Endometrial Receptivity

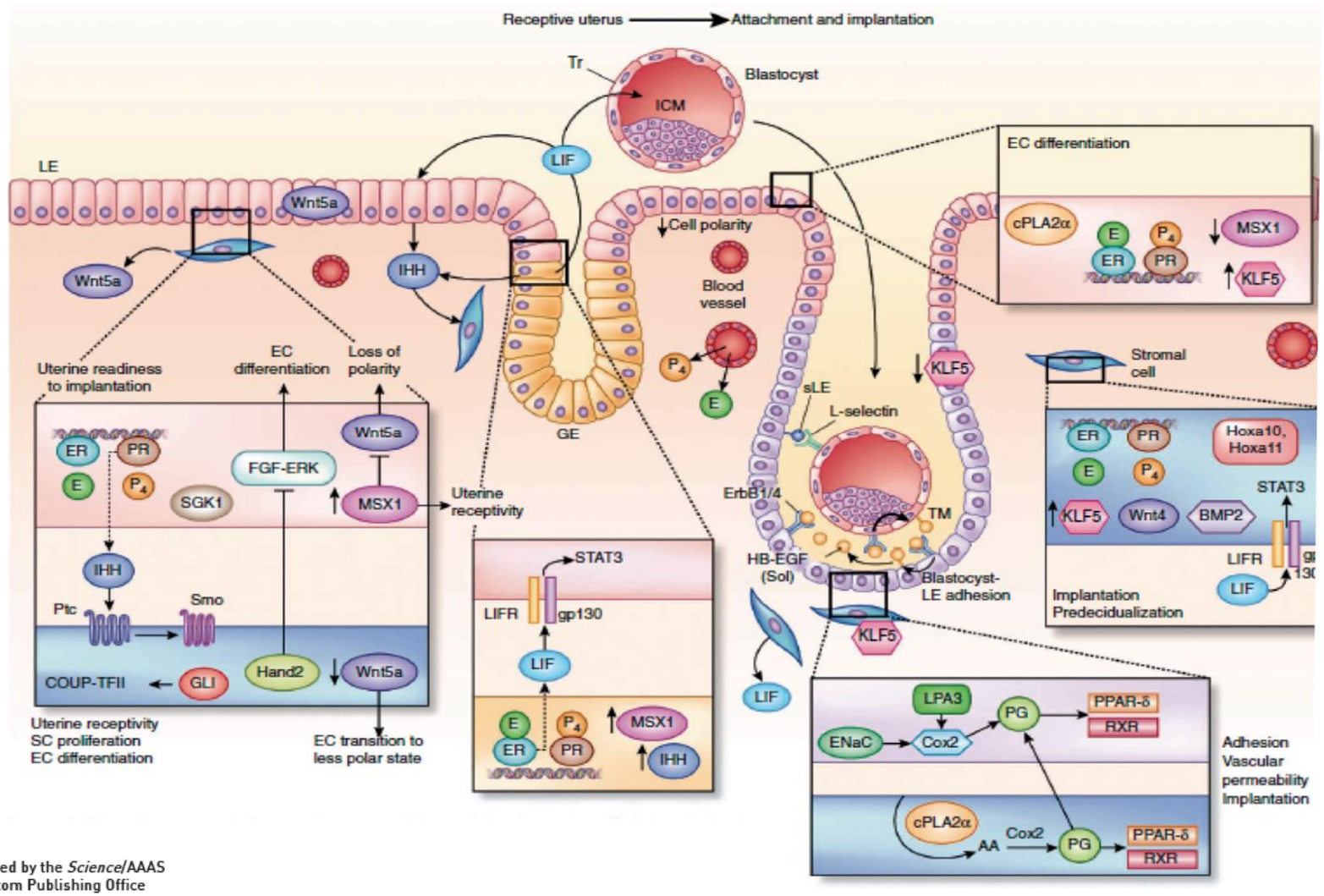
Carlos Simón MD. PhD.

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

Carlos Simon

Scientific Director of Igenomix SL

Molecular interplay in successful implantation



Produced by the *Science*/AAAS Custom Publishing Office

Science AAAS

Cha J*, Vilella F*, Dey SK, Simon C. "Molecular Interplay in Successful Implantation" in Ten Critical Topics in Reproductive Medicine, S. Sanders, Ed. (*Science/AAAS*, Washington, DC, 2013), pp. [44-48].

Efficiency of human embryo implantation

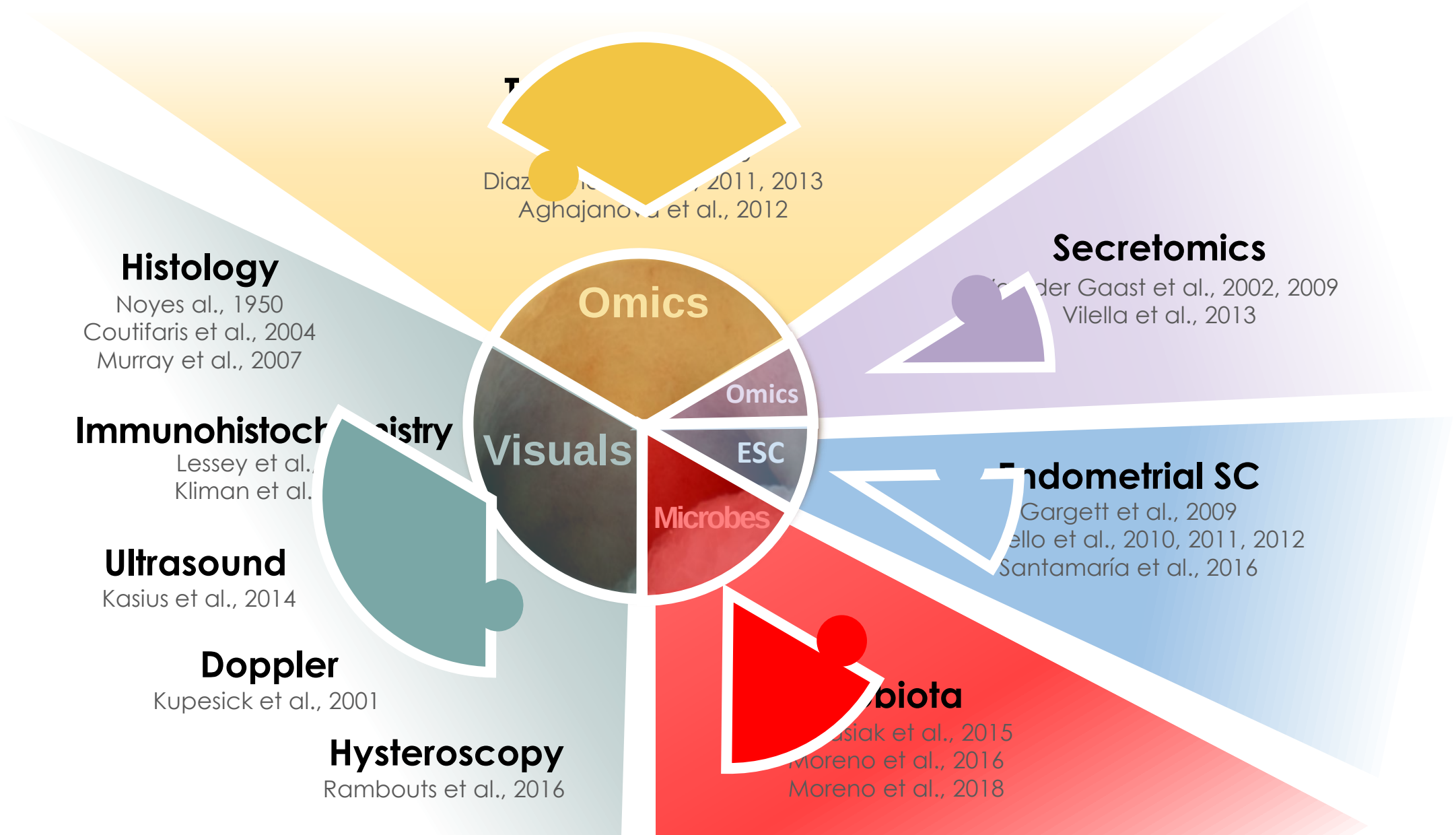
Embryo Aneuploidies	
Drosophila	0.01 %
Mouse	0.01 %
Human	20 – 100 %

Implantation Rate (IR)	
Natural cycle	35 %
Euploid embryos	52 % *(Rubio et al. Fertil Steril 2017)*
Rodents	95 %
Rabbits	96%

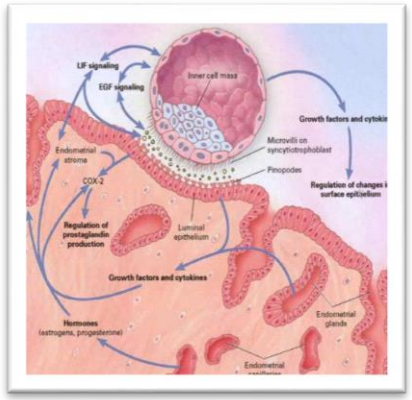
The main difference between humans and rodents lies in

The endometrial/**decidual control** in <u>human implantation</u>
versus
The **embryo control** in <u>rodent implantation</u> (embryonic diapause)

The Puzzle of the Endometrial Factor



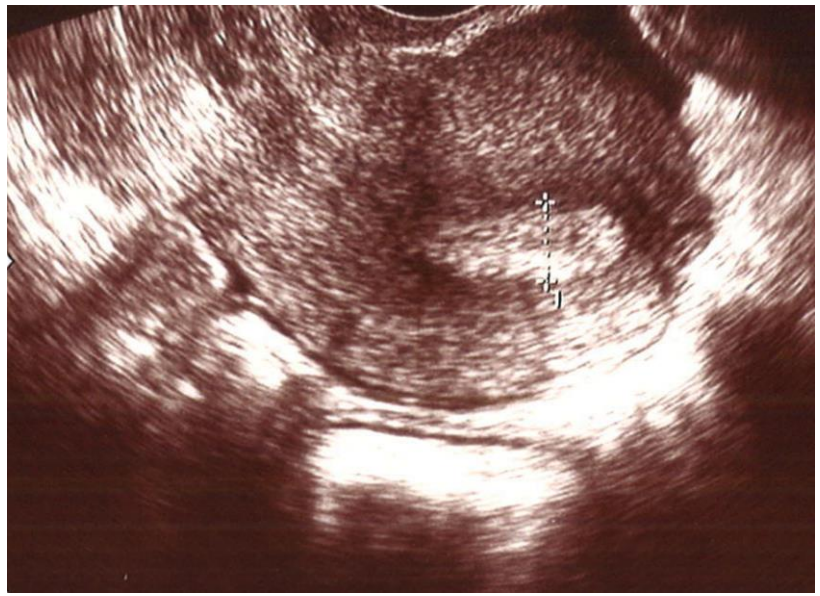
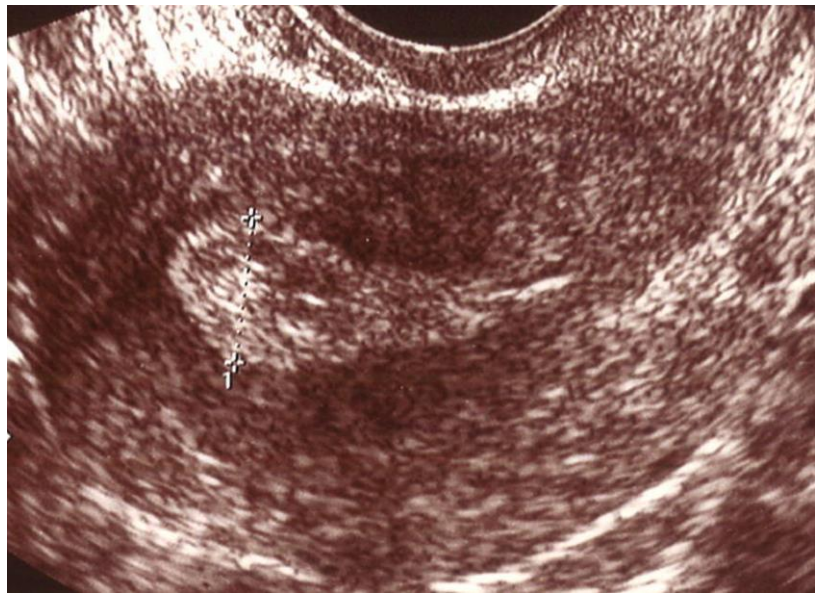
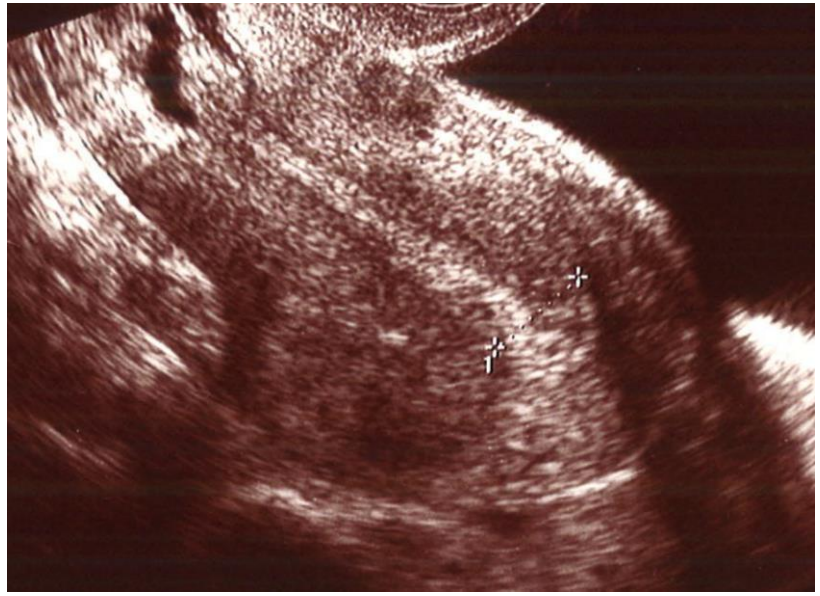
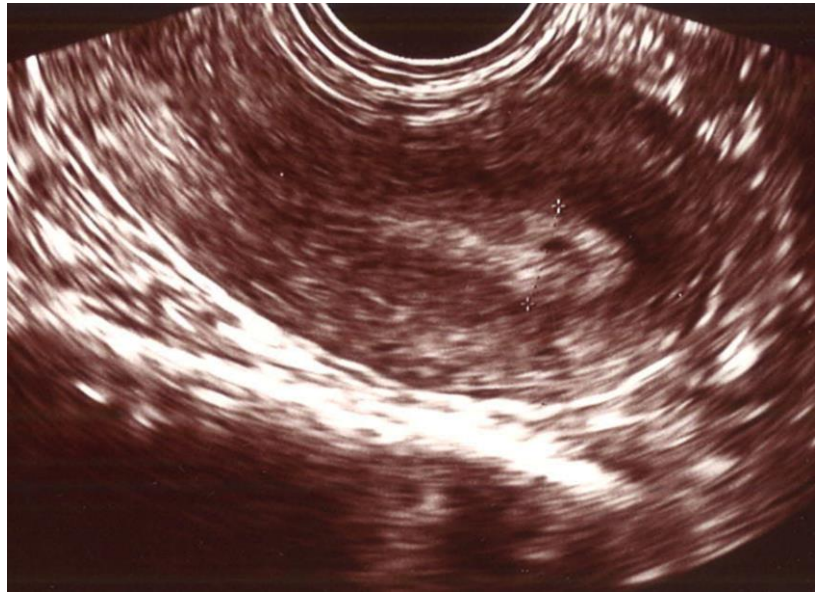
THE WINDOW OF IMPLANTATION



- ✓ 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[1]

- ✓ Randomized studies
  - Interobserver and cycle-to-cycle (60%) variations[2]
  - Endometrial dating is not related to fertility status[3]

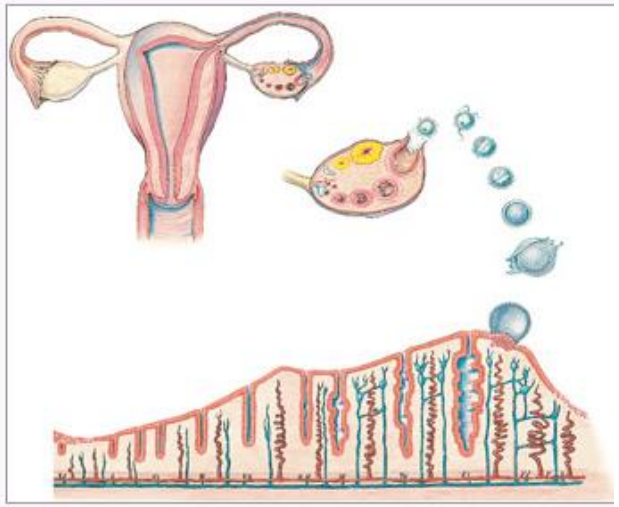
Histological dating is not a valid method for the diagnosis of luteal phase deficiency neither guidance throughout clinical management in infertility

1. Noyes, et al. Fertil Steril 1950
2. Murray, et al. Fertil Steril 2004
3. Coutifaris, et al. Fertil Steril 2004

http://www.etegritytest.com.

Patterns of Integrin Expression

Sample Collection



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).

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.

