Endometrial Receptivity

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Molecular interplay in successful implantation

Efficiency of human embryo implantation

### Embryo Aneuploidies

<table>
<thead>
<tr>
<th>Species</th>
<th>Aneuploidy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>0.01 %</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.01 %</td>
</tr>
<tr>
<td>Human</td>
<td>20 – 100 %</td>
</tr>
</tbody>
</table>

### Implantation Rate (IR)

<table>
<thead>
<tr>
<th>Cycle Type</th>
<th>IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle</td>
<td>35 %</td>
</tr>
<tr>
<td>Euploid embryos</td>
<td>52 %  (Rubio et al. Fertil Steril 2017)</td>
</tr>
<tr>
<td>Rodents</td>
<td>95 %</td>
</tr>
<tr>
<td>Rabbits</td>
<td>96 %</td>
</tr>
</tbody>
</table>

The main difference between humans and rodents lies in

The endometrial/decidual control in human implantationversus

The embryo control in rodent implantation (embryonic diapause)
The Puzzle of the Endometrial Factor

- **Histology**
  - Noyes et al., 1950
  - Coutifaris et al., 2004
  - Murray et al., 2007

- **Immunohistochemistry**
  - Lessey et al.
  - Kliman et al.

- **Ultrasound**
  - Karius et al., 2014

- **Doppler**
  - Kupesick et al., 2001

- **Hysteroscopy**
  - Rambouts et al., 2016

- **Secretomics**
  - der Gaast et al., 2002, 2009
  - Vilella et al., 2013

- **Endometrial SC**
  - Gargett et al., 2009
  - Vilella et al., 2010, 2011, 2012
  - Santamaria et al., 2016

- **Omics**
  - Kasius et al., 2014
  - Murray et al., 2007
  - Coutifaris et al., 2004
  - Gargett et al., 2009

- **Microbes**
  - Franasiak et al., 2015
  - Moreno et al., 2016
  - Moreno et al., 2018

- **Microbiota**
  - Moreno et al., 2016

- **ESC**
  - Diaz et al., 2011, 2013
  - Aghajanova et al., 2012

- **Visuals**
The existence of a WOI (1956 Hertig & Rock)

In the 1990s, the clinical WOI was demonstrated by Navot

In 1999, Wilcox et al popularized the concept that the human embryo implants 8 to 10 days after ovulation. (But ovulation was identified on the basis of changes in urinary presence of estrone 3-glucuronide and pregnanediol 3-glucuronide (RIA))

Wide time frame, with the same success during these 3 days regardless of individual variations or hormonal status (natural cycle, COS, HRT).
Dating the endometrial biopsy

✓ Randomized studies
  - Interobserver and cycle-to-cycle (60%) variations
  - Endometrial dating is not related to fertility status

Histological dating is not a valid method for the diagnosis of luteal phase deficiency neither guidance throughout clinical management in infertility

Endometrial quality is identified during the window of implantation. It is crucial that the patient have a carefully timed endometrial biopsy. The specimen must be collected on cycle days 20–24 (7–11 days post LH surge).

Patterns of Integrin Expression

There are three typical patterns:

1. Beta-3 integrin **POSITIVE** with an "in-phase" endometrium from cycle days 20–24 (7–11 days post LH surge) is a normal pattern of expression.

2. Beta-3 integrin **NEGATIVE** with an "out-of-phase" endometrium occurs in a patient with Luteal Phase Defect, following treatment the patient is advised to undergo a repeat biopsy to confirm diagnosis.¹

3. Beta-3 integrin **NEGATIVE** in a patient with a normal "in phase" endometrium is associated with unexplained infertility,¹ minimal or mild endometriosis,² or hydrosalpinx.³

The E-tegrity test:

- Identifies endometrial quality
- Determines Beta-3 integrin presence
- Provides a histologic evaluation of the endometrium

Endometrial Cycle

Positive

Negative
Figure 3. Panel of markers of endometrial development. Researchers have discovered many products that are made by the endometrium. The most important of these products are only made at particular times of the menstrual cycle. For example, progesterone receptor (PR), mouse ascites Golgi mucin (MAG) and cyclin E are normally only made during the proliferative and early secretory phases (cycle days 5 to ~19), while leukemia inhibitory factor (LIF), αvβ3 integrin (β3), HOXA-10 (HOX) and p27 are normally only expressed in the secretory phase (cycle days ~17 to ~28). Modified from Langman’s Medical Embryology.

Figure 4. Cyclin E and p27 expression in fertile women. Cyclin E first appears at around cycle day 5 and continues to be expressed up until cycle day 19. After day 19, cyclin E normally is absent. p27, on the other hand, is absent until approximately cycle day 17, where it is seen for the remainder of the cycle. Modified from Langman’s Medical Embryology.

