What is new in the endometrial factor

Carlos Simón MD. PhD.

Professor Ob/Gyn, University of Valencia
Adjunct Clinical Professor Ob/Gyn. Stanford University
Adjunct Clinical Professor Ob/Gyn. Baylor College
Scientific Director of Igenomix
Disclosure

Carlos Simon

Founder & SAB Chairman of Igenomix, S.L.
SAB member of Veritas Europe & Latam, S.L.
SAB member of Atlas Molecular Pharma, S.L.

Editorial Editor

External Scientific Advisor:
The Maternal Contribution

Learning objectives

Endometrial Microbiota

Materno-Embryonic Crosstalk

Endometrial Receptivity

Decidualization

Moreno et al., AJOG 2016
Moreno et al., AJOG 2018
Vilella et al., Development 2015
Balaguer et al., MHR 2018

Diaz-Gimeno et al., F&S 2011
Ruiz-Alonso et al., F&S 2013
Garrido-Gomez et al., HR 2014
Von Grothusen et al., HR 2018
Wang W. et al., Cell. 2018 in press

Garrido-Gomez et al., JCEM 2011
Garrido-Gomez et al., Development 2017
Garrido-Gomez et al., PNAS 2017
Endometrial microbiota

Endometrial receptivity

Materno-embryonic crosstalk

The Maternal Contribution

Endometrial receptivity

Plasma membrane transformation

A
The Window of Implantation (WOI)


2-to 12-cell embryos (day 2-4) were transferred between days 16 and 24.

37 transfers within days 17 to 19, 40.5% of conceptions occurred.
15 transferred on days 16 or 20 onwards, NONE CONCEIVED
The Window of Implantation (WOI)

Willcox et al. NEJM 1999

Popularized the concept that the human embryo implants 8 to 10 days after ovulation.

(ovulation was identified by 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).
Anatomical medicine: dating the endometrium

Dating the Endometrial Biopsy

R. W. Noyes, M.D.*, A. T. Hertig, M.D., and J. Rock, M.D.

300 protocols reviewed
40 correlated with basal body temperature
13 were photographed
8 major histologic criteria were described

The most cited paper ever in Obst/Gyn. **2,630 times cited**

Endometrial Thickness (EMT) is not diagnostic of endometrial receptivity

The use of EMT as a tool to decide on cycle cancellation, freezing of all embryos or refraining from further IVF treatment seems not to be justified based on the current meta-analysis.

Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis

Riesewijk et al., 2003 (HG-U133 2.0) WOI
Ponnappalam et al., 2004 (Home-made array) menstrual cycle
Talbi et al., 2005 (HG-U133 2.0) menstrual cycle
ERA classifies the molecular receptivity status of the endometrium.
The symphony of synchronization

Progesterone

Epithelial PR
Personalized embryo transfer (pET) as a treatment for RIF of endometrial origen

- P+3
- P+5
- P+7
- LH+5
- LH+7
- LH+9
## ERA Publications

<table>
<thead>
<tr>
<th>YEAR</th>
<th>TITLE</th>
<th>JOURNAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>Understanding and improving endometrial receptivity</td>
<td>Current Opinion in Obstetrics &amp; Gynecology. 27(3):187-92</td>
</tr>
<tr>
<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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<tr>
<td>2016</td>
<td>Diagnosis of endometrial-factor infertility: current approaches and new avenues for research</td>
<td>Geburts hilfe Frauenheilkd. 76(6): 699-703</td>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
</tr>
<tr>
<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>
Clinical data

**Previous ART treatments**
1. IVF with fresh day-3 ET
2. IVF with fresh day-3 ET

**ART treatments in our center**
3. IVF with fresh day-5 ET
4. IVF with differed day -5 ET in natural cycle
5. OD with day-3 ET in HRT cycle (P+2)
6. OD with day-3 ET in natural cycle
7. OD with day-5 ET in HRT cycle (P+5)

**DIAGNOSTIC INTERVENTION ERA**
pre-receptive at P+5, being receptive at P+7

8. OD with pET using day-5 blastocysts in HRT cycle after 7 days of progesterone (P+7)
Successful twin pregnancy

Ruiz-Alonso et al., Hum Reprod. 2014
The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure

Maria Ruiz-Alonso, M.Sc., David Blesa, Ph.D., Patricia Díaz-Gimeno, Ph.D., Eva Gómez, M.Sc., Manuel Fernández-Sánchez, M.D., Francisco Carranza, M.D., Joan Carrera, M.D., Felip Vilella, Ph.D., Antonio Pellicer, M.D., Ph.D., and Carlos Simón, M.D., Ph.D.