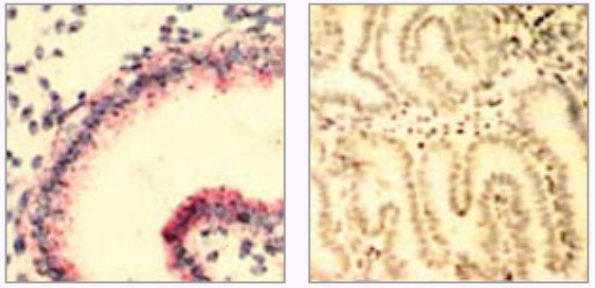
2. Beta-3 integrin NEGATIVE with an "out-of-phase" endometrium occurs in a patient with Luteal Phase Defect; following treatment the patient is advised to undergo a repeat biopsy to confirm diagnosis.[1]

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

Endometrial Function Test® (EFT®)
endometrialfunctiontest.com

The Endometrial Function Test® (EFT®)

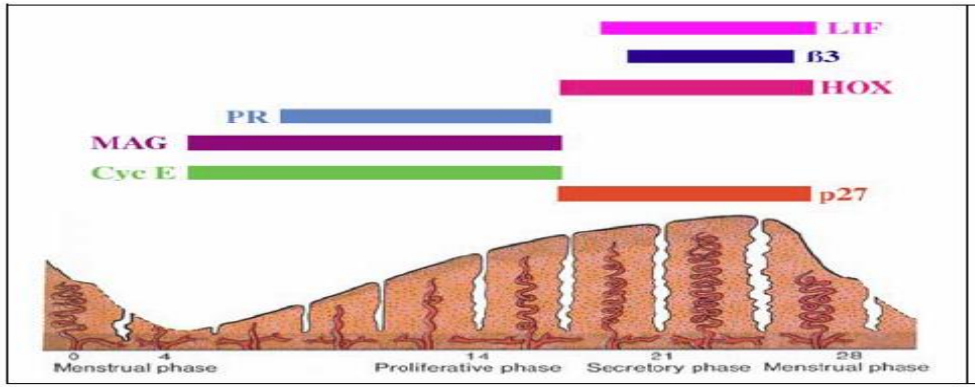


Figure 3. Panel of markers of endometrial development. Researchers have discovered many products that are made by the endometrium. The most important of these products are only made at particular times of the menstrual cycle. For example, progesterone receptor (PR), mouse ascites Golgi mucin (MAG) and cyclin E are normally only made during the proliferative and early secretory phases (cycle days 5 to ~19), while leukemia inhibitory factor (LIF), αvß3 integrin (ß3), HOXA-10 (HOX) and p27 are normally only expressed in the secretory phase (cycle days ~17 to ~28). Modified from *Langman's Medical Embryology*.

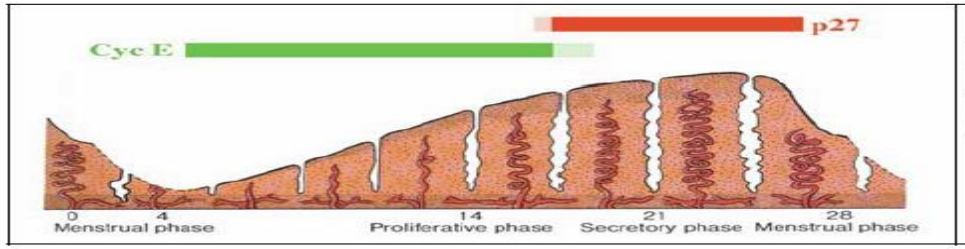


Figure 4. Cyclin E and p27 expression in fertile women. Cyclin E first appears at around cycle day 5 and continues to be expressed up until cycle day 19. After day 19, cyclin E normally is absent. p27, on the other hand, is absent until approximately cycle day 17, where it is seen for the remainder of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

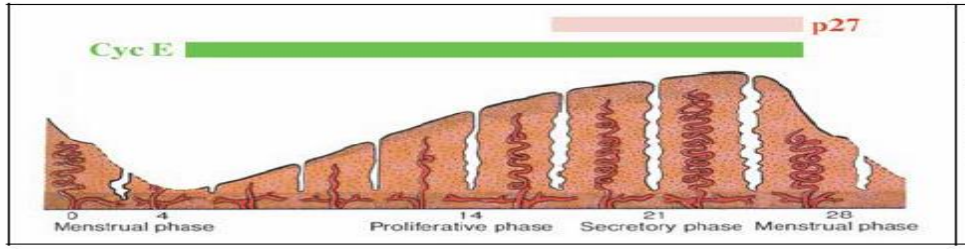
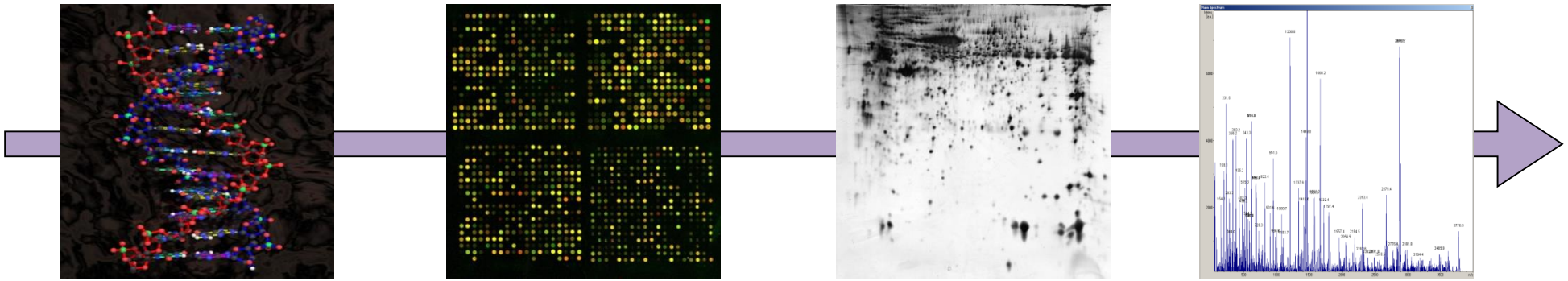
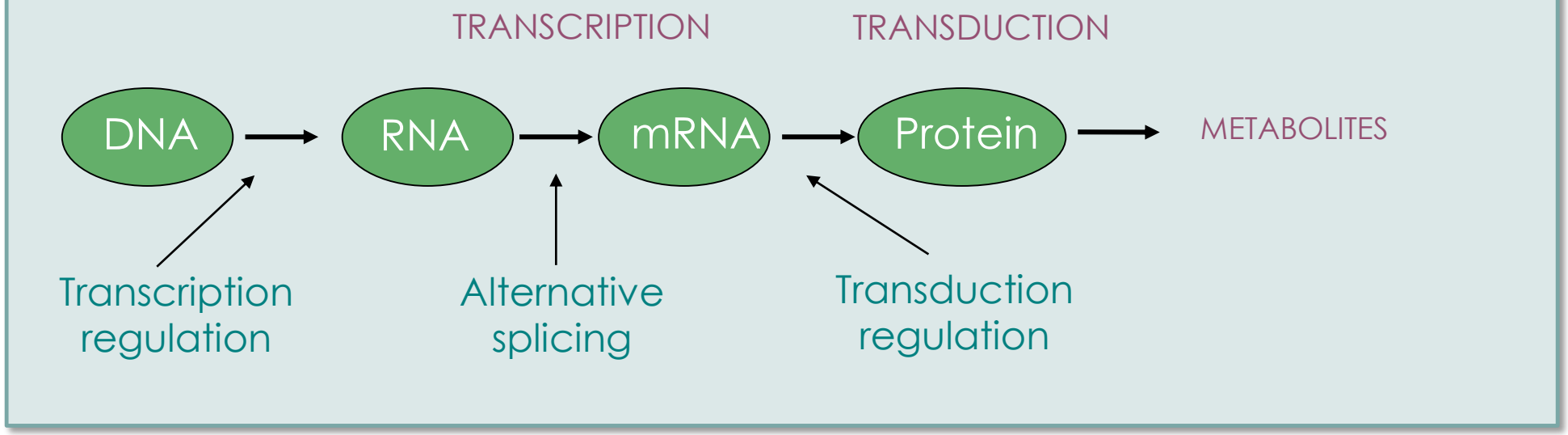


Figure 5. Cyclin E and p27 expression in women with unexplained infertility. The most striking difference between the cyclin expression of fertile women and infertile women is the persistence of cyclin E and decreased presence of p27 into the secretory phase. This finding represents a developmental arrest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

The age of -OMICS



Transcriptome Proteome Metabolome



GENETICS

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

Patricia Díaz-Gimeno, Ph.D.,[a,b] *José A. Horcajadas, Ph.D.,*[c] *José A. Martínez-Conejero, Ph.D.,*[c] *Francisco J. Esteban, Ph.D.,*[d] *Pilar Alamá, M.D.,*[a,b] *Antonio Pellicer, M.D.,*[a,b] *and Carlos Simón, M.D.*[a,b,e]

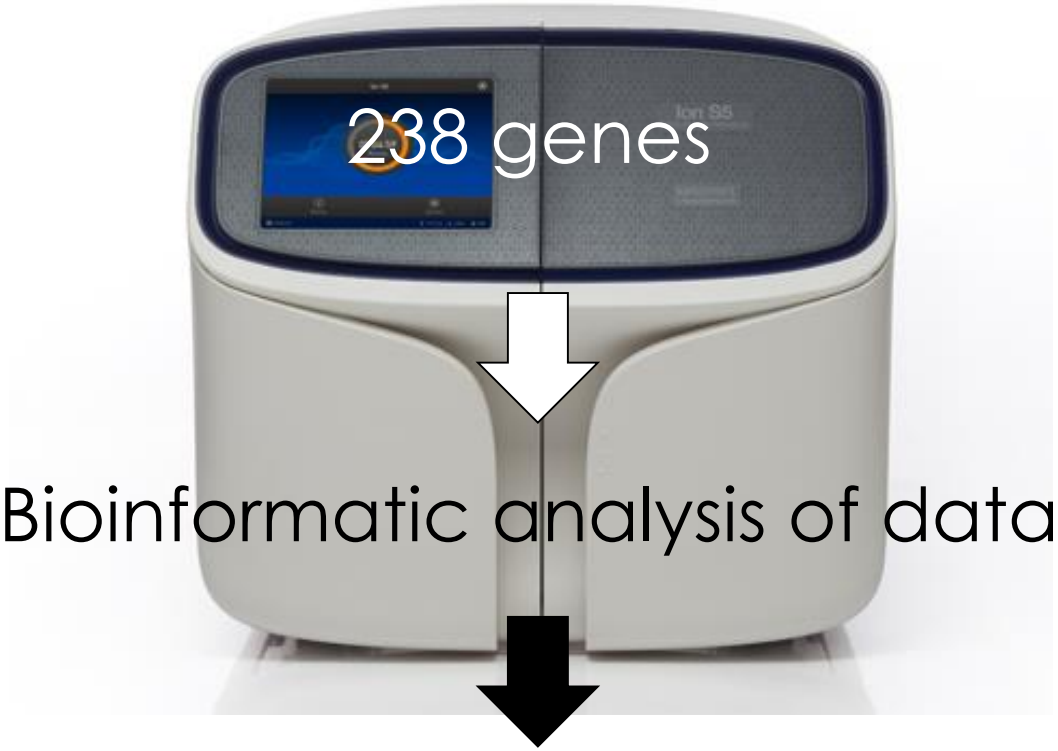
[a] Fundación IVI-Instituto Universitario IVI, University of Valencia, Valencia; [b] Instituto de Investigación, Sanitaria del Hospital Clinico de Valencia, Valencia University, Valencia; [c] iGenomix, Valencia; [d] Department of Experimental Biology, University of Jaén, Jaén; and [e] Centro de Investigación Principe 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 (88) 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

Fertility and Sterility® Vol. 95, No. 1, January 2011
0015-0282/$36.00

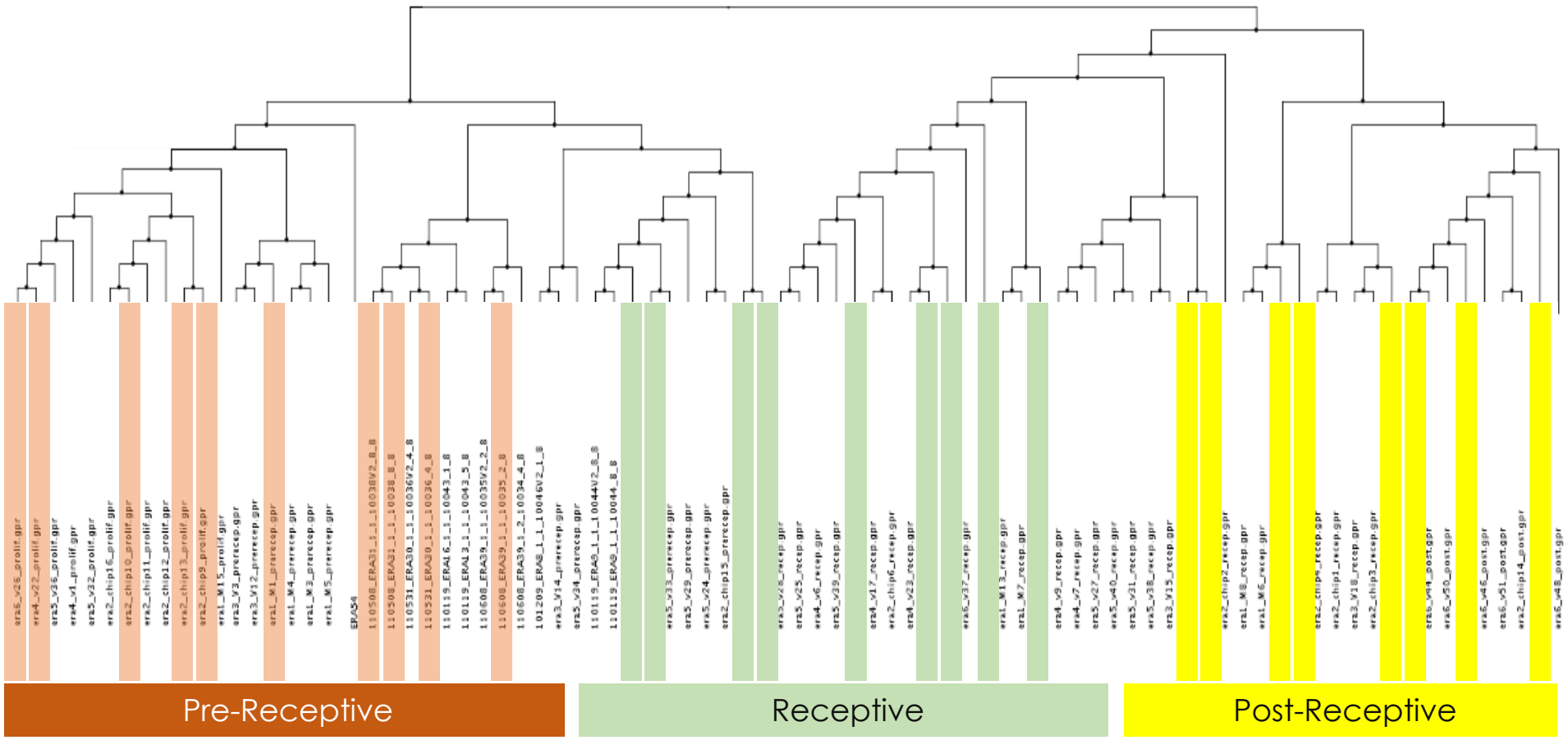
doi:10.1016/j.fertnstert.2010.04.063



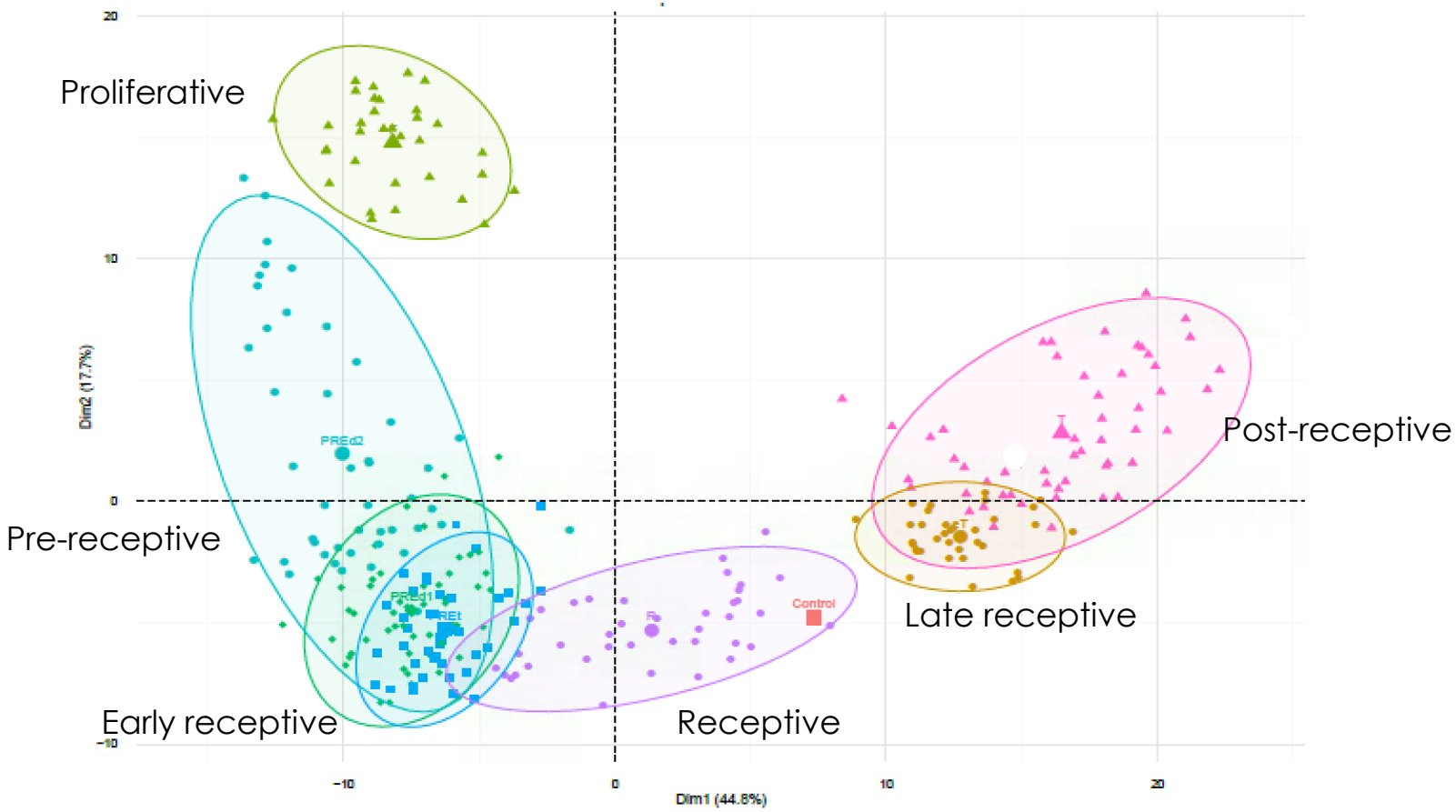
Classification and prediction from gene expression