Figure 5. Cyclin E and p27 expression in women with unexplained infertility. The most striking difference between the cyclin expression of fertile women and infertile women is the persistence of cyclin E and decreased presence of p27 into the secretory phase. This finding represents a developmental arrest of the glands in the endometria of these women. Modified from Langman’s Medical Embryology.
The age of -OMICS

**Transcriptome**
- DNA → RNA → mRNA → Protein
- Transcription regulation
- Alternative splicing

**Proteome**
- Transduction regulation

**Metabolome**
- Metabolites
A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature

Patricia Díaz-Gimeno, Ph.D.,José A. Horcajadas, Ph.D.,José A. Martínez-Conejero, Ph.D.,Francisco J. Esteban, Ph.D.,Pilar Alami, M.D.,Antonio Pellicer, M.D.,and Carlos Simón, M.D.a,b

a Fundación IVI-Instituto Universitario IVI, University of Valencia, Valencia; b Instituto de Investigación, Sanitaria del Hospital Clínico de Valencia, Valencia University, Valencia, Spain; c Igenomix, Valencia; d Department of Experimental Biology, University of Jaén, Jaén; and e Centro de Investigación Príncipe Felipe, Valencia, Spain

Objective: To create a genomic tool composed of a customized microarray and a bioinformatic predictor for endometrial dating and to detect pathologies of endometrial origin. To define the transcriptomic signature of human endometrial receptivity.

Design: Two cohorts of endometrial samples along the menstrual cycle were used: one to select the genes to be included in the customized microarray (endometrial receptivity array [ERA]), and the other to be analyzed by ERA to train the predictor for endometrial dating and to define the transcriptomic signature. A third cohort including pathological endometrial samples was used to train the predictor for pathological classification.

Setting: Healthy oocyte donors and patients.

Patient(s): Healthy fertile women (68) and women with implantation failure (5) or hydrosalpinx (2).

Intervention(s): Human endometrial biopsies.

Main Outcome Measure(s): The gene expression of endometrial biopsies.

Result(s): The ERA included 238 selected genes. The transcriptomic signature was defined by 134 genes. The predictor showed a specificity of 0.8857 and sensitivity of 0.99758 for endometrial dating, and a specificity of 0.1571 and a sensitivity of 0.995 for the pathological classification.

Conclusion(s): This diagnostic tool can be used clinically in reproductive medicine and gynecology. The transcriptomic signature is a potential endometrial receptivity biomarkers cluster. (Fertil Steril® 2011;95:50–60. ©2011 by American Society for Reproductive Medicine.)

Key Words: Endometrial receptivity, endometrial dating, microarray, transcriptomic signature, predictor, diagnostic tool
Endometrial Receptivity Array (ERA)
Endometrial Receptivity Analysis (ERA-NGS)

238 genes

Bioinformatic analysis of data

Classification and prediction from gene expression

Patented in 2009: PCT/ES 2009/000386
LDT with CLIA
Predictor Classifies the Molecular Receptivity Status of the Endometrium
Retrainig the ERA Algorithms

- Proliferative
- Pre-receptive
- Early receptive
- Receptive
- Late receptive
- Post-receptive
The Symphony