Fundación Instituto Valenciano de Infertilitat, and Instituto Universitario IVI/Incliva, Valencia University, Valencia; a b Iviomics, Paterna; c Computational Medicine Institute, Centro de Investigación Príncipe Felipe, Valencia; d Instituto Valenciano de Infertilitat Sevilla, Seville; and e Clínica Girona Unidad de Reproducción Humana, Girona, Spain

Fertility and Sterility® Vol. 100, No. 3, September 2013
# Reproductive outcome. ERA-RCT interim analysis

<table>
<thead>
<tr>
<th></th>
<th>ET</th>
<th>FET</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
Euploid standard ET vs Euploid pET in RIF patients

Tan J et al., J Assist Reprod Genet 2018
Is endometrial receptivity affected in obese women?

Endometrial samples from 200 women according to BMI:
- normal-weight (BMI 19-24.9 kg/m²)
- overweight (BMI 25-29.9 kg/m²)
- obese (BMI 30-34.9 kg/m²)
- morbidly obese (BMI ≥ 35 kg/m²)

ERA results

21 RIF Patients

7 Non receptive
1 Early receptive
1 Late receptive

9 Displaced WOI

8 transferred patients
PR 100% (8/8)

12 Receptive

7 transferred patients
PR 85% (6/7)

Ongoing pregnancy per transfer 67% (10/15)

P-341
Pasternak et al., 2018 ASRM
Cornell NY
Proof of concept of the receptivity transcriptomic signature

Human Reproduction, pp. 1–9, 2018
doi:10.1093/humrep/dey272

Effect of mifepristone on the transcriptomic signature of endometrial receptivity

C. von Grothusen¹, P.G. Lalitkumar¹, M. Ruiz-Alonso², N.R. Boggavarapu¹,*, R. Navarro², J. Miravet-Valenciano², K. Gemzell-Danielsson¹,†, and C. Simon²,3,4,†

¹Division of Obstetrics and Gynecology, Department of Women’s and Children’s Health, Karolinska Institutet, and Karolinska University Hospital, S-171 76 Stockholm, Sweden ²Department of Endometrial Receptivity Analysis, Igenomix S.L., 46980 Valencia, Spain ³Department of Obstetrics and Gynecology, University of Valencia/INCLIVA, 46010 Valencia, Spain ⁴Department of Obstetrics and Gynecology, Stanford University, 94305 CA, USA

*Correspondence address. Department of Women’s and Children’s Health, Akademiska Stråket 1, Bioclinicum, J9:30, 171 64 Solna, Stockholm, Sweden. Tel: +46-73-955-2805; E-mail: nageswara.boggavarapu@ki.se

Submitted on June 5, 2018; resubmitted on June 29, 2018; accepted on July 28, 2018
Effect of mifepristone on the transcriptomic signature of endometrial receptivity

Mifepristone

LH+2

LH+7

Treated Group (n=7)

LH+2

LH+7

Control Group (n=11)

Day 3-9 of cycle

LH+7

Proliferative Group (n=7)

Von Grothusen et al., 2018
Treated group
✓ Estrogen Receptor activated
✓ Progesterone Receptor inactivated
✓ Glucocorticoid Receptor inactivated

Effect of mifepristone on the transcriptomic signature of endometrial receptivity

Von Grothusen et al., 2018
Single cell analysis from endometrial biopsy

Endometrial biopsy

↓

Single cell separation

Stromal cells → Epithelial cells

↓

Single-cell isolation

Wash cells with DMEM medium

↓

Single-cell RNA-seq

Wanxin et al., bioRxiv online Jun. 19, 2018; doi: http://dx.doi.org/10.1101/350538 Cell submitted.