Patented in 2009: PCT/ES 2009/000386
LDT with CLIA

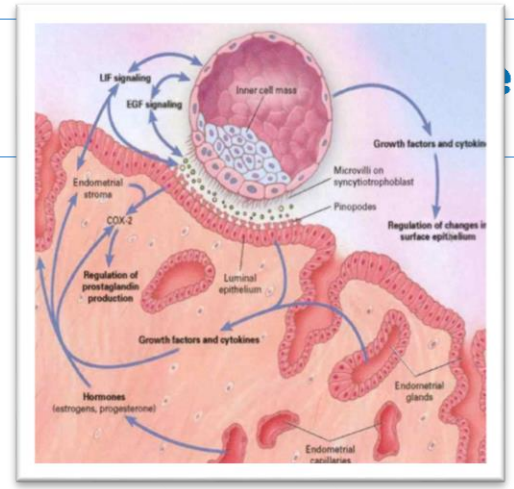
Predictor Classifies the Molecular Receptivity Status of the Endometrium

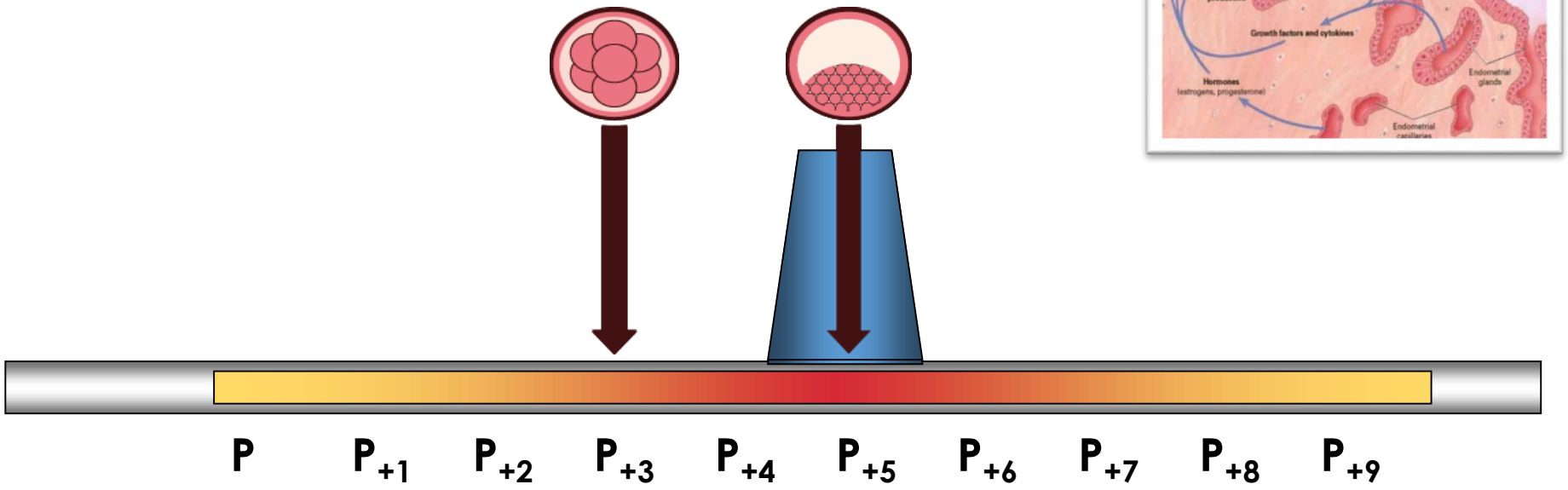


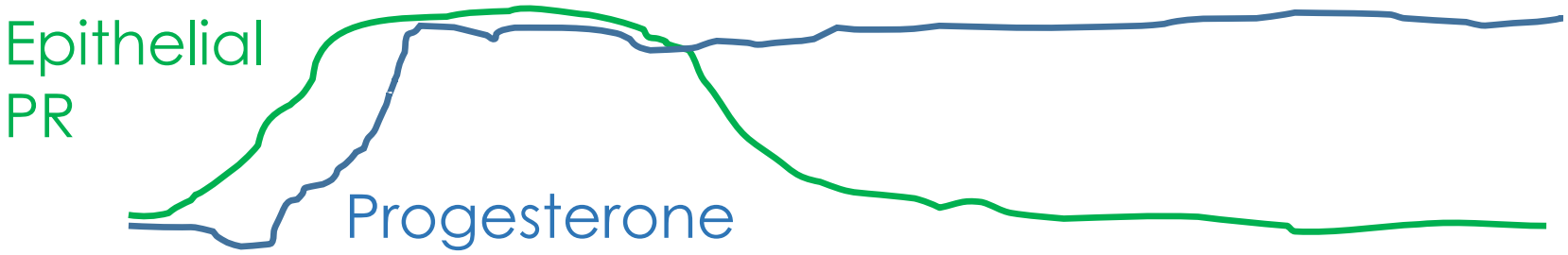
Retrainig the ERA Algorithms



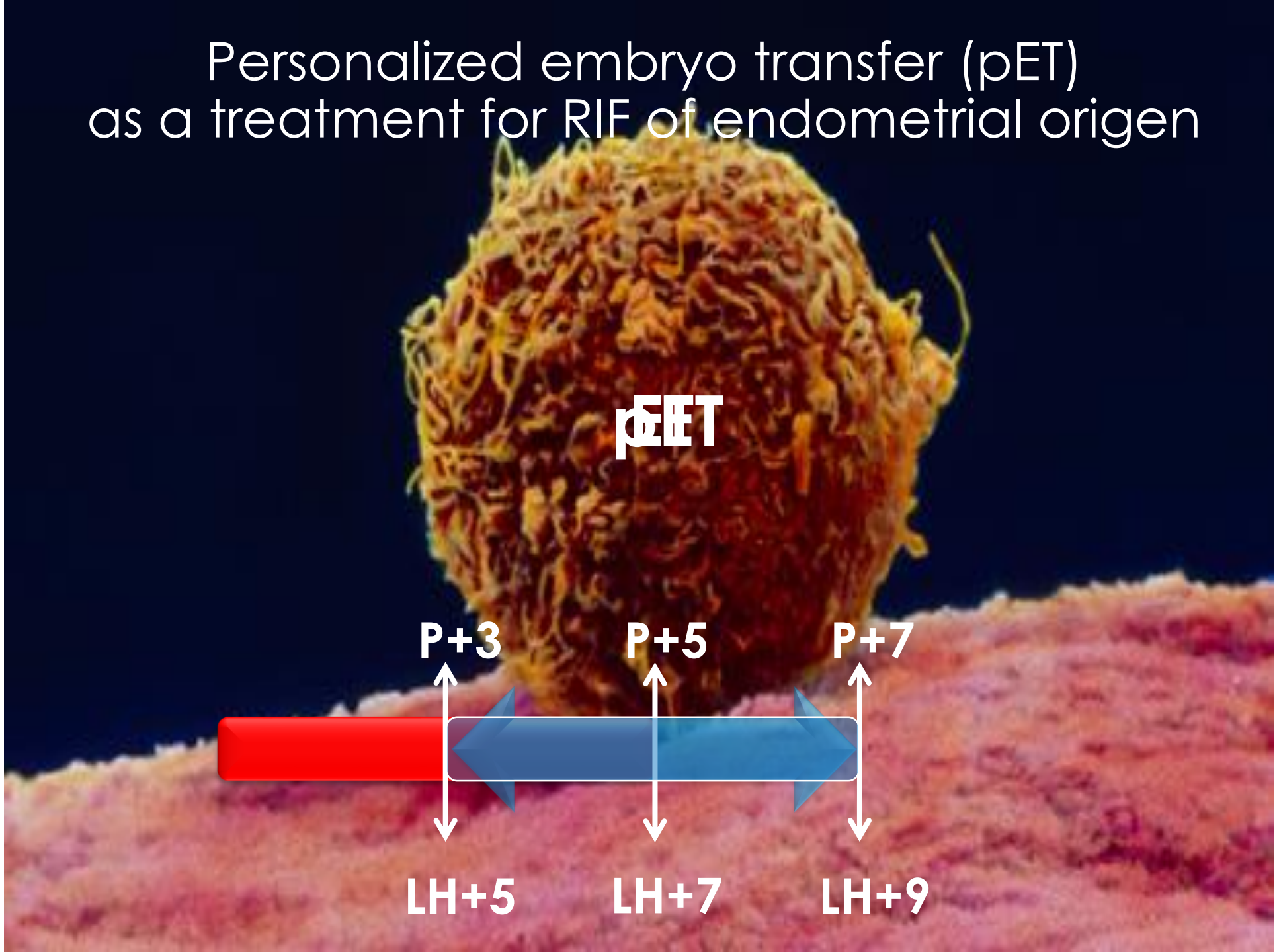
The Symphony







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



ERA Publications

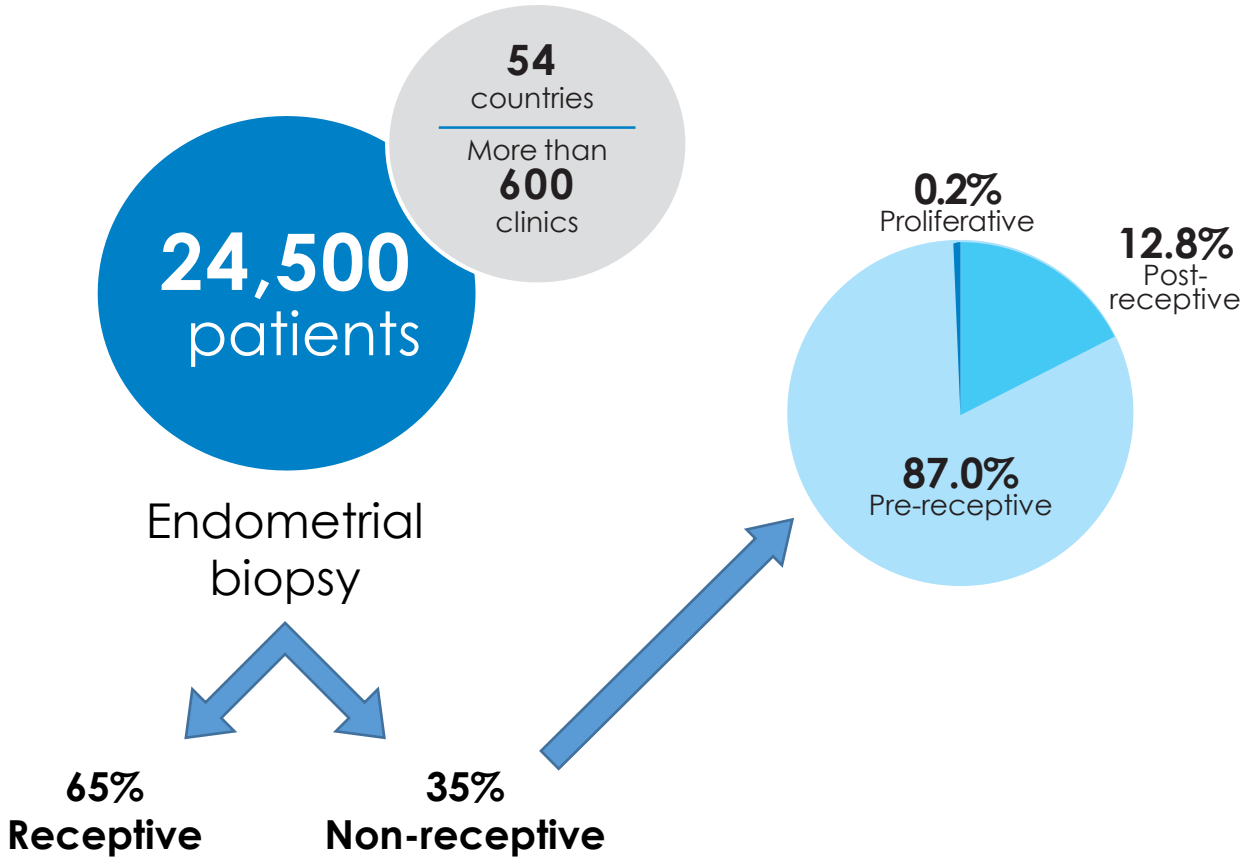
YEAR	TITLE	JOURNAL
2011	A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature	Fertility and Sterility. 95(1): 50-60, 60.e1-15
2012	The genomics of the human endometrium	Biochimica et Biophysica Acta – Molecular Basis Disease. 1822(12):1931-42
2013	The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity	Fertility and Sterility. 99(2):508-17
2013	Profiling the gene signature of endometrial receptivity: clinical results	Fertility and Sterility. 99(4):1078-85
2013	The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure	Fertility and Sterility. 100(3): 818-24
2014	Impact of final oocyte maturation using gonadotropin-releasing hormone agonist triggering and different luteal support protocols on endometrial gene expression	Fertility and Sterility. 101(1):138-46.e3
2014	The impact of using the combined oral contraceptive pill for cycle scheduling on gene expression related to endometrial receptivity	Human Reproduction. 29(6):1271-8
2014	What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study	Human Reproduction. 29(6):1244-7
2014	Scratching beneath 'The Scratching Case': systematic reviews and meta-analyses, the back door for evidence-based medicine	Human Reproduction. 29(8):1618-21
2014	Transcriptomics of the human endometrium	Int J Dev Biol. 58(2-3-4):127-37
2014	Deciphering the proteomic signature of human endometrial receptivity	Human Reproduction. 29(9): 1957-67

ERA Publications

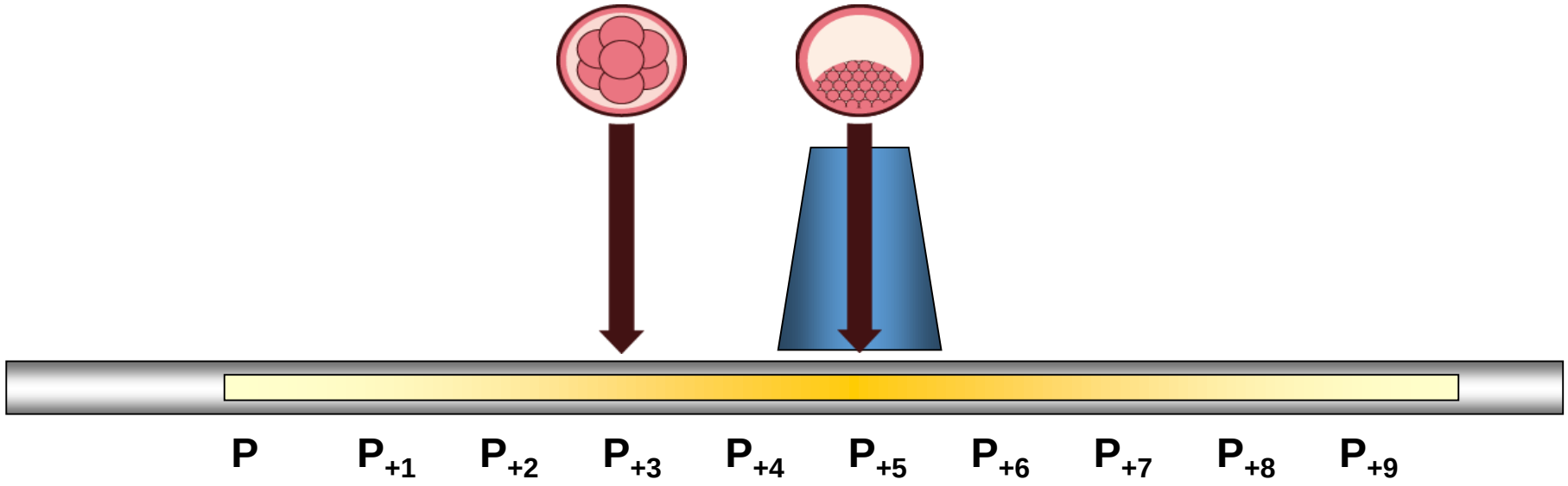
YEAR	TITLE	JOURNAL
2014	Clinical Management of Endometrial Receptivity	Semin Reprod Med. 32(5):410-4
2014	Timing the window of implantation by nucleolar channel system prevalence matches the accuracy of the endometrial receptivity array	Fertility and Sterility. 102(5):1477-81
2015	Human Endometrial Transcriptomics: Implications for Embryonic Implantation	Cold Spring Harb Perspect Med. 5(7):a022996
2015	Understanding and improving endometrial receptivity	Current Opinion in Obstetrics & Gynecology. 27(3):187-92
2015	Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study	Reproductive BioMedicine Online. 31(5):647-54
2016	Diagnosis of endometrial-factor infertility: current approaches and new avenues for research	Geburtshilfe Frauenheilkd. 76(6): 699-703
2017	Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis	Fertility and Sterility. 107(3):740-748.e2
2017	Endometrial function: facts, urban legends, and an eye to the future	Fertility and Sterility. 108(1):4-8
2017	Implantation failure of endometrial origin: it is not pathology, but our failure to synchronize the developing embryo with a receptive endometrium	Fertility and Sterility. 108(1):15-18
2017	Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers	Scientific Reports. 7(1):10077
2017	Window of implantation transcriptomic stratification reveals different endometrial subsignatures associated with live birth and biochemical pregnancy	Fertility and Sterility. 108(4):703-710.e3