Epithelial PR

Progesterone
Personalized embryo transfer (pET) as a treatment for RIF of endometrial origen
<table>
<thead>
<tr>
<th>YEAR</th>
<th>TITLE</th>
<th>JOURNAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature</td>
<td>Fertility and Sterility. 95(1): 50-60, 60.e1-15</td>
</tr>
<tr>
<td>2012</td>
<td>The genomics of the human endometrium</td>
<td>Biochimica et Biophysica Acta - Molecular Basis Disease. 1822(12):1931-42</td>
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<td>2013</td>
<td>The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity</td>
<td>Fertility and Sterility. 99(2):508-17</td>
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<td>2013</td>
<td>The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure</td>
<td>Fertility and Sterility. 100(3): 818-24</td>
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<td>2014</td>
<td>The impact of using the combined oral contraceptive pill for cycle scheduling on gene expression related to endometrial receptivity</td>
<td>Human Reproduction. 29(6):1271-8</td>
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<td>2014</td>
<td>What a difference two days make: “personalized” embryo transfer (pET) paradigm: A case report and pilot study</td>
<td>Human Reproduction. 29(6):1244-7</td>
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<td>2014</td>
<td>Scratching beneath ‘The Scratching Case’: systematic reviews and meta-analyses, the back door for evidence-based medicine</td>
<td>Human Reproduction. 29(8):1618-21</td>
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<tr>
<td>2014</td>
<td>Deciphering the proteomic signature of human endometrial receptivity</td>
<td>Human Reproduction. 29(9): 1957-67</td>
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<td>2014</td>
<td>Clinical Management of Endometrial Receptivity</td>
<td>Semin Reprod Med. 32(5):410-4</td>
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<td>2014</td>
<td>Timing the window of implantation by nucleolar channel system prevalence matches the accuracy of the endometrial receptivity array</td>
<td>Fertility and Sterility. 102(5):1477-81</td>
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<tr>
<td>2015</td>
<td>Human Endometrial Transcriptomics: Implications for Embryonic Implantation</td>
<td>Cold Spring Harb Perspect Med. 5(7):a022996</td>
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<td>2015</td>
<td>Understanding and improving endometrial receptivity</td>
<td>Current Opinion in Obstetrics &amp; Gynecology. 27(3):187-92</td>
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<td>2015</td>
<td>Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study</td>
<td>Reproductive BioMedicine Online. 31(5):647-54</td>
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<td>2016</td>
<td>Diagnosis of endometrial-factor infertility: current approaches and new avenues for research</td>
<td>Geburtshilfe Frauenheilkd. 76(6):699-703</td>
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<td>2017</td>
<td>Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis</td>
<td>Fertility and Sterility. 107(3):740-748.e2</td>
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<td>2017</td>
<td>Endometrial function: facts, urban legends, and an eye to the future</td>
<td>Fertility and Sterility. 108(1):4-8</td>
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<td>2017</td>
<td>Implantation failure of endometrial origin: it is not pathology, but our failure to synchronize the developing embryo with a receptive endometrium</td>
<td>Fertility and Sterility. 108(1):15-18</td>
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<td>2017</td>
<td>Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers</td>
<td>Scientific Reports. 7(1):10077</td>
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<td>2017</td>
<td>Window of implantation transcriptomic stratification reveals different endometrial subsignatures associated with live birth and biochemical pregnancy</td>
<td>Fertility and Sterility. 108(4):703-710.e3</td>
</tr>
</tbody>
</table>
**Results**

- **24,500 patients**
- **54 countries**
- **More than 600 clinics**
- Endometrial biopsy:
  - **65%** Receptive
  - **35%** Non-receptive
- **87.0%** Pre-receptive
- **0.2%** Proliferative
- **12.8%** Post-receptive
Progesterone elevation on the day of hCG
Progesterone

Epithelial
PR

Slow Embryos

Progesterone
Endometrial Thickness versus Molecular Receptivity

<table>
<thead>
<tr>
<th>Endometrial thickness (mm)</th>
<th>Receptive (%)</th>
<th>Non Receptive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;6</td>
<td>6/14 (43%)*</td>
<td>8/14 (57%)*</td>
</tr>
<tr>
<td>6-12</td>
<td>333/431 (77%)*</td>
<td>98/431 (23%)*</td>
</tr>
<tr>
<td>&gt;12</td>
<td>24/37 (65%)</td>
<td>13/37 (35%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>363</td>
<td>119</td>
</tr>
</tbody>
</table>

*P= 0.003 by Chi-square test.

Valbuena D. et al. ESHRE 2016
Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis

Ioanna A. Comstock, M.D.,° Patricia Diaz-Gimeno, Ph.D.,b Sergio Cabanillas, M.D.,b Jose Bellver, M.D.,b Patricia Sebastian-Leon, Ph.D.,b Meera Shah, M.D.,a Amy Schutt, M.D.,f Cecilia T. Valdes, M.D.,f Maria Ruiz-Alonso, M.Sc.,d Diana Valbuena, M.D., Ph.D.,d Carlos Simon, M.D., Ph.D.,d,e,f and Ruth B. Lathi, M.D.a

pET outcome after receptive ERA in patients with RIF (n=310)

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>NR (52)</th>
<th>R (205)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR First attempt</td>
<td>13% (12/90)</td>
<td>45% (161/355)</td>
</tr>
<tr>
<td>IR Total attempts</td>
<td>10% (17/174)</td>
<td>41% (182/441)</td>
</tr>
<tr>
<td>PR First attempt</td>
<td>23% (12/52)</td>
<td>60% (123/205)</td>
</tr>
<tr>
<td>PR Total attempts</td>
<td>17% (17/100)</td>
<td>55% (140/253)</td>
</tr>
<tr>
<td><strong>OPR First attempt</strong></td>
<td><strong>0% (0/12)</strong></td>
<td><strong>74% (91/123)</strong></td>
</tr>
<tr>
<td>OPR Total attempts</td>
<td>0% (0/100)</td>
<td>74% (103/140)</td>
</tr>
<tr>
<td><strong>Clinical efficiency</strong></td>
<td>Positive (52)</td>
<td>Negative (205)</td>
</tr>
<tr>
<td>True</td>
<td>40</td>
<td>123</td>
</tr>
<tr>
<td>False</td>
<td>12</td>
<td>82</td>
</tr>
<tr>
<td>Sensitivity (TP/TP+FN)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Specificity (TN/TN+FP)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>PPV (TP/TP+FP)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>NPV (TN/TN+FN)</td>
<td>0.60</td>
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</tbody>
</table>