Data: 2,149 cells from 19 healthy ovum donors throughout their natural menstrual cycle.
113 ± 6 single cells analyzed per donor:
- 48.23% epithelial cells (2.19% ciliated and 46.04% non-ciliated)
- 43.76% stromal fibroblasts,
- 1.44% endothelial,
- 5.91% lymphocytes
- 0.65% macrophages
Temporal Transcriptome Dynamics

Unciliated epithelia

Stromal fibroblasts

Decidualization

Phase

- menstrual
- early-pro
- late-pro
- early-sec
- mid-sec
- late-sec

WOI

log2 (rpm+1)

0 5 10 15

PLAU, MTP3, MPZL1, CAPR1, SERPINF1, ATP1A1, C12orf70, TTP, TLR4, COX15, MT1M, MT1G, TLR3, OPN, CYP3A4, GDF15, FGF2, FGFR1, LMC01

STC1, NPYTC2, BMP7, PMAIP1, SPRED1, SRPRB, VNTSA, ZP4, E2, BL2, MATZN2, S100A4, DKK1, FOXO1, IL1B, FGF7, LMCO1

Early-pregnancy
Viable term pregnancy despite “subluteal” serum progesterone levels in the first trimester

Khalid M. Sultan, M.D.
Owen K. Davis, M.D.
Hung-Ching Liu, Ph.D.
Zev Rosenwaks, M.D.*
It is not about the route of administration IM vs vaginal progesterone. Clinical Pregnancy Rate.

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Vaginal</th>
<th>Intramuscular</th>
<th>Odds Ratio M H(1)Random,95% CI</th>
<th>Weight</th>
<th>Odds Ratio M H(1)Random,95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Intramuscular Progesterone versus Vaginal Gel Progesterone (Oocyte donation)</td>
<td>26/54</td>
<td>5/18</td>
<td>10.6%</td>
<td>2.41 [0.76, 7.71]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>54</td>
<td>18</td>
<td>10.6%</td>
<td>2.41 [0.76, 7.71]</td>
<td></td>
</tr>
<tr>
<td>Total events: 26 (Vaginal), 5 (Intramuscular)</td>
<td></td>
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</tr>
<tr>
<td>Heterogeneity: not applicable</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Test for overall effect: Z = 1.49 (P = 0.14)</td>
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</tr>
<tr>
<td>2 Intramuscular Progesterone versus Vaginal Ring Progesterone (Oocyte donation)</td>
<td>33/83</td>
<td>20/70</td>
<td>31.0%</td>
<td>1.65 [0.84, 3.26]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>83</td>
<td>70</td>
<td>31.0%</td>
<td>1.65 [0.84, 3.26]</td>
<td></td>
</tr>
<tr>
<td>Total events: 33 (Vaginal), 20 (Intramuscular)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: not applicable</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Test for overall effect: Z = 1.44 (P = 0.15)</td>
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<tr>
<td>3 Intramuscular Progesterone versus Vaginal Suppositories Progesterone (Frozen-embryo transfer)</td>
<td>31/184</td>
<td>27/170</td>
<td>45.1%</td>
<td>1.07 [0.61, 1.89]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>184</td>
<td>170</td>
<td>45.1%</td>
<td>1.07 [0.61, 1.89]</td>
<td></td>
</tr>
<tr>
<td>Total events: 31 (Vaginal), 37 (Intramuscular)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: not applicable</td>
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<tr>
<td>Test for overall effect: Z = 0.25 (P = 0.81)</td>
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<tr>
<td>4 Intramuscular Progesterone versus Vaginal Suppositories Progesterone (Oocyte donation)</td>
<td>31/39</td>
<td>25/37</td>
<td>13.3%</td>
<td>1.86 [0.66, 5.25]</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>39</td>
<td>37</td>
<td>13.3%</td>
<td>1.86 [0.66, 5.25]</td>
<td></td>
</tr>
<tr>
<td>Total events: 31 (Vaginal), 25 (Intramuscular)</td>
<td></td>
<td></td>
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<tr>
<td>Heterogeneity: not applicable</td>
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<tr>
<td>Test for overall effect: Z = 1.17 (P = 0.24)</td>
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</tr>
<tr>
<td>Total (95% CI)</td>
<td>360</td>
<td>295</td>
<td>100.0%</td>
<td>1.44 [0.98, 2.10]</td>
<td></td>
</tr>
<tr>
<td>Total events: 121 (Vaginal), 77 (Intramuscular)</td>
<td></td>
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<tr>
<td>Heterogeneity: Tau² = 0.0, Chi² = 2.19, df = 3 (P = 0.53); R² =0.0%</td>
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<tr>
<td>Test for overall effect: Z = 1.88 (P = 0.060)</td>
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</tbody>
</table>