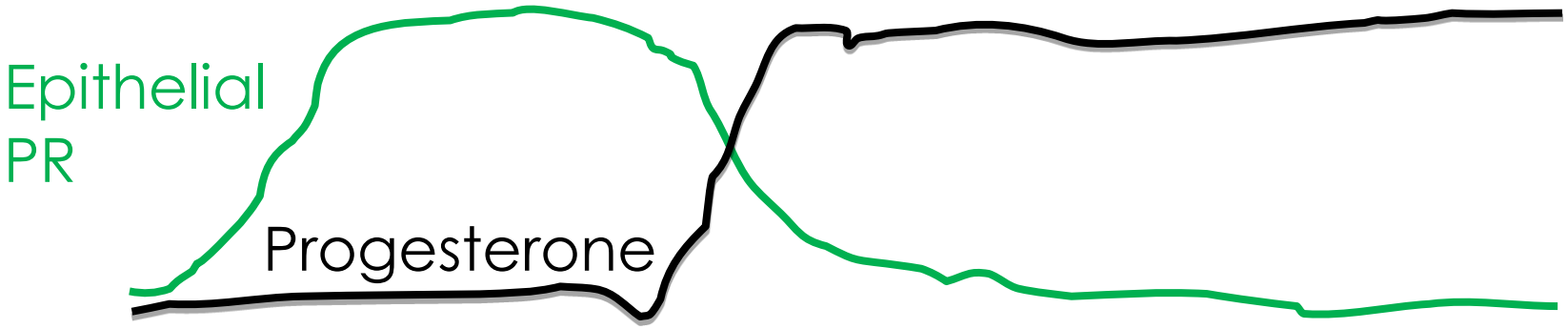
ERA in numbers

✓ Results

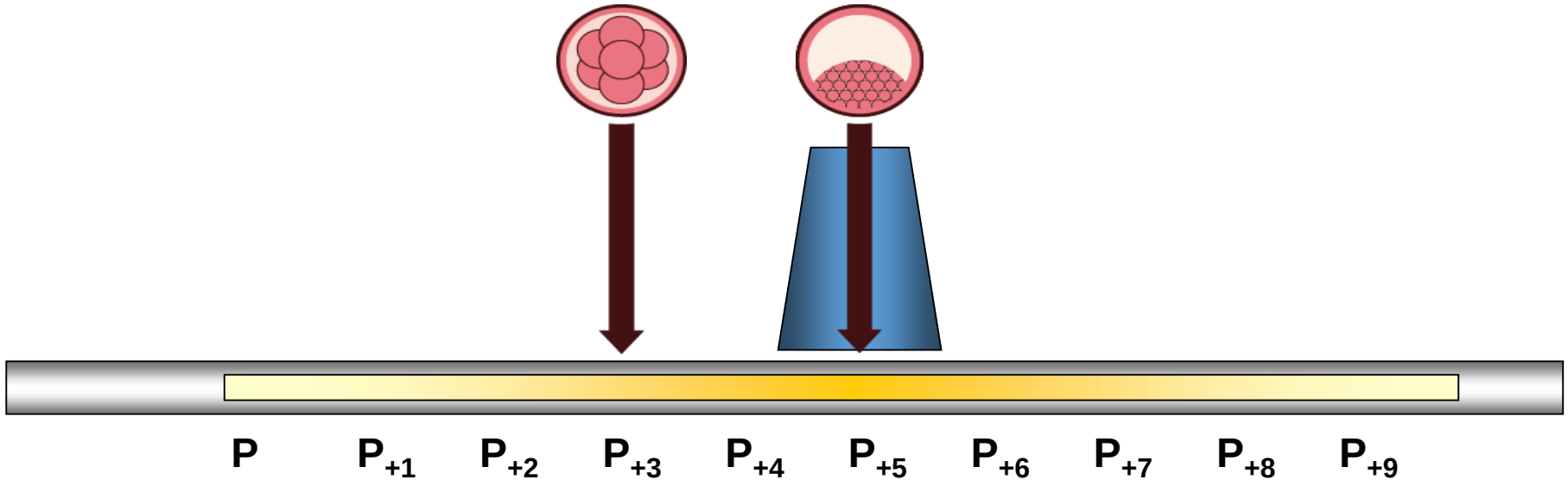


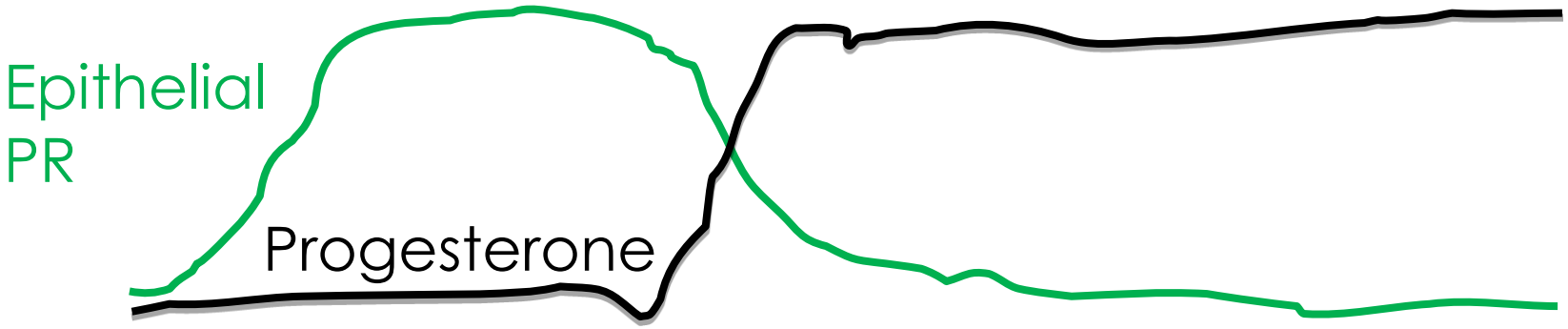
Progesterone elevation on the day of hCG





Slow Embryos





Endometrial Thickness versus Molecular Receptivity

Endometrial thickness (mm)	Receptive (%)	Non Receptive (%)
<6	6/14 **(43%)***	8/14 **(57%)***
6-12	333/431 **(77%)***	98/431 **(23%)***
>12	24/37 **(65%)**	13/37 **(35%)**
TOTAL	363	119

*P= 0,003 by Chi-square test.

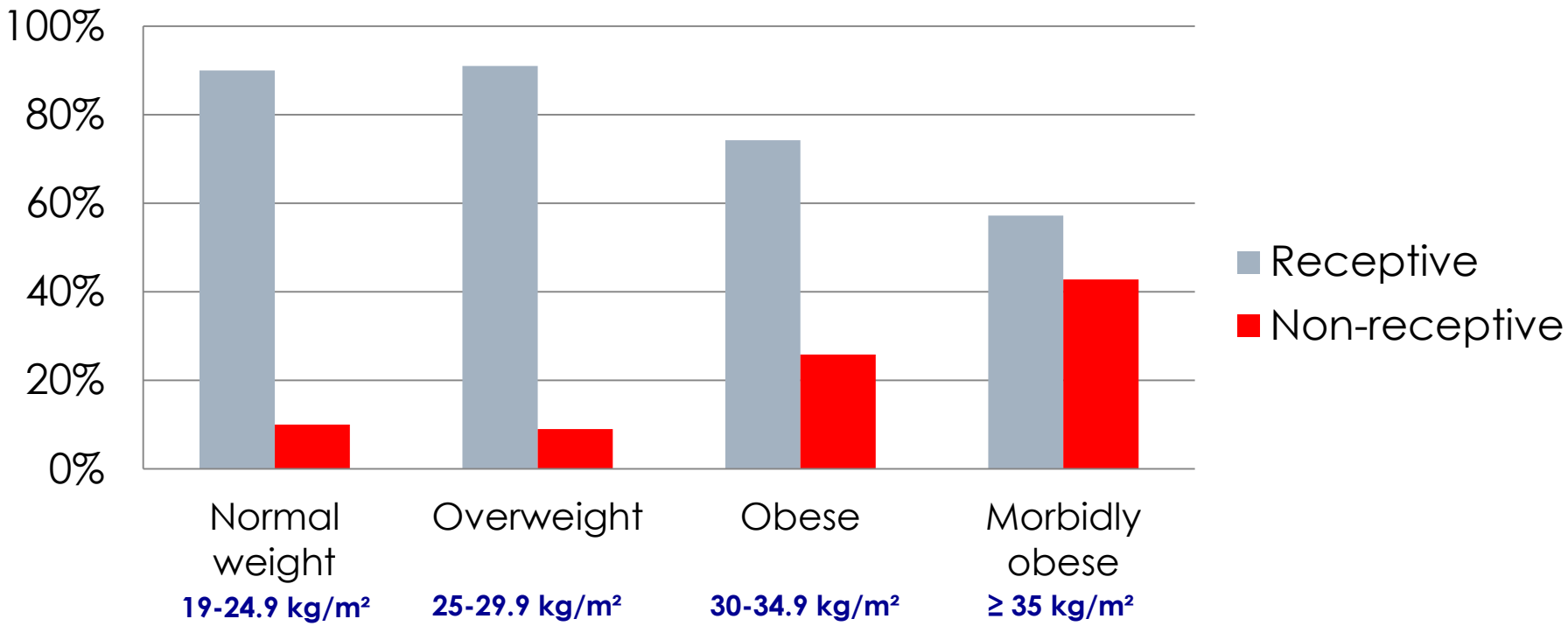
Valbuena D. et al. ESHRE 2016

ORIGINAL ARTICLES: INFERTILITY

Fertility and Sterility

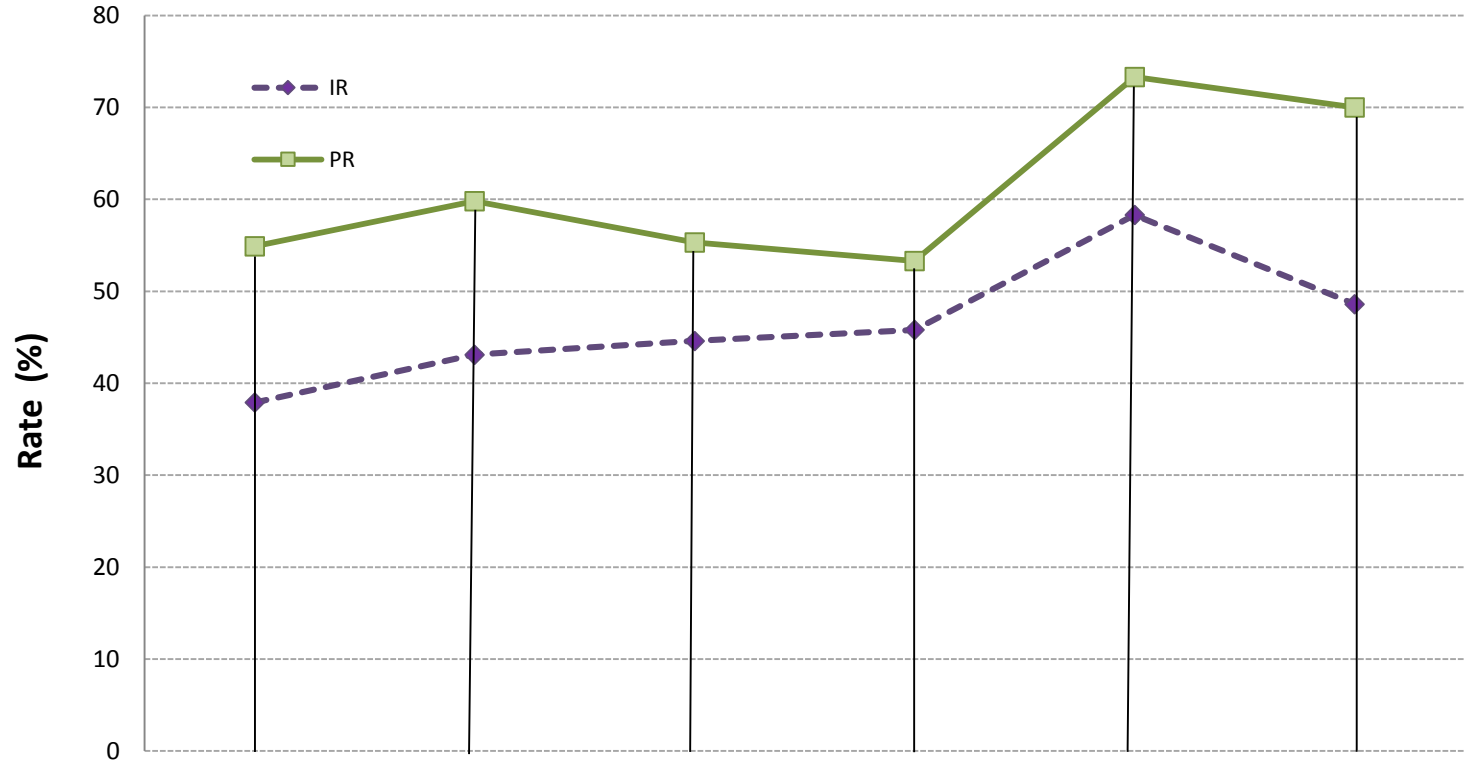
Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis

Ioanna A. Comstock, M.D.,[a] 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.,[c] Cecilia T. Valdes, M.D.,[c] Maria Ruiz-Alonso, M.Sc.,[d] Diana Valbuena, M.D., Ph.D.,[d] Carlos Simon, M.D., Ph.D.,[a,b,c,d] and Ruth B. Lathi, M.D.[a]



Comstock, et al. Fertil Steril 2017

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



Months after ERA test	1	2	3	4	5	6
Number of patients	91	87	47	30	15	40
Implantation Rate (%)	37.9	43.1	44.6	45.8	58.3	48.6
Pregnancy Rate (%)	54.9	59.8	55.3	53.3	73.3	70.0

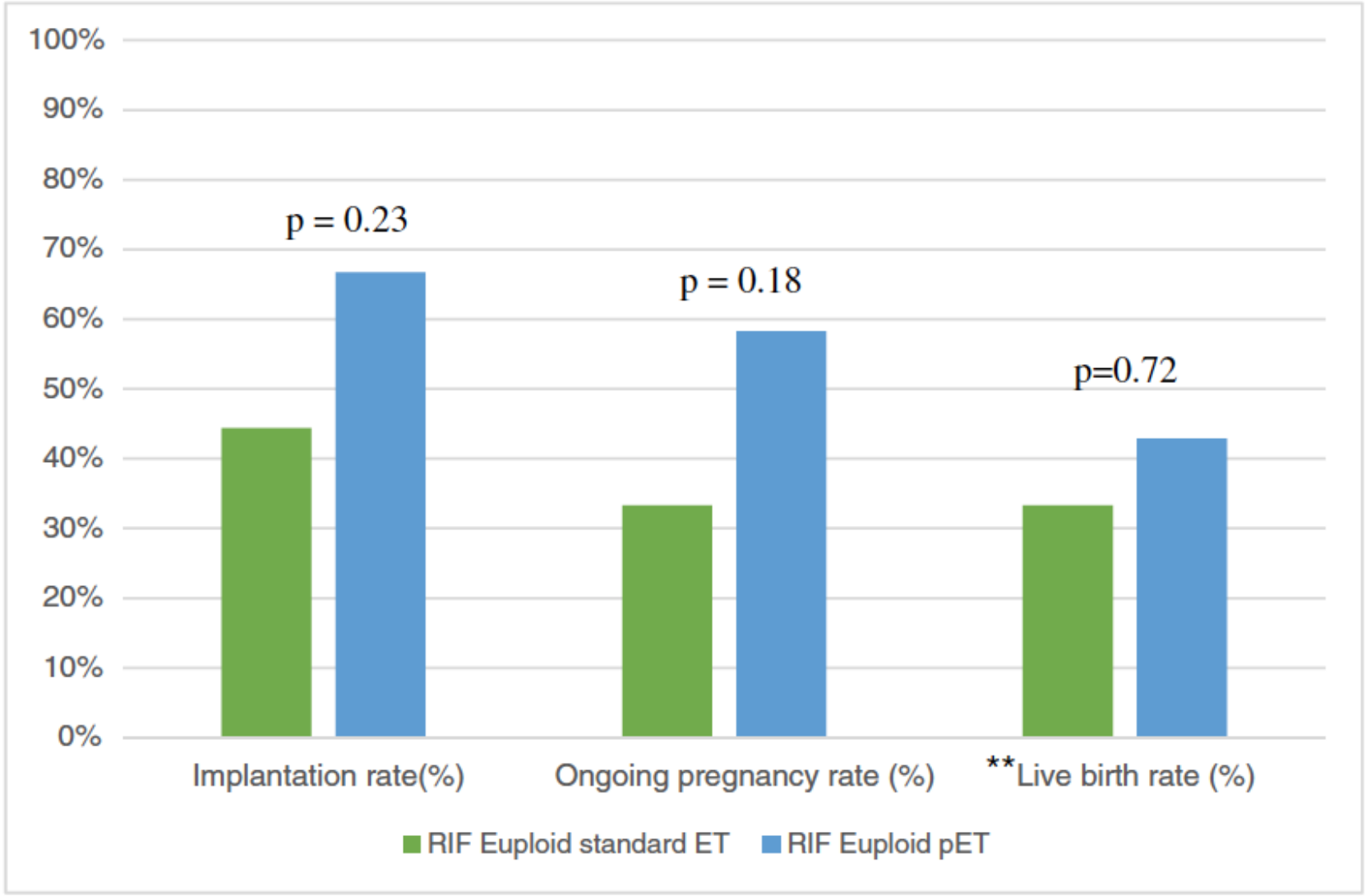
Ruiz-Alonso, et al. Fertil Steril 2013

CLINICAL OUTCOME AND EFFICIENCY OF ET ACCORDING ERA DIAGNOSIS

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

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

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

(ClinicalTrials.gov Identifier: NCT01954758)