*P-466* Ruiz-Alonso et al, *Clinical efficiency of embryo transfer performed in receptive vs non-receptive endometrium diagnosed by the endometrial receptivity array (ERA) (70th ASRM Annual Meeting, Honolulu, Hawaii. 2014)*
RIF Euploid standard ET vs RIF Euploid pET

Tan J et al., J Assist Reprod Genet 2018 Jan 11
Study Design

Prospective, Randomized, Multicenter, International, Open label, Controlled trial

(ClinicalTrials.gov Identifier: NCT01954758)

**Estimated number size: 546**

**Inclusion criteria**

- Patients undergoing IVF/ICSI with their own oocytes
- Age ≤ 37 years
- BMI: 18.5-30
- Normal ovarian reserve (AFC ≥ 8; FSH < 8)
- Blastocyst transfer (day 5/6)
- PGS was not an inclusion criteria
- Pathology affecting the endometrial cavity must be previously operated
To assess whether personalized embryo transfer (pET) guided by endometrial receptivity analysis (ERA) improves the reproductive outcome compared to fresh embryo transfer (FET), or deferred embryo transfer (DET) in infertile women undergoing IVF.
ERA RCT CONSORTIUM PARTICIPANT SITES

Stanford University, USA
IECH Monterrey, Mexico
IVI, Spain (11 sites)
ProcreaTec, Spain
Instituto Vida Matamoros, Mexico
IVI Panama
ReproTec, Colombia
Centro de Infertilidade e Medicina Fetal do Norte Fluminense, Brazil
Embriofert, Brazil
Centro Reprodução Nilo Frantz, Brazil
Centro Reprodução G Mario Covas, Brazil
Bahceci Health Group, Turkey
UZ Brussels, University Fertility Center, Belgium
Genesis, IVF, Serbia
Sbalagrm Sofia, Bulgaria
Oak Clinic, Japan
KKH, Singapore
Genesis IVF, Serbia
ReproTec, Spain
ProcreaTec, Spain

Active Sites with EC/IRB approval (28)
Recruiting Sites at the Interim (12)
ClinicalTrials.gov Identifier: NCT01954758

Approved by the protocol review service of the Lancet in 2012

Trial started in October 2013

Interim outcome evaluated in April 2016

Estimated n=546
Interim n=356 (April 2016)

Group A
FRESH CYCLES
N= 117

Group B
ALL FROZEN
N= 122

Group C
ALL FROZEN & ERA
N= 117

FET

DET

pET
<table>
<thead>
<tr>
<th>Reproductive Outcome / Interim study</th>
<th>FET</th>
<th>DET</th>
<th>pET</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate/ET (%)</td>
<td>61.7 (37/60)</td>
<td>60.8 (45/74)</td>
<td>85.7* (42/49)</td>
<td>0.003</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>35.3 (36/102)</td>
<td>41.4 (53/128)</td>
<td>47.8 (43/90)</td>
<td>0.21</td>
</tr>
<tr>
<td>Biochemical pregnancies (%)</td>
<td>21.6 (8/37)</td>
<td>6.7 (3/45)</td>
<td>11.9 (5/42)</td>
<td>0.13</td>
</tr>
<tr>
<td>Ectopic pregnancies (%)</td>
<td>2.7 (1/37)</td>
<td>0 (0/45)</td>
<td>2.4 (1/42)</td>
<td>0.55</td>
</tr>
<tr>
<td>Clinical miscarriages (%)</td>
<td>5.4 (2/37)</td>
<td>20.0 (9/45)</td>
<td>21.4 (9/42)</td>
<td>0.10</td>
</tr>
<tr>
<td>Ongoing pregnancy/ET (%)</td>
<td>43.3 (26/60)</td>
<td>44.6 (33/74)</td>
<td>55.1 (27/49)</td>
<td>0.24</td>
</tr>
<tr>
<td>Twins (%)</td>
<td>28.6 (8/28)</td>
<td>26.2 (11/42)</td>
<td>19.4 (7/36)</td>
<td>0.66</td>
</tr>
<tr>
<td>Singleton (%)</td>
<td>71.4 (20/28)</td>
<td>73.8 (31/42)</td>
<td>80.6 (29/36)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*p value <0.05 by Chi-Square test
Endometrial Microbiota

