highly subjective, meaning that they have not clinical application. The ERA test has been shown to be highly sensitive and specific in the detection of genetic expression profiles associated with receptivity. It allows the personalized window of implantation to be detected before the patient starts using assisted reproduction techniques.

5. Sample extraction and shipment
   An endometrial biopsy taken from the uterine fundus must be immediately introduced into an ERA cryotube and preserved in a refrigerator (4-8ºC) for at least 4 hours.

   Shipment to our laboratory, at room temperature, should not take longer than 72-96 hours and the sample should not be allowed to reach more than 35˚C.

6. Methodology
   MAIN STAGES OF THE ASSAY

   - Messenger RNA (mRNA) is obtained
   - The quality of the extracted mRNA is determined
   - Sample labeling and purification
   - Hybridization of the labeled mRNA with the ERA array
   - Washing and scanning of the array
   - The signal intensity is measured and is classified by the predictor computer

7. Limits of the technique. The ERA test has a specificity of 0.8857 and a sensitivity of 0.9975 for receptivity profile classification. The biopsy procedure, though simple, has a risk (less than 5%) of not obtaining a sufficient quantity and/or quality of endometrial tissue, in which case it is impossible to perform the test and a new sample extraction would be required.
When to perform the embryo transfer

The blastocyst transfer should be performed in the same type of cycle and on the same day on which a receptive result was obtained. A receptive result implies that this is the day on which the blastocyst transfer (D5-6) should be performed. If a day 3 embryo is going to be transferred then the transfer should be performed 2 days earlier.

Comparison of clinical results

### CLINICAL OUTCOME

<table>
<thead>
<tr>
<th></th>
<th>ET</th>
<th>pET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Source of oocytes</td>
<td>Ovum</td>
<td>donation</td>
</tr>
<tr>
<td>Age</td>
<td>40.7 ± 4.7 (32-49)</td>
<td></td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>12.9% (4/31)</td>
<td>34.5% (10/29)</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>23.5% (4/17)</td>
<td>52.9% (9/17)</td>
</tr>
<tr>
<td>Ongoing pregnancy rate</td>
<td>0% (0/4)</td>
<td>66.7% (6/9)</td>
</tr>
<tr>
<td>Clinical abortion</td>
<td>100% (4/4)</td>
<td>0% (0/9)</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td>0.0% (0/4)</td>
<td>33.3% (3/9)</td>
</tr>
</tbody>
</table>

Classification of ERA patients according to their endometrial receptivity

- 24% of patients are non-receptive

Data from a pilot study done with patients undergoing embryo transfer (ET) before their first ERA test (on a day later diagnosed as non-receptive by the ERA) who subsequently did a 2nd biopsy and ERA test and received personalized embryo transfer (pET) on a day which the ERA gave a receptive diagnosis.