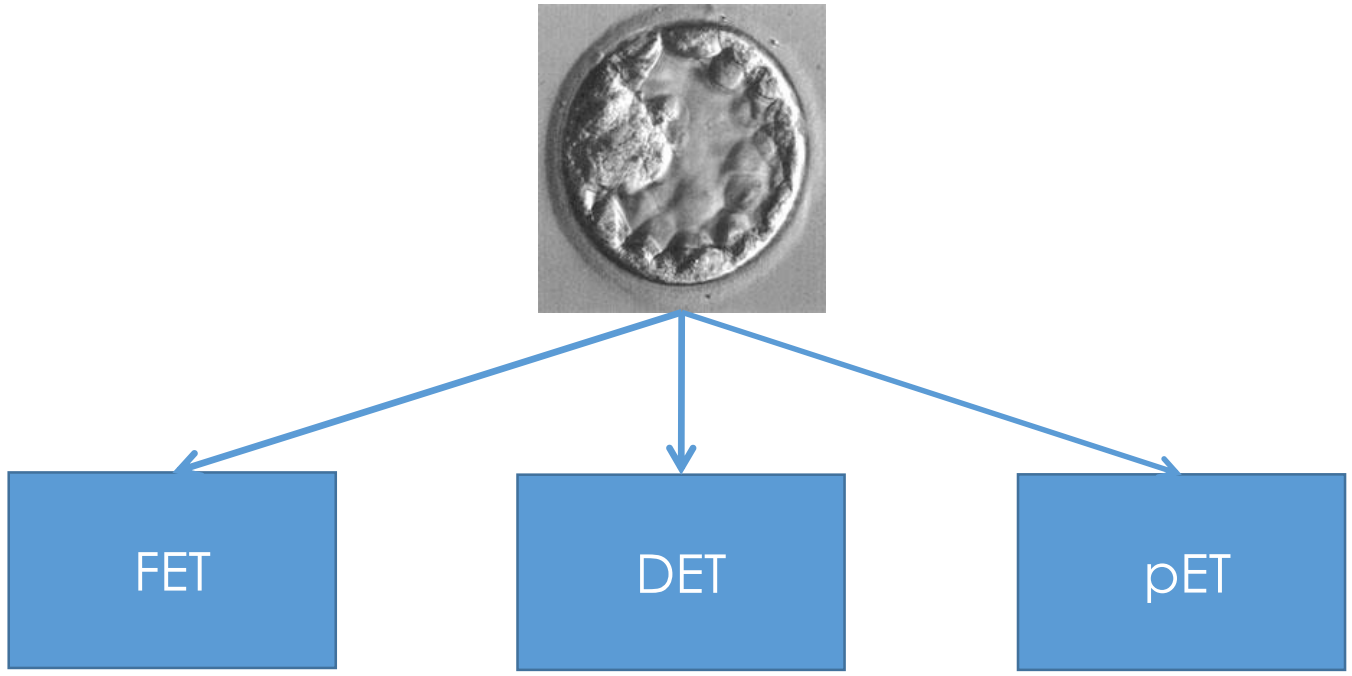
Stimated number size: 546

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

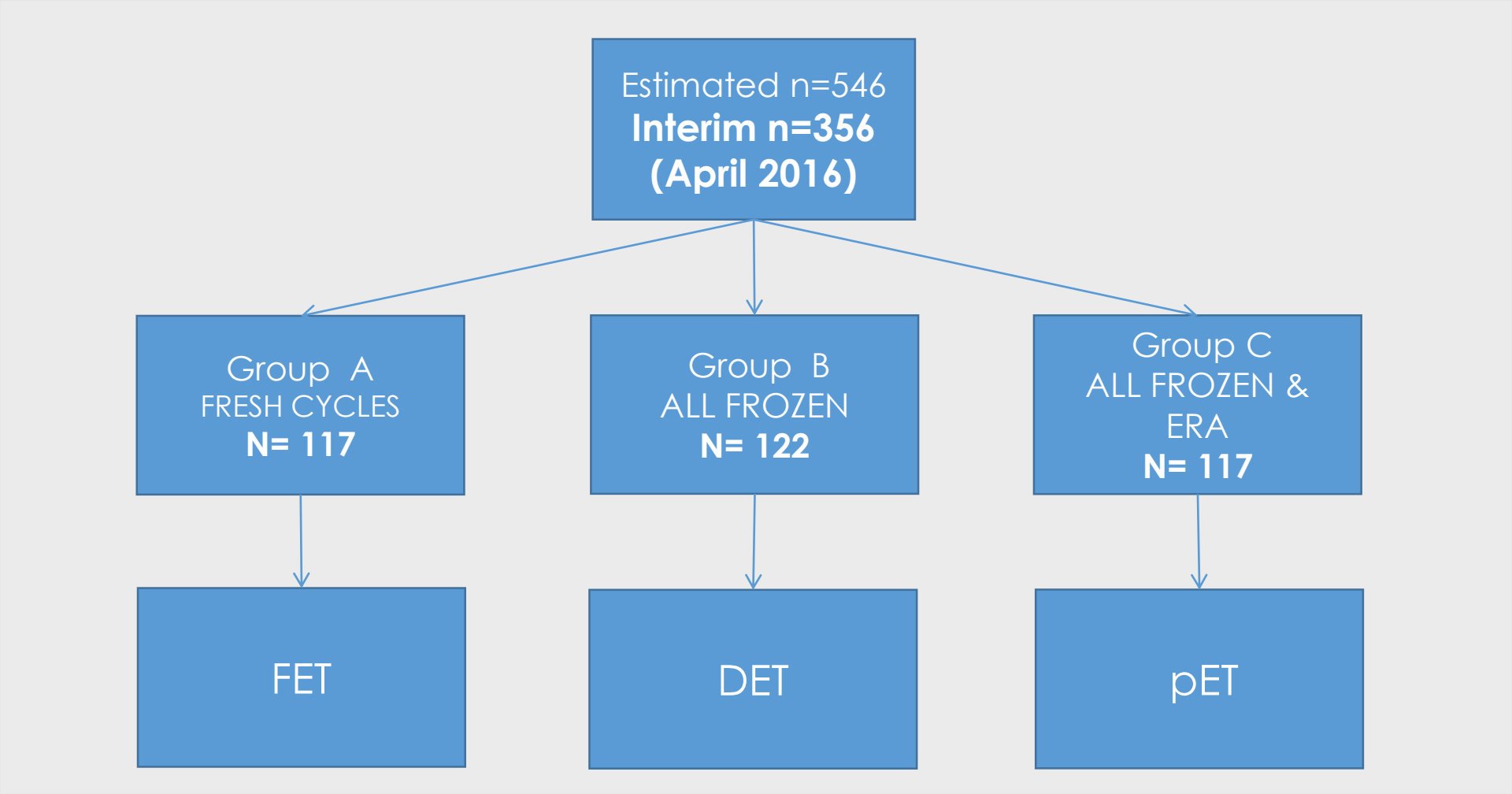
Objective

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




UZ Brussels, University Fertility Center, Belgium
Genesis IVF, Serbia
IVI, Spain (11 sites)
Sbalagrm Sofia, Bulgary
Oak Clinic, Japan
ProcreaTec, Spain
Bahceci Health Group, Turkey
Stanford University, USA
Instituto Vida Matamoros, Mexico
IECH Monterrey, Mexico
IVI Panama
KKH, Singapore
ReproTec, Colombia
Centro de Infertilidade e Medicina Fetal
do Norte Fluminense, Brazil
Centro Reproduçao G Mario Covas, Brazil
Huntington Medicina Reprodutiva, Brazil
Embriofert, Brazil
Centro Reproduçao Nilo
Frantz, Brazil
Recruiting Sites at the Interim (12)
Active Sites with EC/IRB approval (28)
igenomix
PIONEERS IN REPRODUCTIVE GENETICS

Aproved by the protocol review service of the Lancet in 2012
Trial started in October 2013
Interim outcome evaluated in April 2016



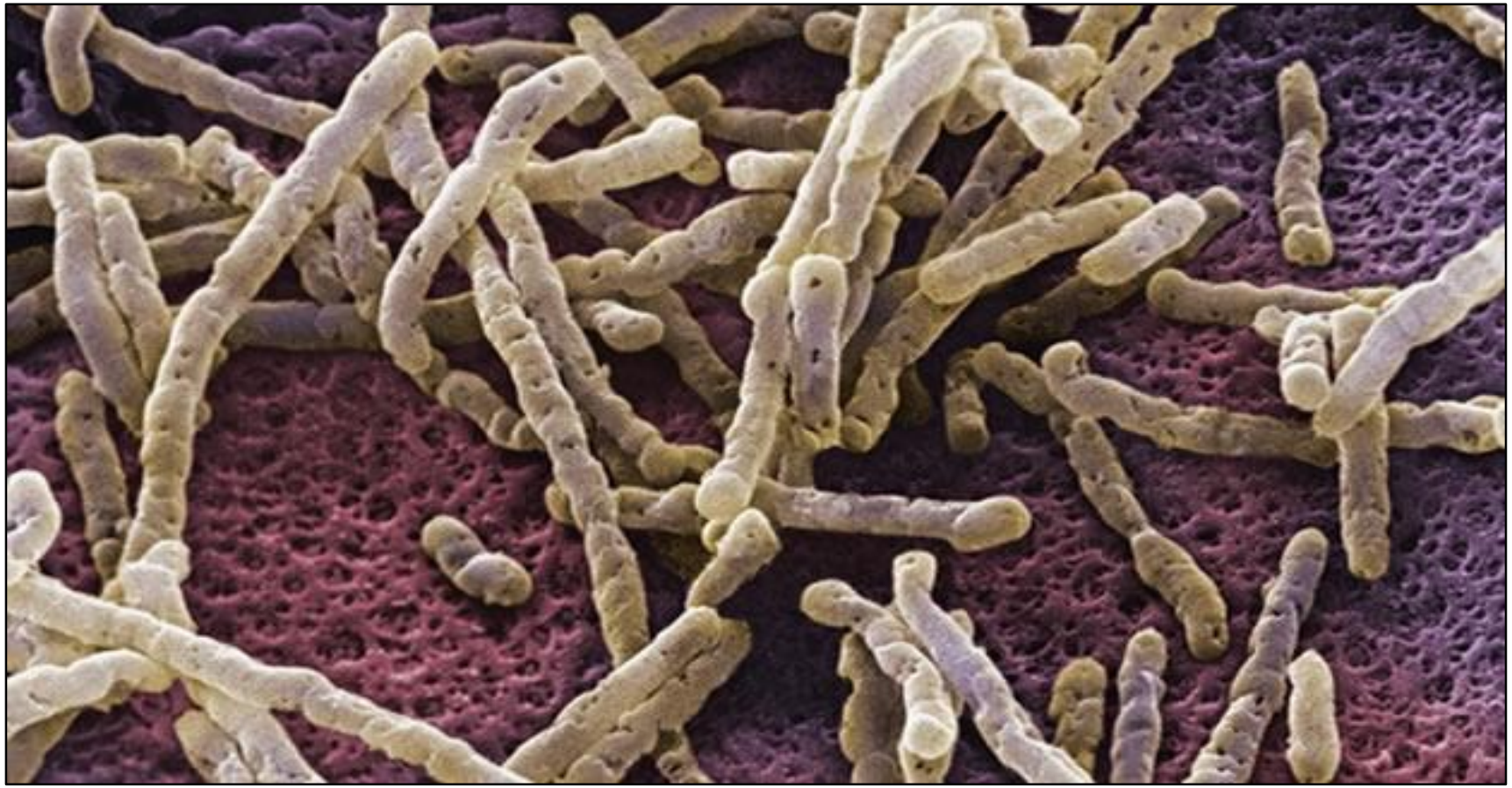
Reproductive Outcome / Interim study)

igenomix

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

* p value <0.05 by Chi-Square test

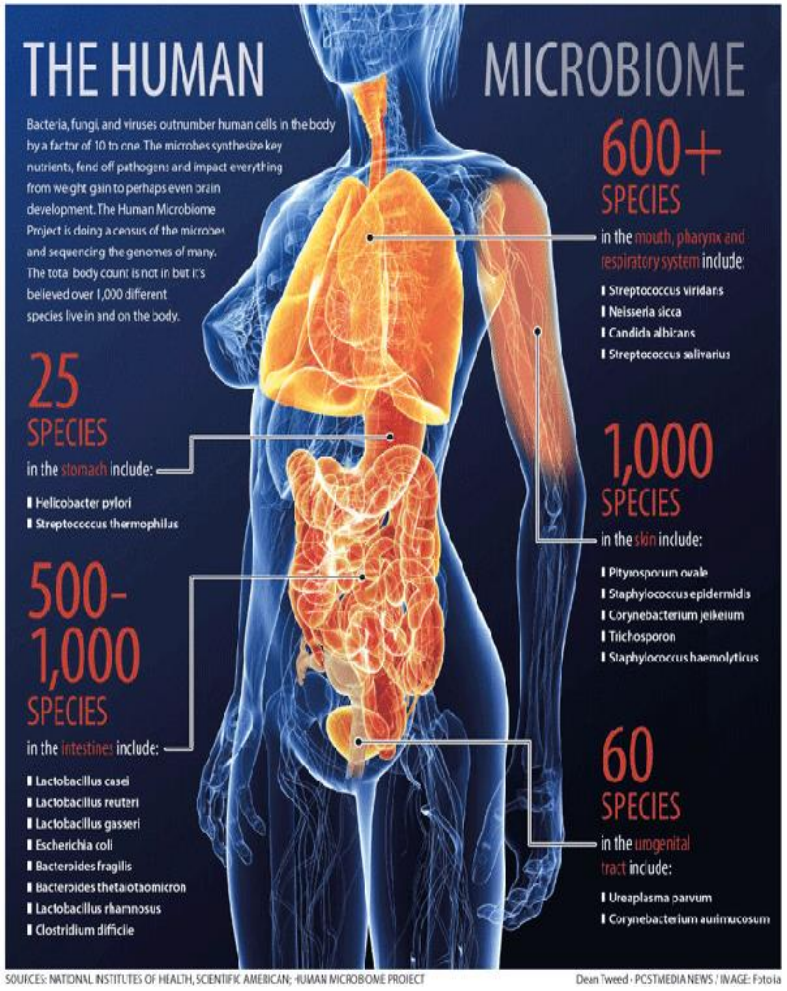
Simon C , et al. 2016 Awarded Oral Presentation ASRM



Bacteria: an invisible universe also in

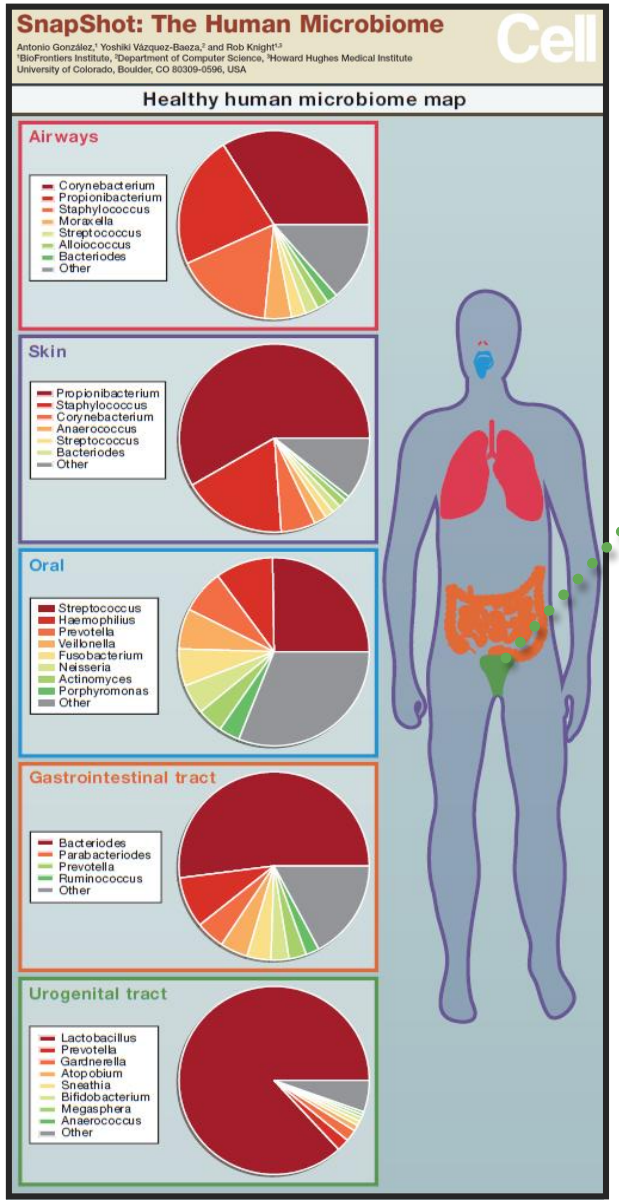
Reproductive Medicine

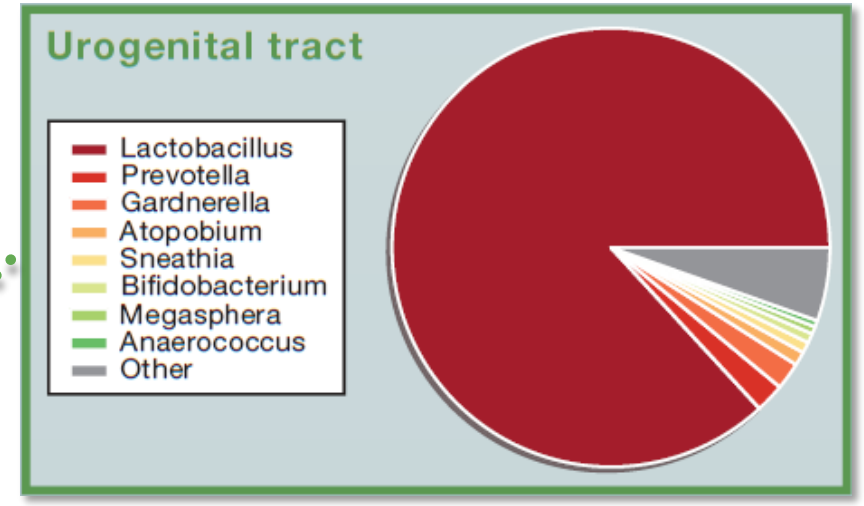
The Human Microbiome



- 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

The Human Microbiome





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.