Bacteria: an invisible universe also in Reproductive Medicine
Humans have 10x > bacteria than cells

A person of 70 kg weight has 1 Kg of bacteria cohabitants

Our body contains bacteria, particularly abundant in the skin and digestive tract

Between 20 and 60% of these bacteria (depending on location) can not be cultured
VAGINAL BACTERIA & HUMAN REPRODUCTION

- Up to 40% of patients undergoing IVF treatments present abnormal vaginal microbiota.
- Bacterial vaginosis (BV) is responsible for:
  - 2-fold increase risk of early miscarriage.
  - >5-fold increased risk of late miscarriage.
  - >3-fold increased risk of premature rupture of membranes.
  - Up to 2-fold increased risk of preterm labor.

Sirotta et al. 2014. Semin Reprod Med. 32:35–42
Krauss-Silva et al. 2010. Reprod Health 7:14
Is there a specific human endometrial microbiota?

And, if this is so...

Could the endometrial microbiota promote/impair endometrial receptivity and pregnancy outcomes?
Evidence that the endometrial microbiota has an effect on implantation success or failure

Inmaculada Moreno, PhD; Francisco M. Codoñer, PhD; Felipe Viella, PhD; Diana Valbuena, MD, PhD; Juan F. Martínez-Blanch, PhD; Jorge Jiménez-Almazán, PhD; Roberto Alonso; Pilar Alamá, MD, PhD; Jose Remohí, MD, PhD; Antonio Pellicer, MD, PhD; Daniel Ramon, PhD; Carlos Simon, MD, PhD

BACKGROUND: Bacterial cells in the human body account for 1—3% of total body weight and are at least equal in number to human cells. Recent research has focused on understanding how the different bacterial communities in the body (eg, gut, respiratory, skin, and vaginal microbiomes) predispose to health and disease. The microbiota of the reproductive tract has been inferred from the vaginal bacterial communities, and the uterus has been classically considered a sterile cavity. However, while the vaginal microbiota has been investigated in depth, there is a paucity of consistent data regarding the existence of an endometrial microbiota and its possible impact in reproductive function.

OBJECTIVE: This study sought to test the existence of an endometrial microbiota that differs from that in the vagina, assess its hormonal regulation, and analyze the impact of the endometrial microbial community on reproductive outcome in infertile patients undergoing in vitro fertilization.

STUDY DESIGN: To identify the existence of an endometrial microbiota, paired samples of endometrial fluid and vaginal aspirates were obtained simultaneously from 13 fertile women in pre-ovulatory and receptive phases within the same menstrual cycle (total samples analyzed n = 52). To investigate the hormonal regulation of the endometrial microbiota during the acquisition of endometrial receptivity, endometrial fluid was collected at pre-ovulatory and receptive phases within the same cycle from 22 fertile women (n = 44). Finally, the reproductive impact of an altered endometrial microbiota in endometrial fluid was assessed by implantation, ongoing pregnancy, and live birth rates in 35 infertile patients undergoing in vitro fertilization (total samples n = 41) with a receptive endometrium diagnosed using the endometrial receptivity array.

RESULTS: The 16S ribosomal RNA (rRNA) gene; the resulting sequences were taxonomically assigned using QIIME. Data analysis was performed using R packages. The χ² test, Student t test, and analysis of variance were used for statistical analyses.

RESULTS: When bacterial communities from paired endometrial fluid and vaginal aspirate samples within the same subjects were interrogated, different bacterial communities were detected between the uterine cavity and the vagina of some subjects. Based on its composition, the microbiota in the endometrial fluid, comprising up to 191 operational taxonomic units, was defined as a Lactobacillus-dominated microbiota (>90% Lactobacillus spp.) or a non-Lactobacillus-dominated microbiota (<90% Lactobacillus spp. with >10% of other bacteria). Although the endometrial microbiota was not hormonally regulated during the acquisition of endometrial receptivity, the presence of a non-Lactobacillus-dominated microbiota in a receptive endometrium was associated with significant decreases in implantation (60.7% vs 23.1% (P = .02)), pregnancy (70.6% vs 33.3% (P = .03)), ongoing pregnancy (58.8% vs 13.3% (P = .02)), and live birth (58.8% vs 6.7% (P = .002)) rates.

CONCLUSION: Our results demonstrate the existence of an endometrial microbiota that is highly stable during the acquisition of endometrial receptivity. However, pathological modification of its profile is associated with poor reproductive outcomes for in vitro fertilization patients. This finding adds a novel microbiological dimension to the reproductive process.