Glujovsky, et al., Cochrane Database Syst Rev 2010
It is about timing of P in the system and E/E syncronization

63 studies: 55,199 fresh IVF cycles

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of studies</th>
<th>Odds ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4–0.6 ng/mL</td>
<td>5 studies (n=1659)</td>
<td>0.39 (0.14–1.08)</td>
</tr>
<tr>
<td>0.8–1.1 ng/mL</td>
<td>40 studies (n=16,304)</td>
<td>0.79 (0.67–0.95)</td>
</tr>
<tr>
<td>1.2–1.4 ng/mL</td>
<td>19 studies (n=5885)</td>
<td>0.67 (0.53–0.84)</td>
</tr>
<tr>
<td>1.5–1.75 ng/mL</td>
<td>26 studies (n=21,647)</td>
<td>0.64 (0.54–0.76)</td>
</tr>
<tr>
<td>1.9–3.0 ng/mL</td>
<td>12 studies (n=15,091)</td>
<td>0.68 (0.51–0.91)</td>
</tr>
</tbody>
</table>

*Odds ratio compare women with progesterone elevation with those without progesterone elevation (defined as ≥0.8 ng/mL)

The Incomplete Symphony

Epithelial PR

Progesterone
Active management of embryo-endometrial synchrony increases implantation rates

<table>
<thead>
<tr>
<th>SIR by ET Day (Fresh D5, Fresh D6, Cryo) and D5 Expansion</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Age Group</td>
<td>D5 ET Slow</td>
<td>D5 ET Normal</td>
<td>P-value</td>
</tr>
<tr>
<td>&lt;35</td>
<td>45/102 (44%)</td>
<td>493/771 (64%)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>≥35</td>
<td>16/90 (18%)</td>
<td>286/511 (56%)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>D6 ET Slow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>52/101 (52%)</td>
<td>212/339 (63%)</td>
<td>.05</td>
</tr>
<tr>
<td>≥35</td>
<td>37/116 (32%)</td>
<td>99/207 (48%)</td>
<td>.005</td>
</tr>
<tr>
<td>D6 ET Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryo ET Slow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>236/416 (57%)</td>
<td>145/243 (60%)</td>
<td>.5</td>
</tr>
<tr>
<td>≥35</td>
<td>140/378 (37%)</td>
<td>49/117 (42%)</td>
<td>.3</td>
</tr>
</tbody>
</table>

Franasiak, et al. 2013 ASRM Annual Meeting
The Incomplete Symphony

Progesterone

Epithelial

PR

Progesterone
When points out the moon... do not look at the finger.

Endometrial Receptivity

PR Activation

Route of Administration

Types of Progesterone

Levels in serum
Endometrial microbiome

Moreno I. et al., AJOG 2016
Moreno I. et al., AJOG 2018

Residents, Tourists, or Invaders?
The sterility of a healthy uterine cavity

Molecular assessment of endometrial microbiota by NGS

1. ENDOMETRIAL/VAGINAL ASPIRATION
2. gDNA PURIFICATION
3. 16S rRNA gene BARCODED BACTERIAL 16S rRNA PCR
4. SEQUENCING
5. DATA ANALYSIS & TAXONOMICAL ASSIGNMENT

Propionibacteria spp.
Corynebacteria spp.
Other Actinobacteria
Staphylococci spp.
Lactobacillales
Clostridiales
α-Proteobacteria
β-Proteobacteria
γ-Proteobacteria
Endometrial vs vaginal microbiota in fertile subjects

Moreno et al., Am J Obstet Gynecol 2016

Pre-receptive (LH+2)
Receptive (LH+7)
E: Endometrial fluid
V: Vaginal aspirate
Average bacterial communities in endometrial and vaginal microbiota of fertile subjects

Endometrial fluid
Vaginal aspirate

Report of Major Impact

Evidence that the endometrial microbiota has an effect on implantation success or failure

Only found in endometrial samples of these women

Moreno et al., Am J Obstet Gynecol 2016
IVF PATIENTS (n=35)
Endometrial Fluid
Endometrial Biopsy