Leitich et al. 2003. Am J Obstet Gynecol. 189:139–47
Sirota et al. 2014. Semin Reprod Med. 32:35-42
Mangot-Bertrand et al. 2013. Eur J Clin Microbiol Infect Dis 32: 535-41
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[1]; Francisco M. Codoñer, PhD[1]; Felipe Vilella, PhD[1]; Diana Valbuena, MD, PhD; Juan F. Martinez-Blanch, PhD; Jorge Jimenez-Almazán, PhD; Roberto Alonso; Pilar Alamá, MD, PhD; Jose Remohí, MD, PhD; Antonio Pellicer, MD, PhD; Daniel Ramon, PhD[2]; Carlos Simon, MD, PhD[2]

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 prereceptive 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 prereceptive 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. Genomic DNA was obtained either from endometrial fluid or vaginal aspirate and sequenced by 454 pyrosequencing of the V3—V5 region of the *16S* ribosomal RNA (rRNA) gene; the resulting sequences were taxonomically assigned using QIIME. Data analysis was performed using R packages. The χ^2 test, Student *t* test, and analysis of variance were used for statistical analyses.

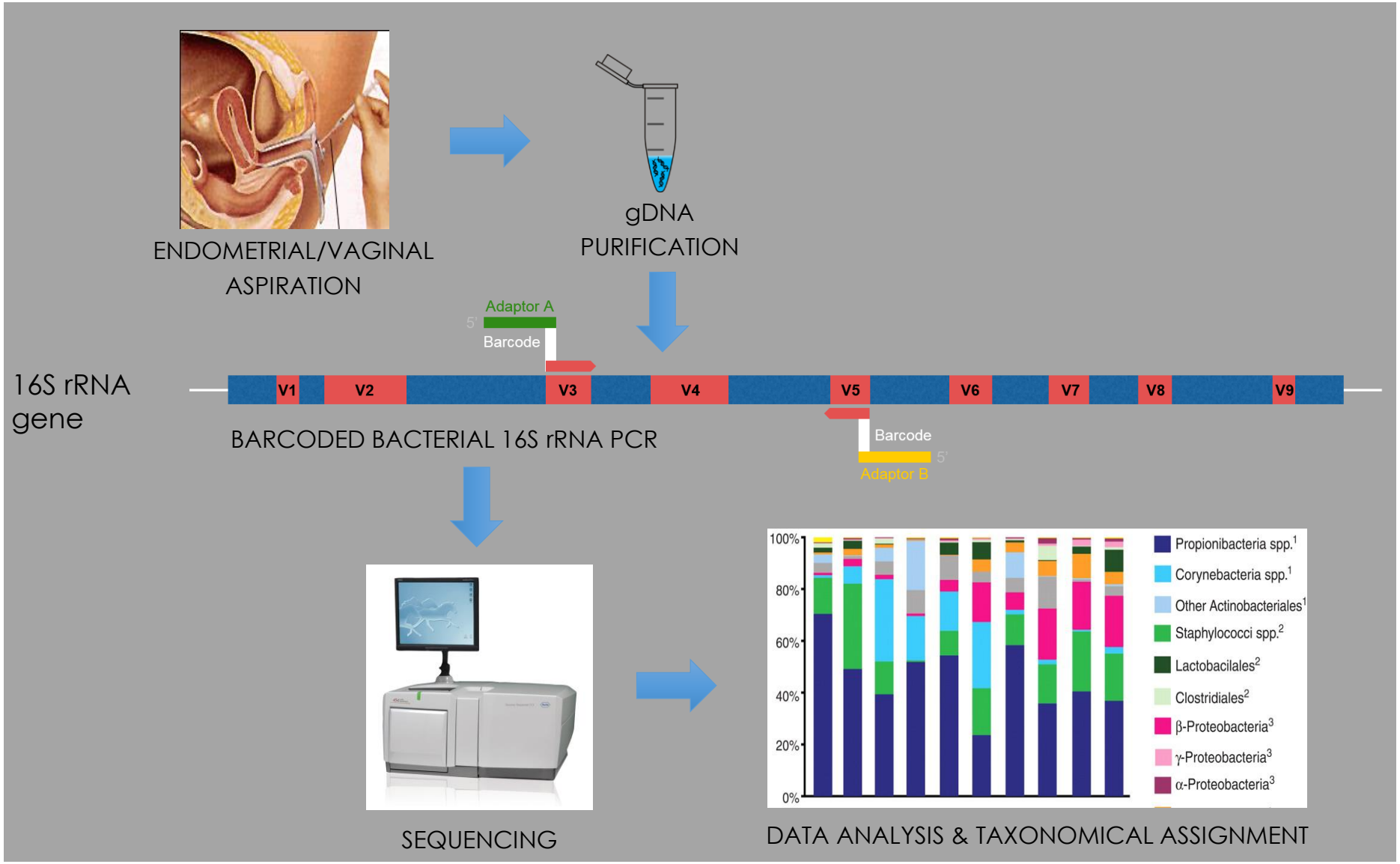
RESULTS: When bacterial communities from paired endometrial fluid and vaginal aspirate samples within the same subjects were interrogated, different bacterial communities were detected between the uterine cavity and the vagina of some subjects. Based on its composition, the microbiota in the endometrial 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

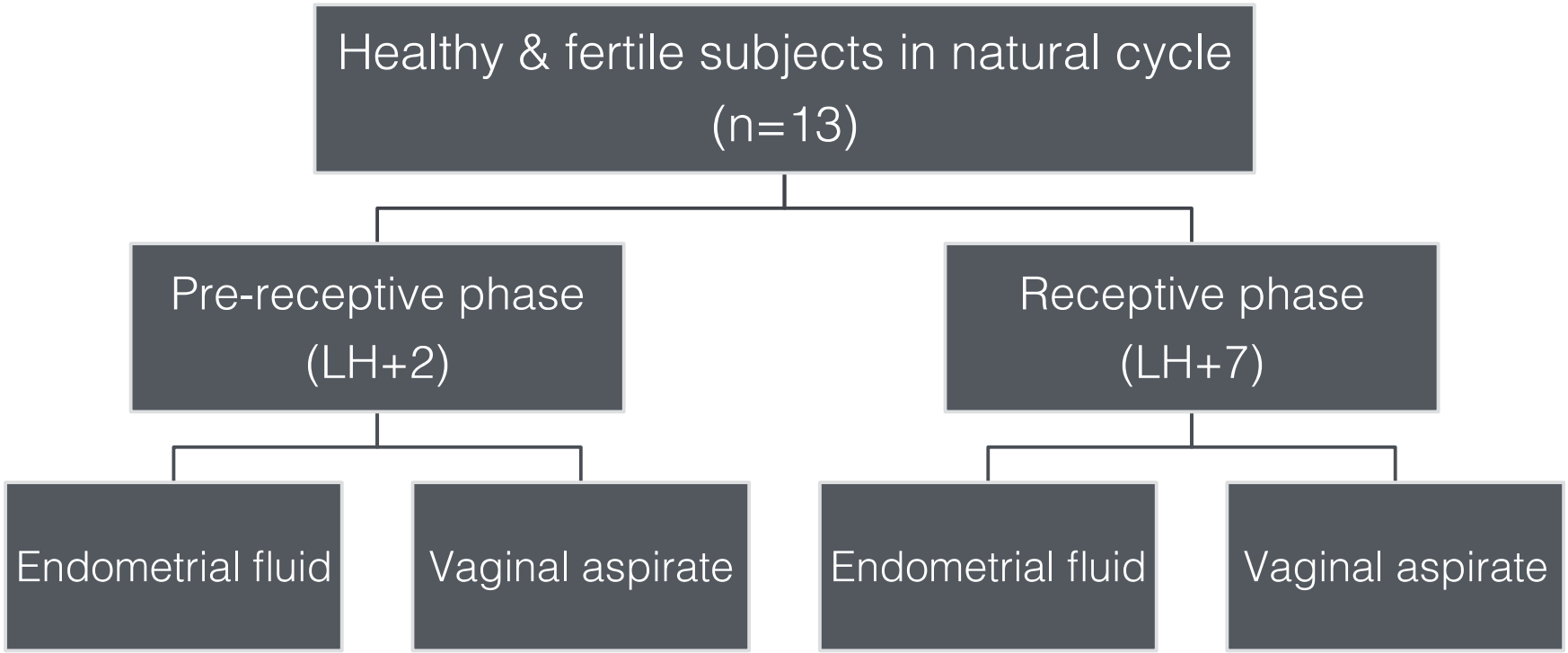
Methods

Molecular assessment of endometrial microbiota by NGS



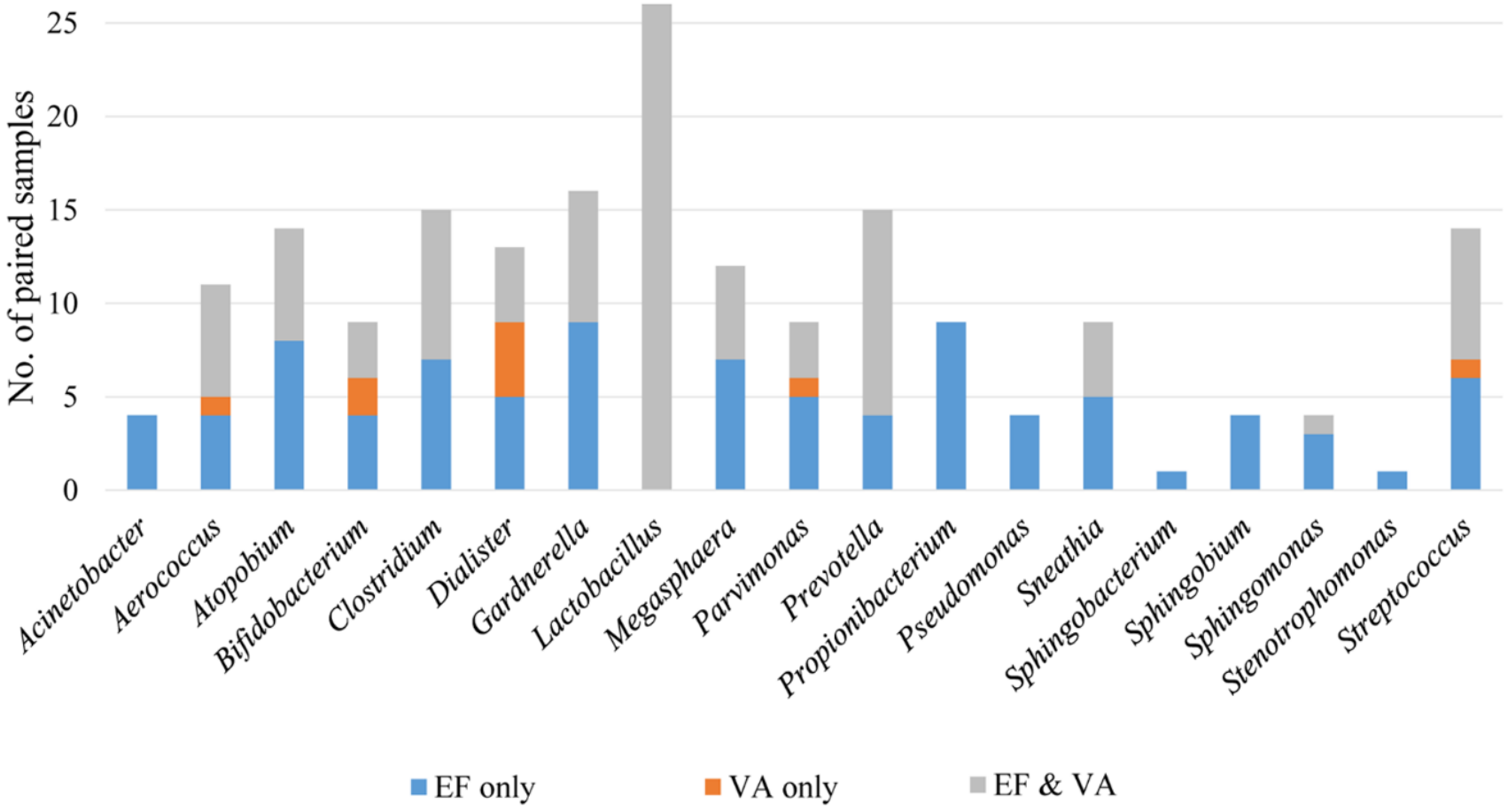
STUDY 1

Endometrial and vaginal microbiota differ in some asymptomatic subjects



Subjects: n=13
Paired samples Endometrium-Vagina: n=26
Total samples analyzed: n=52

Distribution of endometrial and vaginal microbiota in paired samples

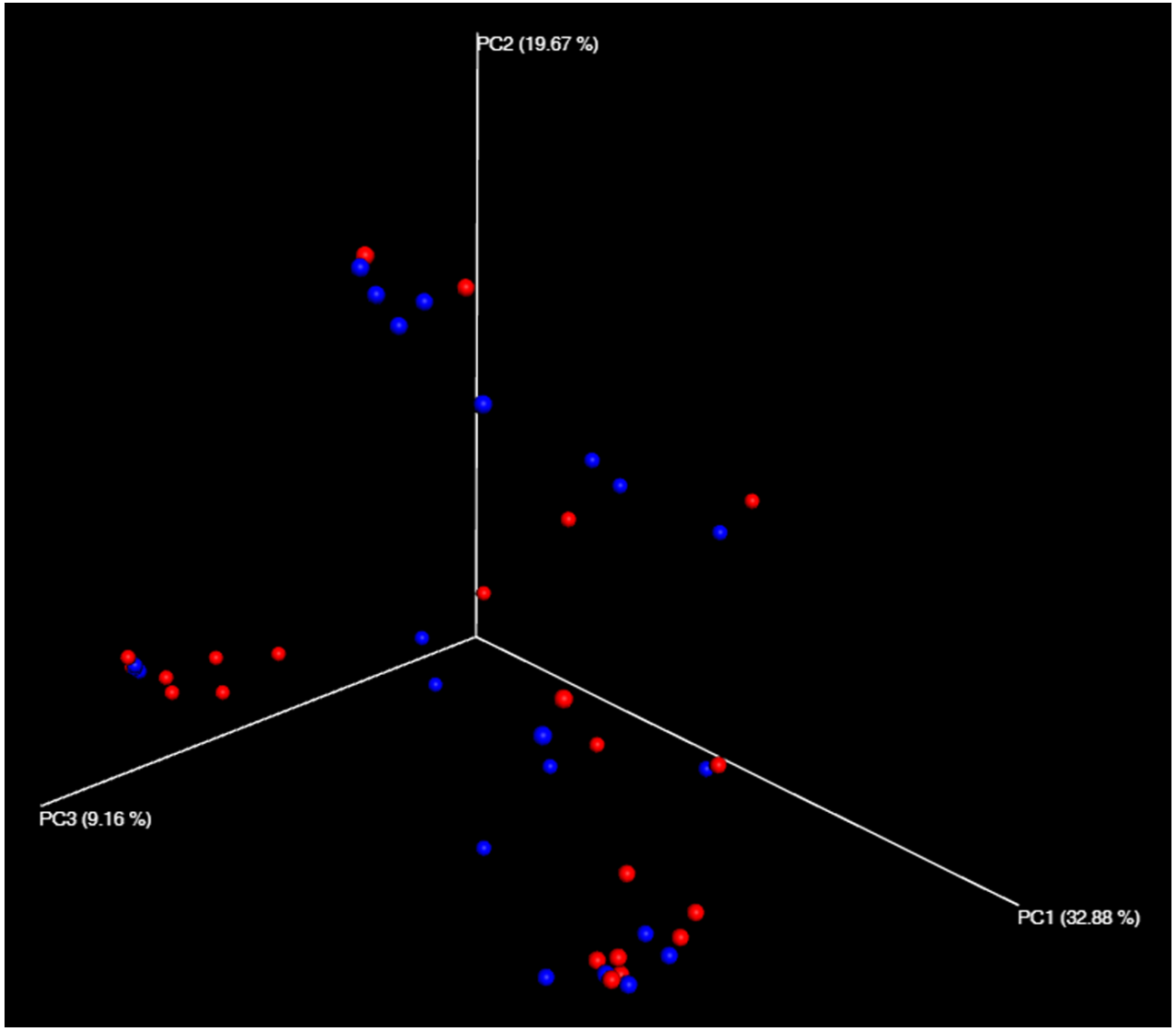


Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

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

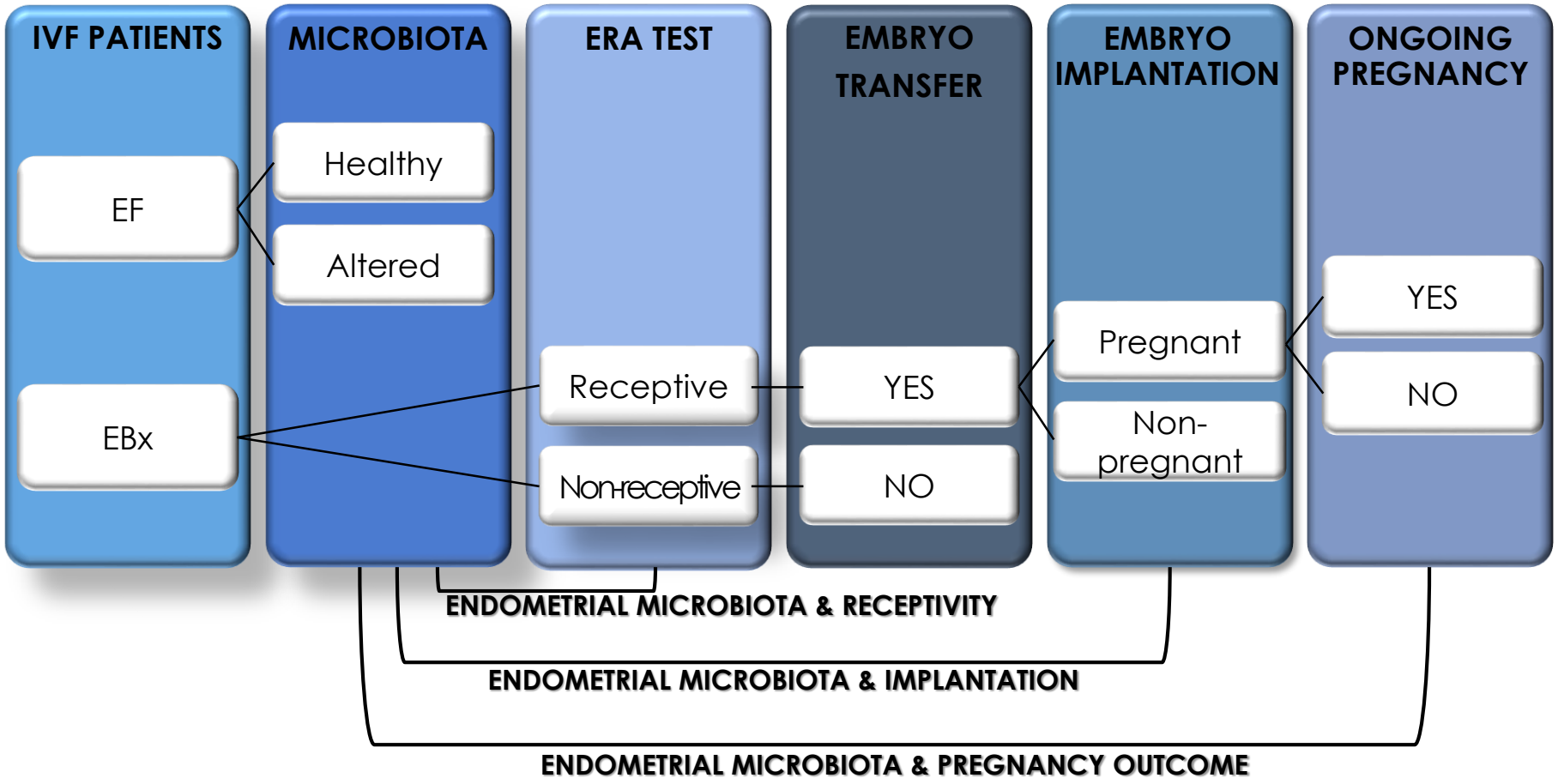


- Pre-receptive (LH+2)
- Receptive (LH+7)