Key words: assisted reproductive techniques, bacterial pathogens, embryo implantation, endometrial microbiota, endometrial receptivity array
Methods

Molecular assessment of endometrial microbiota by NGS

1. **Endometrial/Vaginal Aspiration**
2. **gDNA Purification**
3. **16S rRNA Gene**
4. **Barcoded Bacterial 16S rRNA PCR**
5. **Sequencing**
6. **Data Analysis & Taxonomical Assignment**

**Diagram Details**
- **Adaptor A**
- **Adaptor B**
- **Proportion**
- **Proportions**

**Taxonomic Assignment**
- Propionibacteria spp.
- Corynebacteria spp.
- Other Actinobacteria
- Staphylococci spp.
- Lactobacillales
- Clostridiales
- α-Proteobacteria
- γ-Proteobacteria
- α-Proteobacteria
**STUDY 1**

Endometrial and vaginal microbiota differ in some asymptomatic subjects

Healthy & fertile subjects in natural cycle (n=13)

- Pre-receptive phase (LH+2)
  - Endometrial fluid
  - Vaginal aspirate

- Receptive phase (LH+7)
  - Endometrial fluid
  - Vaginal aspirate

Subjects: n=13
Paired samples Endometrium-Vagina: n=26
Total samples analyzed: n=52
Distribution of endometrial and vaginal microbiota in paired samples

Conclusion Study 1

The uterine cavity is not sterile. Endometrial and Vaginal Microbiomes are different in asymptomatic women.
Bacterial communities in EF during the acquisition of endometrial receptivity in healthy asymptomatic women

IVF PATIENTS

EF

EBx

MICROBIOTA

Healthy

Altered

ERA TEST

Receptive

Non-receptive

EMBRYO TRANSFER

YES

NO

EMBRYO IMPLANTATION

Pregnant

Non-pregnant

ONGOING PREGNANCY

YES

NO

ENDOMETRIAL MICROBIOTA & RECEPTIVITY

ENDOMETRIAL MICROBIOTA & IMPLANTATION

ENDOMETRIAL MICROBIOTA & PREGNANCY OUTCOME

Design
Endometrial microbiota profile of infertile patients


Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer
Endometrial microbiota profile of infertile patients

≥90% Lactobacillus

*Lactobacillus*-dominated Microbiota (LDM)

Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer

Endometrial microbiota profile of infertile patients

- Samples 1 to 41
- LB: Live birth
- MISC: Miscarriage
- NP: No Pregnant
- NoET: No embryo transfer

Low abundance of Lactobacillus in endometrium is associated with poor reproductive IVF outcomes

<table>
<thead>
<tr>
<th>Characteristics and Outcomes</th>
<th>LDM (n=17)</th>
<th>NLDM (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>40.06±3.47</td>
<td>39.00±5.09</td>
<td>0.49</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.18±5.18</td>
<td>22.45±4.02</td>
<td>0.30</td>
</tr>
<tr>
<td>Previous pregnancies (n)</td>
<td>1.71±2.44</td>
<td>1.53±2.32</td>
<td>0.84</td>
</tr>
<tr>
<td>Previous miscarriages (n)</td>
<td>1.53±2.21</td>
<td>1.14±1.56</td>
<td>0.58</td>
</tr>
<tr>
<td>Metaphase II oocytes per cycle (n)</td>
<td>11.94±4.27</td>
<td>10.20±4.81</td>
<td>0.28</td>
</tr>
<tr>
<td>Fertilization rate per cycle</td>
<td>157/203 (77.34%)</td>
<td>118/153 (77.12%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Transferred embryos per cycle (n)</td>
<td>1.65±0.49</td>
<td>1.73±0.59</td>
<td>0.65</td>
</tr>
<tr>
<td>Months between EF and transfer (n)</td>
<td>2.82±2.55</td>
<td>1.80±1.08</td>
<td>0.16</td>
</tr>
<tr>
<td>Pregnancy rate per transfer</td>
<td>12/17 (70.6%)</td>
<td>5/15 (33.3%)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Implantation rate per transfer</td>
<td>17/28 (60.7%)</td>
<td>6/26 (23.1%)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Ongoing pregnancy per transfer</td>
<td>10/17 (58.5%)</td>
<td>2/15 (13.3%)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Miscarriage rates (%)</td>
<td>2/10 (16.7%)</td>
<td>3/5 (60.0%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Live birth rate per transfer</td>
<td>10/17 (58.8%)</td>
<td>1³/15 (6.7%)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

BMI: body mass index; LDM: Lactobacillus-dominated microbiota; NLDM: non-Lactobacillus-dominated microbiota; *Chi Square ($\chi^2$ test) and Student’s t-test were performed; *p-value<0.05; §: Voluntary termination of pregnancy.