MICROBIOTA
Healthy
Altered

ERA TEST
Receptive
Non-receptive

EMBRYO TRANSFER
YES
NO

EMBRYO IMPLANTATION
Pregnant
Non-pregnant

ONGOING PREGNANCY
YES
NO

Clinical impact of the endometrial microbiome

ENDOMETRIAL MICROBIOTA & RECEPTIVITY
ENDOMETRIAL MICROBIOTA & IMPLANTATION
ENDOMETRIAL MICROBIOTA & PREGNANCY OUTCOME
Endometrial microbiota profile of infertile patients

Moreno et al., Am J Obstet Gynecol 2016

Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer
Classification of endometrial microbiota profiles

PREDICTION MODEL:

- 2 target classes
  - Live birth (LB)
  - No Live birth (No LB)

- 4 variables
  - % Lactobacillus
  - % Bifidobacterium
  - % Gardnerella
  - % Streptococcus

Both models presented a similar conclusion in establishing 90% of Lactobacillus as the cut-off value to predict reproductive outcomes.

METHOD A: Classification and Regression Trees (CART)

<table>
<thead>
<tr>
<th></th>
<th>No LB</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB</td>
<td>0.62</td>
<td>50%</td>
</tr>
<tr>
<td>No LB</td>
<td>0.88</td>
<td>50%</td>
</tr>
</tbody>
</table>

METHOD B: Generalized linear model (GLM)

Logistic regression: 

\[ P(LB) = \frac{e^x}{1 + e^x} \]

where:

\[ x = \frac{\ln p}{1 - p} = -2.359 + 2.554 \times (\% \text{ Lactobacillus}) \]

| COEFFICIENTS | Estimate | Std. Error | Z value | Pr(>|z|) |
|--------------|----------|------------|---------|----------|
| (Intercept)  | -2.359   | 1.100      | -2.145  | *0.0320  |
| Lactobacillus| 2.554    | 1.277      | 2.001   | *0.0454  |

Moreno et al., Am J Obstet Gynecol 2016
Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer

 Moreno et al., Am J Obstet Gynecol 2016
Endometrial microbiota profile of infertile patients

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

Lactobacillus
Roseburia
Ruminococcus
Faecalibacterium
Lachnospiraceae
Blaustia
Pseudomonas
Escherichia
Bacteroides
[Ruminococcus]
Allobaculum
Lactococcus
Clostridium[Clostridiaceae]
Clostridiales
Bacillus
Propionibacterium
Veillonella
Streptococcus
Bifidobacterium
Gardnerella

Moreno et al., Am J Obstet Gynecol 2016
## 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>1§/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.

*Moreno et al., Am J Obstet Gynecol 2016*
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

Chen et al., Nat Commun 2017
G protein-coupled receptors (GPCRs) are the pharmacological target for 35% of approved drugs (Sriram and Insel, 2018).
## GPCRs ligands

- Sensory signal mediators (e.g., light and olfactory stimulatory molecules)
  - adenosine, bombesin, bradykinin, endothelin, GABA.
- Hepatocyte growth factor (HGF), melanocortins neuropeptide Y, opioid peptides, opsins, somatostatin, GH, tachykinins.
- Members of the VIP family, and vasopressin
- Biogenic amines (e.g., dopamine, epinephrine, norepinephrine, histamine, serotonin, melatonin);
- Glutamate (metabotropic effect)
- Glucagon; acetylcholine (muscarinic effect)
- Chemokines; lipid mediators of inflammation (e.g., prostaglandins, prostanoids, platelet-activating factor, and leukotrienes)
- Peptide hormones (e.g., calcitonin, C5a anaphylatoxin, follicle-stimulating hormone [FSH], gonadotropin-releasing hormone [GnRH], neurokinin, thyrotropin-releasing hormone [TRH], oxytocin)
- Endocannabinoids.