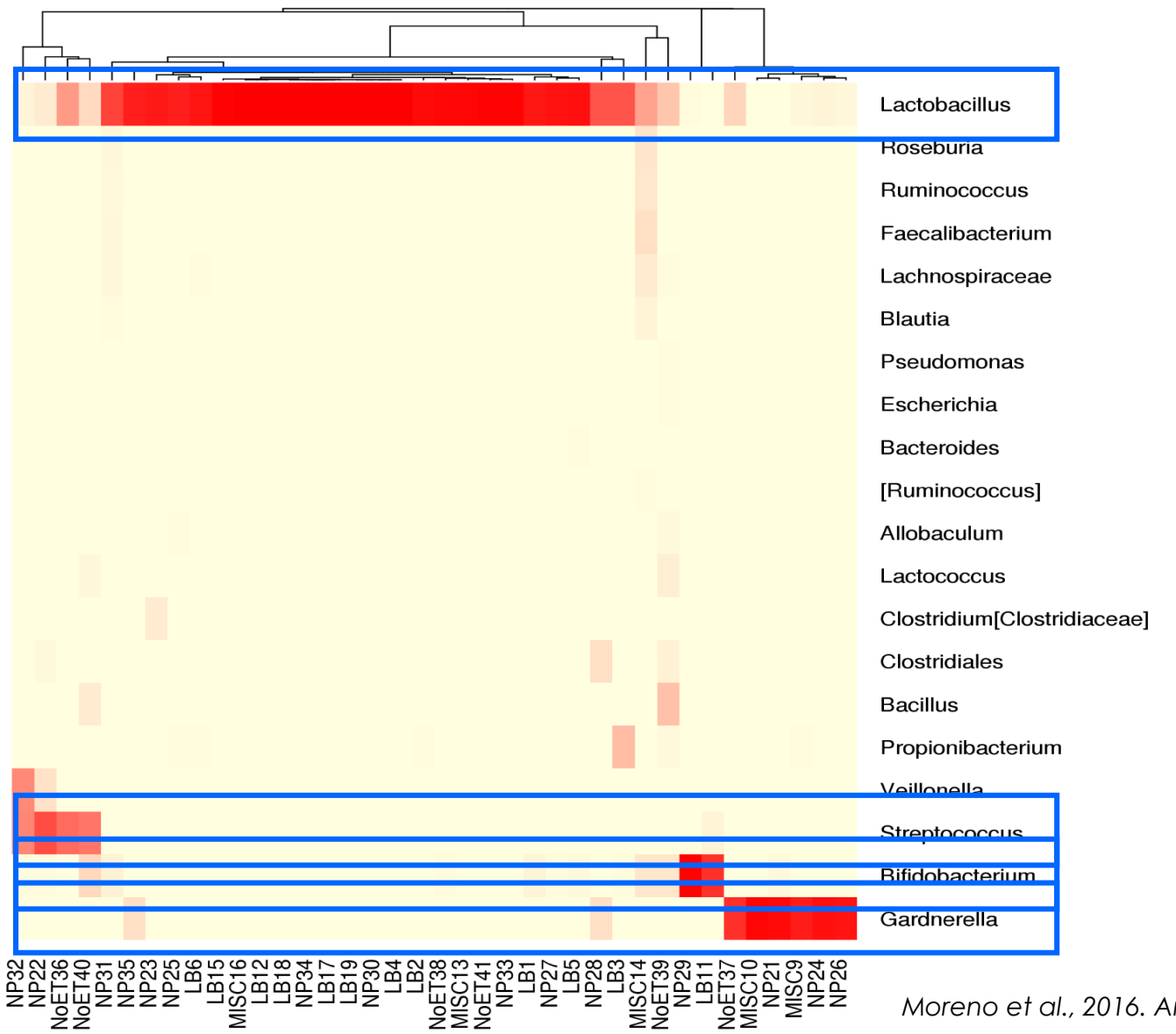
Subjects 1 to 22
Pre-receptive (LH+2)
Receptive (LH+7)

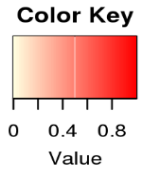
Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

Design


IVF PATIENTS
MICROBIOTA
ERA TEST
EMBRYO TRANSFER
EMBRYO IMPLANTATION
ONGOING PREGNANCY
EF
EBx
Healthy
Altered
Receptive
Non-receptive
YES
NO
Pregnant
Non-pregnant
YES
NO
ENDOMETRIAL MICROBIOTA & RECEPTIVITY
ENDOMETRIAL MICROBIOTA & IMPLANTATION
ENDOMETRIAL MICROBIOTA & PREGNANCY OUTCOME

Endometrial microbiota profile of infertile patients

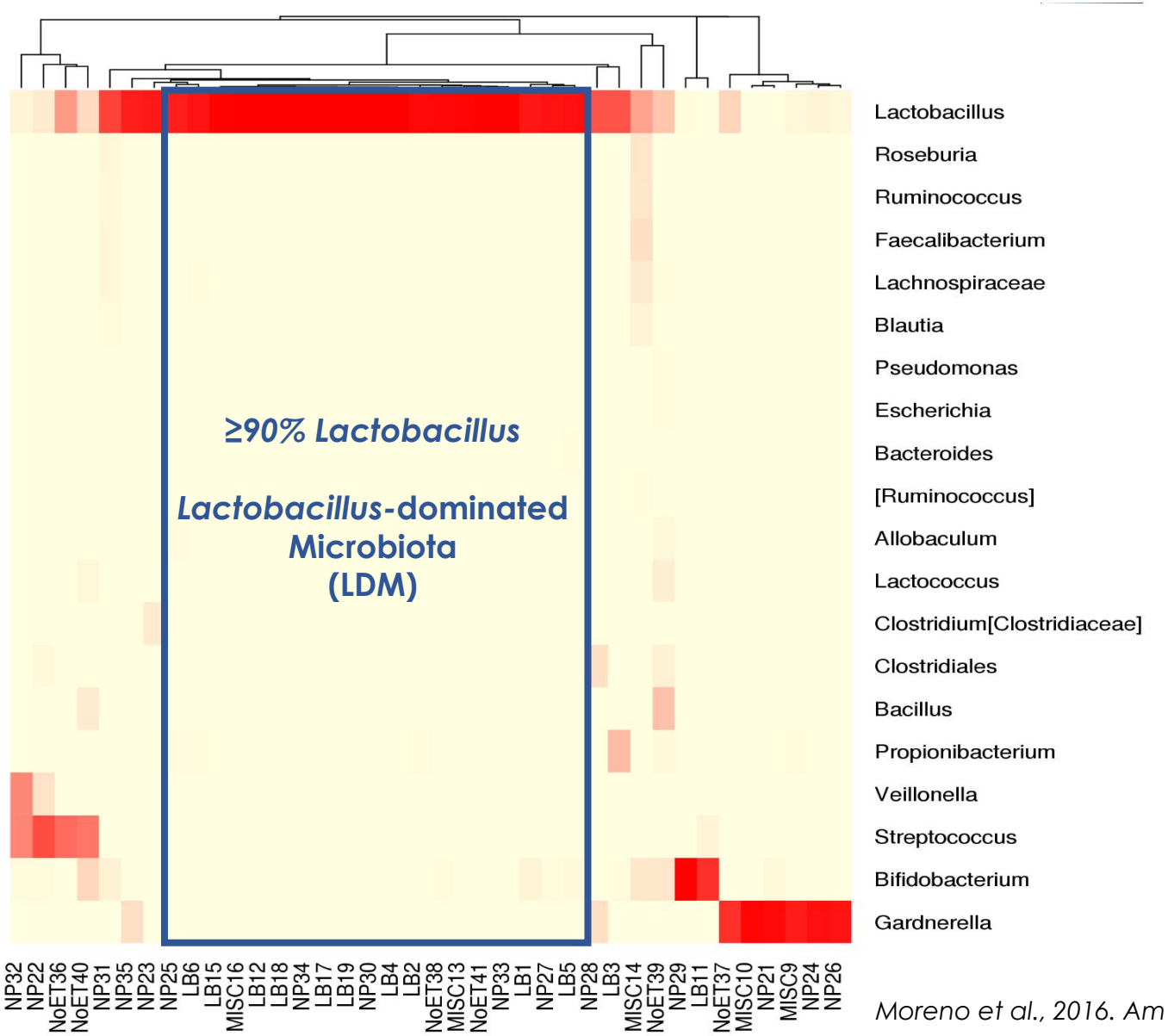




Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer

Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

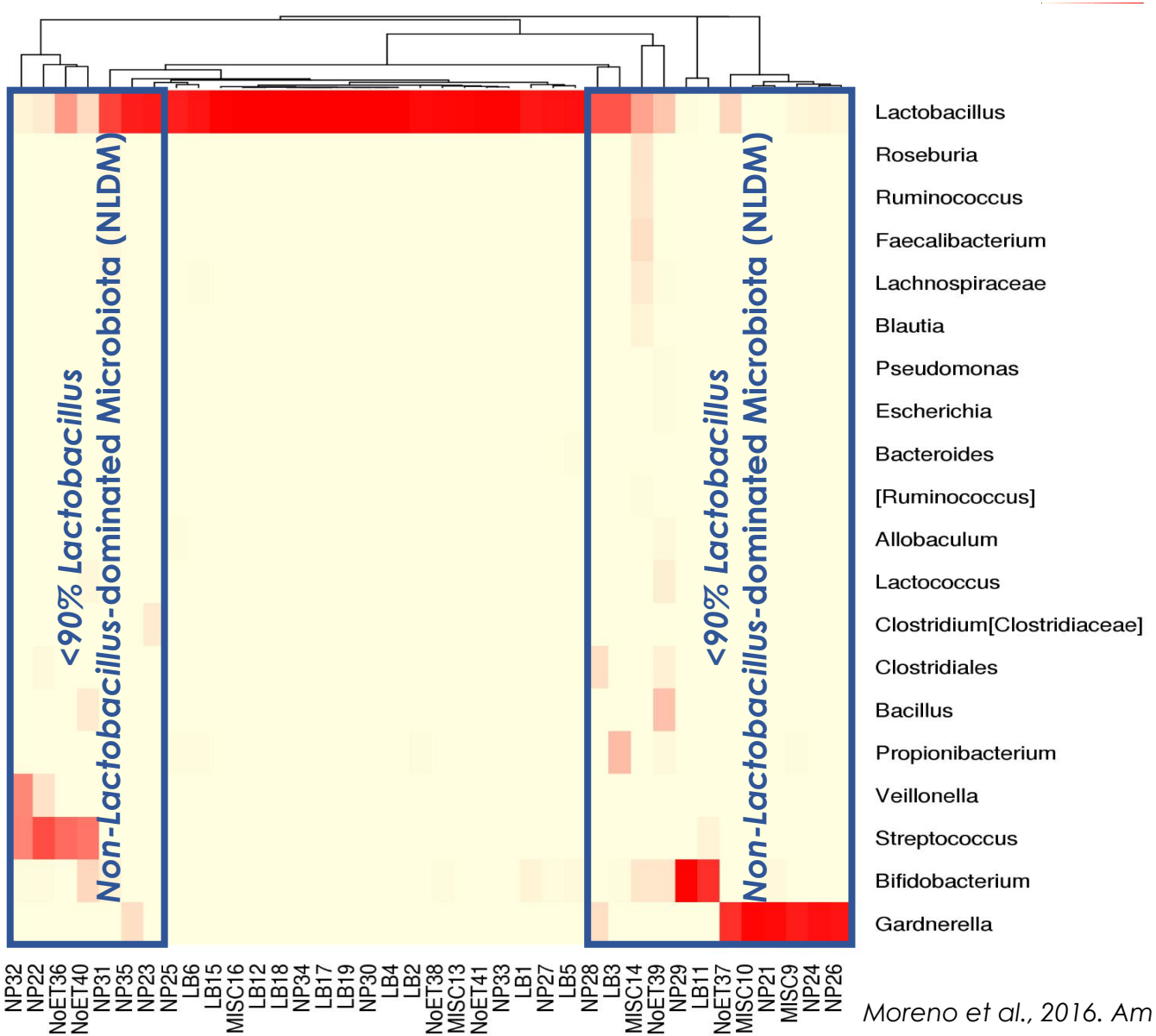
Endometrial microbiota profile of infertile patients

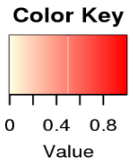


Samples 1 to 41
LB: Live birth
MISC: Miscarriage
NP: No Pregnant
NoET: No embryo transfer

Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

Endometrial microbiota profile of infertile patients





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

Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

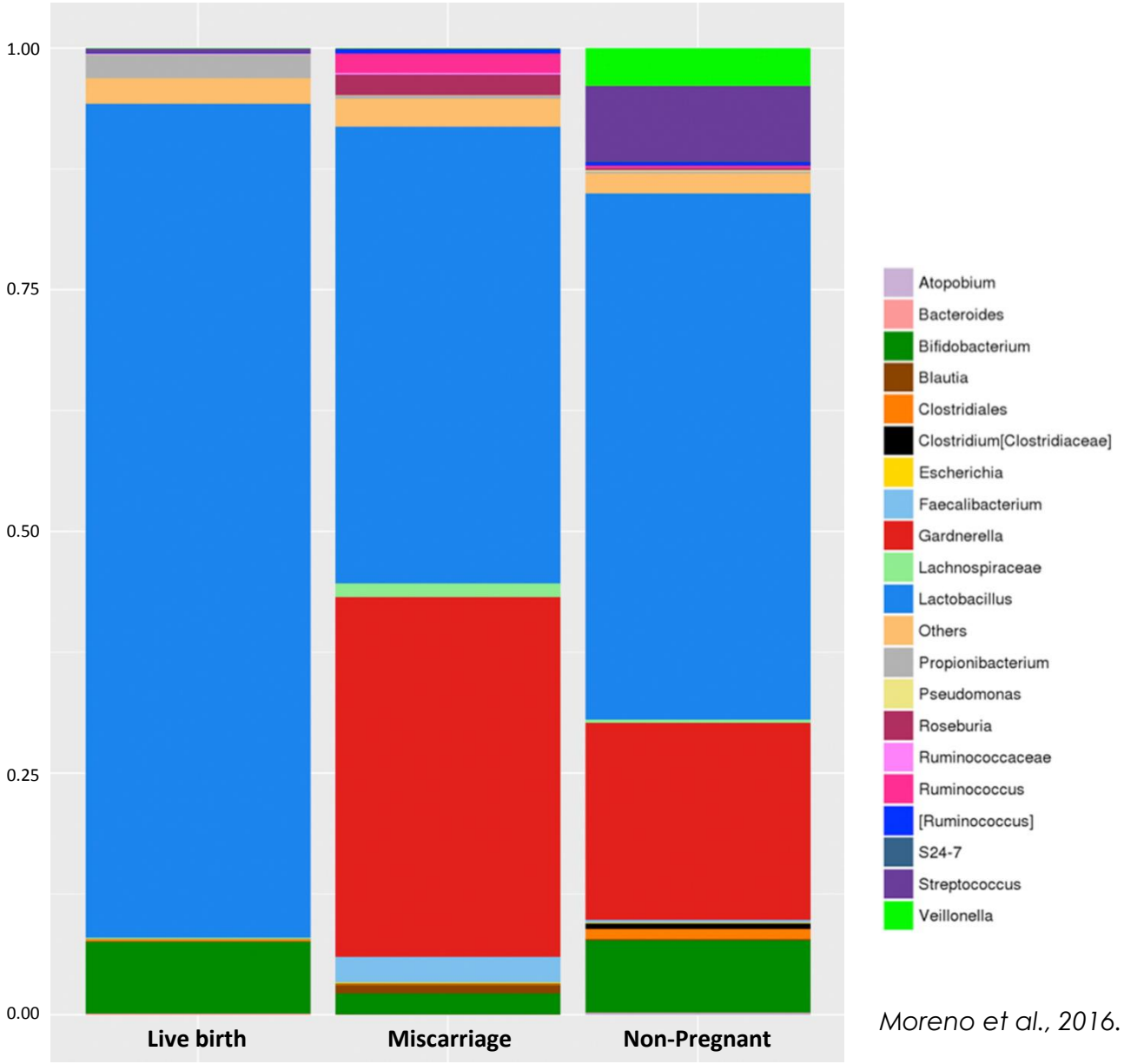
Low abundance of Lactobacillus in endometrium is associated with poor reproductive IVF outcomes