Low abundance of *Lactobacillus* in endometrium is associated with poor reproductive IVF outcomes

THE MICROBIOTA OF THE FEMALE REPRODUCTIVE TRACT


The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases

CL: Lower third of vagina
CU: Posterior fornix
CV: Cervical mucus from the cervical canal
ET: Endometrium
FL: Fallopian tubes (left and right)
PF: Peritoneal fluid from the pouch of Douglas
Commensal bacteria make GPCR ligands that mimic human signalling molecules


Commensal bacteria are believed to have important roles in human health. The mechanisms by which they affect mammalian physiology remain poorly understood, but bacterial metabolites are likely to be key components of host interactions. Here we use bioinformatics and synthetic biology to mine the human microbiota for N-acyl amides that interact with G-protein-coupled receptors (GPCRs). We found that N-acyl amide synthase genes are enriched in gastrointestinal bacteria and the lipids that they encode interact with GPCRs that regulate gastrointestinal tract physiology. Mouse and cell-based models demonstrate that commensal GPR119 agonists regulate metabolic hormones and glucose homeostasis as efficiently as human ligands, although future studies are needed to define their potential physiological role in humans. Our results suggest that chemical mimicry of eukaryotic signalling molecules may be common among commensal bacteria and that manipulation of microbiota genes encoding metabolites that elicit host cellular responses represents a possible small-molecule therapeutic modality (microbiome–biosynthetic gene therapy).
BIOMEDICAL STUDY PROTOCOL

Development of a non-invasive diagnosis tool for the simultaneous analysis of endometrial receptivity and microbiota to improve reproductive outcomes in infertile patients.

niERA-MIC

IGX1-MIC-CS-17-05

Principal Investigator and Coordinator: Dr. Carlos Simon

Sponsor of the study: Igenomix
<table>
<thead>
<tr>
<th>OTU ID</th>
<th>k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus</th>
<th>77.15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k__Bacteria;p__Actinobacteria;c__Actinobacteridae;f__Actinobacteriales;g__Actinobacteria</td>
<td>4.60</td>
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<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae;g__Pseudoalteromonas</td>
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<td>k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella</td>
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<td></td>
<td>k__Bacteria;p__Actinobacteria;c__Actinobacteridae;f__Actinobacteriales;g__Bifidobacterium</td>
<td>1.70</td>
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<tr>
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<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrio</td>
<td>0.83</td>
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<tr>
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<td>k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella</td>
<td>0.68</td>
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<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas</td>
<td>0.57</td>
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<tr>
<td></td>
<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__</td>
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</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Shuttleworthia</td>
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<tr>
<td></td>
<td>k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Streptococcaceae;g__Streptococcus</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Megasphaera</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Ureaplasma</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Enhydrobacter</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Escherichia</td>
<td>0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>DNA PRESERVANT</th>
<th>SAMPLE</th>
<th>Cycle day</th>
<th>ERA test</th>
<th>niERA test</th>
<th>16S Reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>niERA2</td>
<td>YES, 50 µL</td>
<td>EF</td>
<td>d27</td>
<td>Post-R</td>
<td>Post-R</td>
<td>274.065</td>
</tr>
</tbody>
</table>
Molecular microbiologically diagnosis of chronic endometritis

ASSESSED FOR ELIGIBILITY (n=113)
- Not DNA for RT-PCR diagnosis (n=18)

MOLECULAR DIAGNOSIS OF CE (n=95)
- Excluded for incomplete clinical diagnosis:
  - Non informative (n=9)
  - Only 1 classical diagnosis method (n=3)
  - Only 2 classical diagnosis methods (n=18)

CE DIAGNOSED BY 3 CLASSICAL METHODS (n=65)

Concordant CE diagnosis by the 3 classical methods (n=13)
- Discordant histology + hysteroscopy with microbial culture (n=14)
- Discordant histology + microbial culture with hysteroscopy (n=17)
- Discordant hysteroscopy + microbial culture with histology (n=21)

Discordant CE diagnosis by the 3 classical methods (n=52)

Moreno et al., AJOG 2018
METHODS

ENDOMETRIAL TISSUE

TOTAL DNA

RT-PCR

Specific amplification of CE pathogens

16S METAGENOMICS

16S bacterial library

Enterobacteria
Escherichia coli
Enterococcus faecalis
Staphylococcus spp.
Streptococcus spp.
Gardnerella vaginalis
Neisseria gonorrhoeae
Chlamydia trachomatis
Ureaplasma urealyticum
Mycoplasma hominis