Cryan et al., Cell Host & Microbe 2018
The uterine microbiome in miscarriage and early pregnancy

**Primary infertility - 2 years**
1 failed IVF cycle

- **27th Feb 2017**: 1st ICSI cycle: 13 fertilized embryos - 10 blastocysts/6 Euploids - Vitrification
- **28th Apr 2017**: 1st ET w/ 2 frozen euploid blastocysts
  - ERA: Receptive in P+5.
  - MICROBIOME: NLD (56% Lactobacilli, 12% Enterobacteriaceae, 8% Streptococci, 8% Staphylococci, etc)

- **15th May 2017**: 2nd ET w/ 2 frozen euploid blastocysts
  - β-HCG: Positive

- **26th Jun 2017**: Curetage & POC: 46, XX (fetal origin)
  - Clinical Miscarriage (8 wks + 3d)

- **4th Aug 2017**: Last Menstruation
- **4th Sep 2017**: MICROBIOME: LD (99% Lactobacillus). Gestational age 4 wks

- **26th Jun 2018**: Live birth XY (C-section. No obstetrical/labor complications)

- **8th May 2018**: AKA 1

- **28th Apr 2018**: AKA 2
The uterine microbiome in miscarriage

16S sequencing - Bacterial composition

- g__Lactobacillus
- f__Aeromonadaceae
- g__Gardnerella
- g__Gluconacetobacter
- g__Prevotella
- g__Corynebacterium
- g__Enterococcus
- g__Micrococcus
- g__Klebsiella
- g__Granulicatella
- g__Dyella
- g__Streptococcus
- g__Staphylococcus
- g__Citrobacter
- g__Propionibacterium
- g__Kocuria
- f__Sinobacteraceae
- g__Morganella
- g__Agrobacterium

AKA 1
Miscarriage
The uterine microbiome in miscarriage and early pregnancy

16S sequencing - Bacterial composition

AKA 1
Miscarriage

AKA 2
Early successful pregnancy (4 weeks)
Take home message:
ENDOMETRIAL MICROBIOTA

✓ The uterine cavity is not a sterile site.
✓ Endometrial microbiota is not a carry-over from the vagina.
✓ The percentage of Lactobacillus together with specific pathogens are significant variables to predict reproductive success.
The Uterine Microbiome Team

**Research Director**
Felipe Vilella, PhD

**Research Manager**
Inmaculada Moreno, PhD

**Researchers**
Tamara Garrido, PhD
Aymara Mas, PhD

**Medical Manager**
Diana Valbuena, MD, PhD

**PhD Students**
Nuria Balaguer
Alessia Grasso
Iolanda Garcia
David Bolumar

**Lab. Technicians**
Patricia Escorcia
Maria Herrero
Marta Gonzalez

**Bioinformatic Team**
Jorge Jimenez
Roberto Alonso
Roser Navarro
Monica Clemente
Davide Bau

FINANCIAL SUPPORT
Materno-embryonic crosstalk

**Senior Researchers**
Felipe Vilella, PhD
Inmaculada Moreno, PhD
Tamara Garrido, PhD
Aymara Mas, PhD

**Bioinformatic Team**
Jorge Jimenez
Roberto Alonso
Roser Navarro

**Medical Department**
Diana Valbuena, MD, PhD

**PhD Students**
Nuría Balaguer
Alessia Grasso
Jose M. Miguez
Iolanda Garcia
David Bolumar

**Lab. Technicians**
Patricia Escorcia
Maria Herrero
Marta Gonzalez

**FINANCIAL SUPPORT**
ERA Team

Research Director
Felipe Vilella, PhD

Research Manager
Inmaculada Moreno, PhD

Researchers
Tamara Garrido, PhD
Aymara Mas, PhD

Medical Manager
Diana Valbuena, MD, PhD

COLLABORATORS

Steve Quake LAB
Steve Quake
Wanxin Wang
Wenying Pan

Ruth Lathi, Stanford University

FINANCIAL SUPPORT

Unión Europea
Stanford University
INCLIVA
Universitat
Valencia
Stanford University
BCM
Barclay College
of Medicine

Instituto de Salud Carlos III
Collaborators

**Steve Quake LAB**
- Steve Quake
- Wanxin Wang
- Wenying Pan

**Susan Fisher LAB**
- Susan J Fisher
- Olga Genvacev
- Michael McMaster
- Matthew Gormley
- Mirhan Kapidzic
- Katherine Ona

**Hospital La Fe**
- Alfredo Perales
- Laura Rubert
- Joana Dasí
- Julia Escrig

**Stanford University**

**University of California**

**UCSF**

**INCLIVA**

**Universitat de València**

**Ministerio de Ciencia, Innovación y Universidades**

**Instituto de Salud Carlos III**