MICROBIOME PROFILE
### RESULTS: MOLECULAR MICROBIOLOGY vs CLASSICAL DX for CE

<table>
<thead>
<tr>
<th>Molecular Microbiology compared to classical methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>PPV</th>
<th>NPV</th>
<th>FPR</th>
<th>FNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR vs Histology (n=65)</td>
<td>56.00%</td>
<td>40.00%</td>
<td>46.15%</td>
<td>36.84%</td>
<td>59.26%</td>
<td>60.00%</td>
<td>44.00%</td>
</tr>
<tr>
<td>RT-PCR vs Hysteroscopy (n=65)</td>
<td>58.73%</td>
<td>50.00%</td>
<td>58.46%</td>
<td>97.37%</td>
<td>3.70%</td>
<td>50.00%</td>
<td>41.27%</td>
</tr>
<tr>
<td>RT-PCR vs Microbial culture (n=65)</td>
<td>71.43%</td>
<td>56.67%</td>
<td>66.15%</td>
<td>65.79%</td>
<td>62.96%</td>
<td>43.33%</td>
<td>28.57%</td>
</tr>
<tr>
<td>RT-PCR vs Histology &amp; Hysteroscopy (only concordant results, n=27)</td>
<td>56.00%</td>
<td>50.00%</td>
<td>55.55%</td>
<td>93.33%</td>
<td>8.33%</td>
<td>50.00%</td>
<td>44.00%</td>
</tr>
<tr>
<td>RT-PCR vs Histology, Hysteroscopy &amp; Microbial culture (only concordant results, n=13)</td>
<td>75.00%</td>
<td>100.00%</td>
<td>76.92%</td>
<td>100.00%</td>
<td>25.00%</td>
<td>0.00%</td>
<td>25.00%</td>
</tr>
</tbody>
</table>

PPV: Positive predictive value; NPV: Negative predictive value; FPR: False positive rate; FNR: False negative rate

Moreno et al., AJOG 2018
Concordant chronic endometritis results in patients/samples analysed by the four methods compared in this study (three classical methods and the RT-PCR method)

<table>
<thead>
<tr>
<th>Patient 10</th>
<th>Histology/CD138</th>
<th>Hysteroscopy</th>
<th>Microbial culture</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 8</th>
<th>Histology/CD138</th>
<th>Hysteroscopy</th>
<th>Microbial culture</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>S. agalactiae</td>
<td>Streptococcus spp. G. vaginalis</td>
<td></td>
</tr>
</tbody>
</table>
Discordant chronic endometritis results in patients/samples analysed by the four methods compared in this study. Black arrows show CD138 positive cells.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology/CD138</th>
<th>Hysteroscopy</th>
<th>Microbial culture</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>Negative</td>
<td>Negative</td>
<td><em>E. coli</em></td>
<td><em>Streptococcus</em> spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Ureaplasma</em></td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>65</td>
<td>Negative</td>
<td>Positive</td>
<td><em>S. agalactiae</em></td>
<td><em>Streptococcus</em> spp.</td>
</tr>
<tr>
<td>Patient</td>
<td>Microbial culture</td>
<td>RT-PCR</td>
<td>16s rRNA sequencing (genera percentage)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------------</td>
<td>--------</td>
<td>----------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lactobacillus</td>
<td>Enterococcus</td>
</tr>
<tr>
<td>8</td>
<td><em>Streptococcus agalactiae</em></td>
<td></td>
<td>10.2</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td></td>
<td>99.9</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td><em>Escherichia coli</em></td>
<td></td>
<td>61.0</td>
<td>0.3</td>
</tr>
<tr>
<td>17</td>
<td><em>Enterococcus faecalis</em>, <em>Ureaplasma</em></td>
<td></td>
<td>94.4</td>
<td>0.0</td>
</tr>
<tr>
<td>18</td>
<td><em>Streptococcus agalactiae</em></td>
<td></td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>19</td>
<td><em>Escherichia coli</em></td>
<td></td>
<td>61.5</td>
<td>0.1</td>
</tr>
<tr>
<td>24</td>
<td>Ureaplasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td><em>Enterococcus faecium</em></td>
<td></td>
<td>98.8</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td><em>Enterococcus faecalis</em>, <em>Streptococcus mitis</em></td>
<td></td>
<td>93.2</td>
<td>0.2</td>
</tr>
<tr>
<td>31</td>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td>7.4</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>83.1</td>
<td>0.0</td>
</tr>
</tbody>
</table>

ND: Not determined
Conclusions

✓ Molecular microbiology effectively detects and quantifies bacterial DNA from chronic endometritis-causing pathogens.

✓ The microbiome results using NGS were concordant with RT-PCR in 91.67% of cases and coincide with the microbial culture in 75% allowing for the detection of culturable and non-culturable bacteria.

✓ The molecular diagnosis of CE is equivalent to using the histology, hysteroscopy and microbial culture together, overcoming the bias of using any of them alone.
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FINANCIAL SUPPORT

Unión Europea
Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"

Instituto de Salud Carlos III