Characteristics and Outcomes	LDM (n=17)	NLDM (n=15)	p-value
Age (y)	40.06±3.47	39.00±5.09	0.49
BMI (kg/m^2)	24.18±5.18	22.45±4.02	0.30
Previous pregnancies (n)	1.71±2.44	1.53±2.32	0.84
Previous miscarriages (n)	1.53±2.21	1.14±1.56	0.58
Metaphase II oocytes per cycle (n)	11.94±4.27	10.20±4.81	0.28
Fertilization rate per cycle	157/203 (77.34%)	118/153 (77.12%)	0.62
Transferred embryos per cycle (n)	1.65±0.49	1.73±0.59	0.65
Months between EF and transfer (n)	2.82±2.55	1.80±1.08	0.16
Pregnancy rate per transfer	12/17 (70.6%)	5/15 (33.3%)	0.03*
Implantation rate per transfer	17/28 (60.7%)	6/26 (23.1%)	0.02*
Ongoing pregnancy per transfer	10/17 (58.5%)	2/15 (13.3%)	0.02*
Miscarriage rates (%)	2/10 (16.7%)	3/5 (60.0%)	0.07
Live birth rate per transfer	10/17 (58.8%)	1[§]/15 (6.7%)	0.002*

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

Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

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

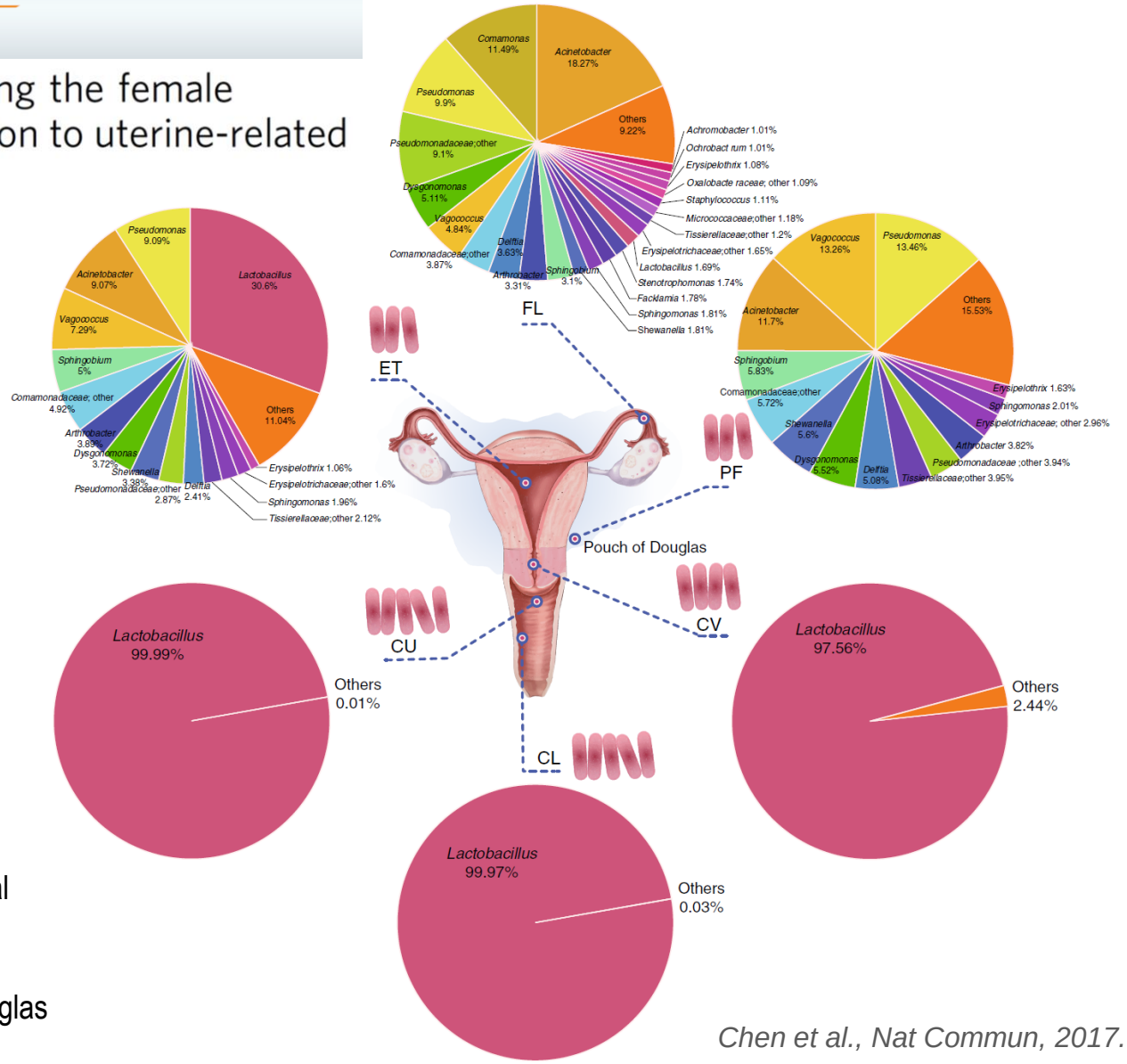


Moreno et al., 2016. Am J Obstet Gynecol. 215:684-703

THE MICROBIOTA OF THE FEMALE REPRODUCTIVE TRACT

nature COMMUNICATIONS

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.

Commensal bacteria make GPCR ligands that mimic human signalling molecules

Louis J. Cohen[1,2], Daria Esterhazy[3], Seong-Hwan Kim[1], Christophe Lemetre[1], Rhiannon R. Aguilar[1], Emma A. Gordon[1], Amanda J. Pickard[4], Justin R. Cross[4], Ana B. Emiliano[5], Sun M. Han[1], John Chu[1], Xavier Vila-Farres[1], Jeremy Kaplitt[1], Aneta Rogoz[3], Paula Y. Calle[1], Craig Hunter[6], J. Kipchirchir Bitok[1] & Sean F. Brady[1]

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).

[1]Laboratory of Genetically Encoded Small Molecules, Rockefeller University, New York, New York 10065, USA. [2]Division of Gastroenterology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA. [3]Laboratory of Mucosal Immunology, Rockefeller University, New York, New York 10065, USA. [4]Donald B. and Catherine C. Marron Cancer Metabolism Center, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA. [5]Laboratory of Molecular Genetics, Rockefeller University, New York, New York 10065, USA. [6]Comparative Biosciences Center, Rockefeller University, New York, New York 10065, USA.

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

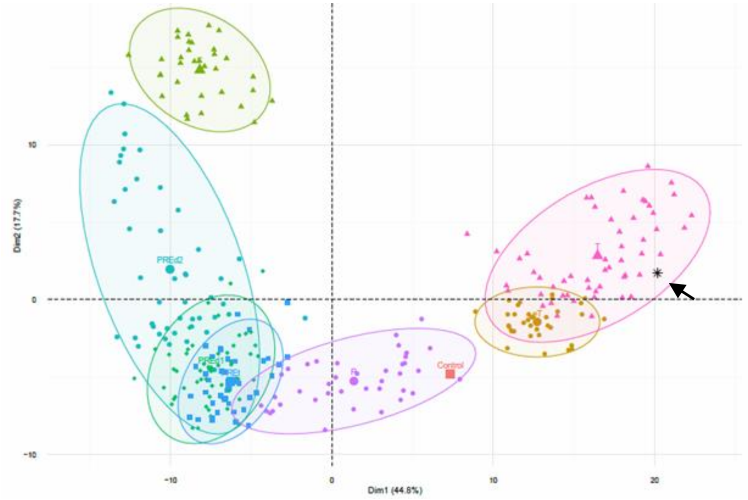
IGX1-MIC-CS-17-05

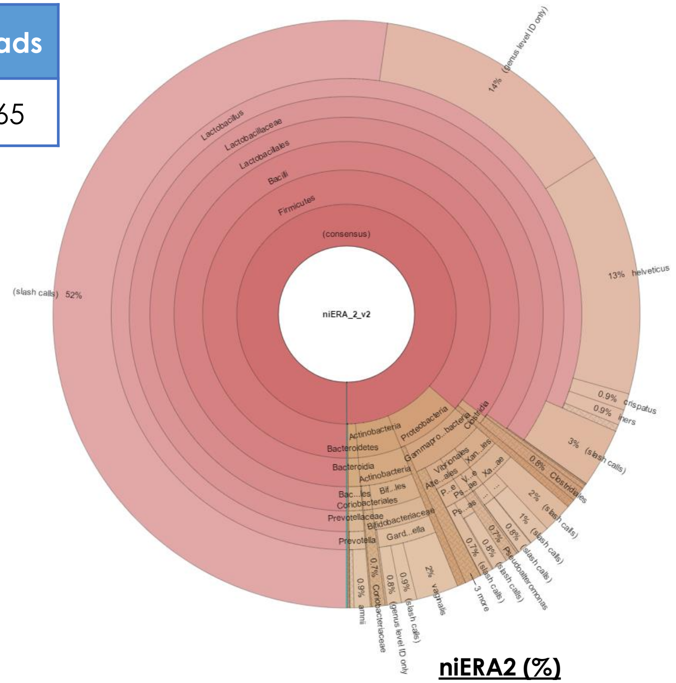
Principal Investigator and Coordinator: Dr. Carlos Simon

Sponsor of the study: Igenomix

niERA-MICROBIOME – Example

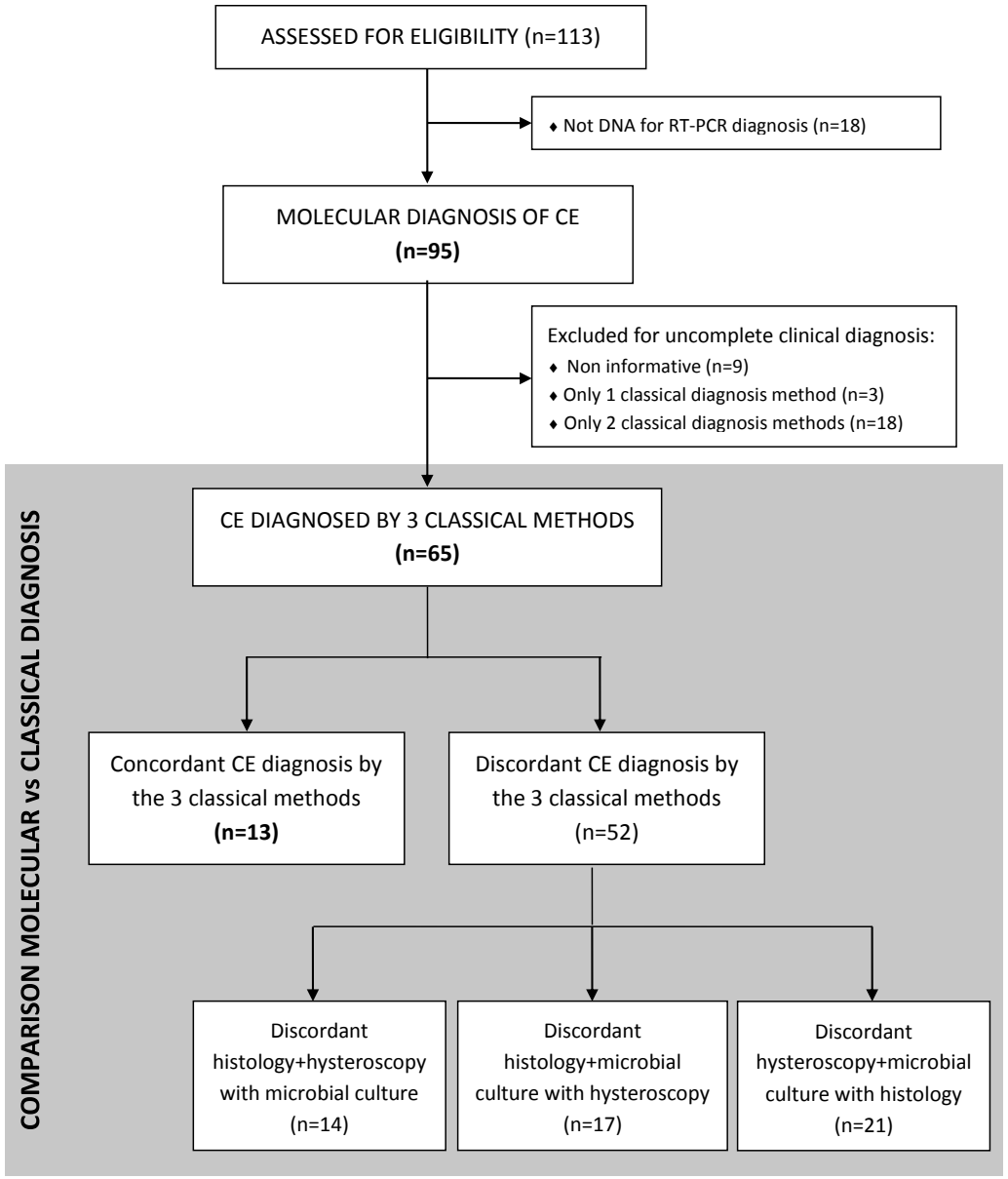
PATIENT	DNA PRESERVANT	SAMPLE	Cycle day	ERA test	niERA test	16S Reads
niERA2 (205)	YES, 50 µL	EF	d27	Post-R	Post-R	274.065





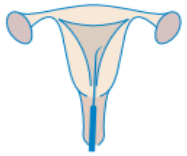
#OTU ID	niERA2 (%)
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae;g__Lactobacillus	**77.15**
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;Other	4.60
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae;g__Pseudoalteromonas	2.63
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae;g__Prevotella	2.05
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	1.70
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae;g__Vibrio	0.83
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella	0.68
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.57
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	0.48
k__Bacteria;p__Firmicutes;c__Bacilli;o__Gemellales;f__Gemellaceae;g__Gemella	0.34
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Tissierellaceae];g__Parvimonas	0.31
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Shuttleworthia	0.28
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	0.21
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megasphaera	0.16
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Mycoplasmatales;f__Mycoplasmataceae;g__Ureaplasma	0.14
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Enhydrobacter	0.13
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.11
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Escherichia	0.10

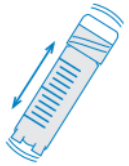
Molecular microbiologycal diagnosis of chronic endometritis



Moreno et al., AJOG 2018

METHODS



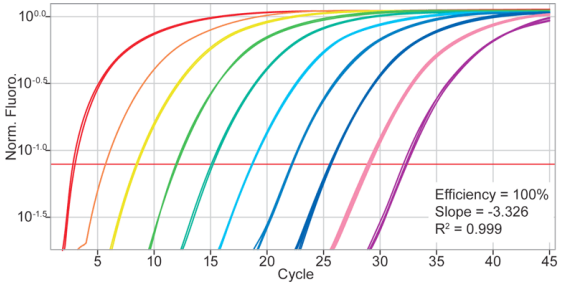


ENDOMETRIAL TISSUE

TOTAL DNA

RT-PCR

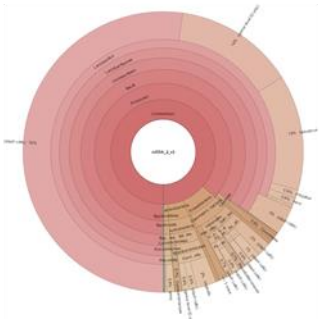
Specific amplification of CE pathogens



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

16S METAGENOMICS

16S bacterial library



MICROBIOME PROFILE

RESULTS: MOLECULAR MICROBIOLOGY vs CLASSICAL DX for CE

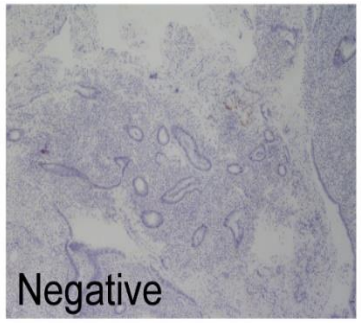
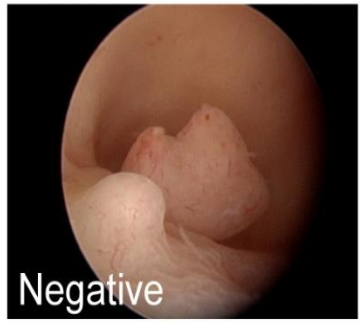
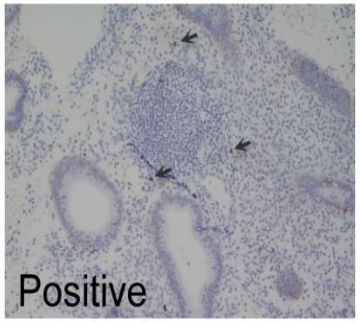
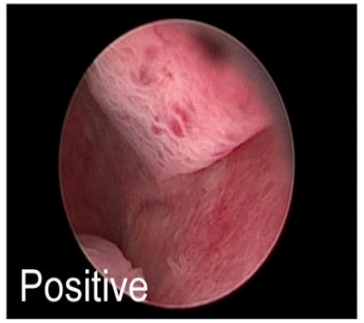
Molecular Microbiology compared to classical methods	Sensitivity	Specificity	Accuracy	PPV	NPV	FPR	FNR
RT-PCR vs Histology (n=65)	56.00%	40.00%	46.15%	36.84%	59.26%	60.00%	44.00%
RT-PCR vs Hysteroscopy (n=65)	58.73%	50.00%	58.46%	97.37%	3.70%	50.00%	41.27%
RT-PCR vs Microbial culture (n=65)	71.43%	56.67%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

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

Moreno et al., AJOG 2018

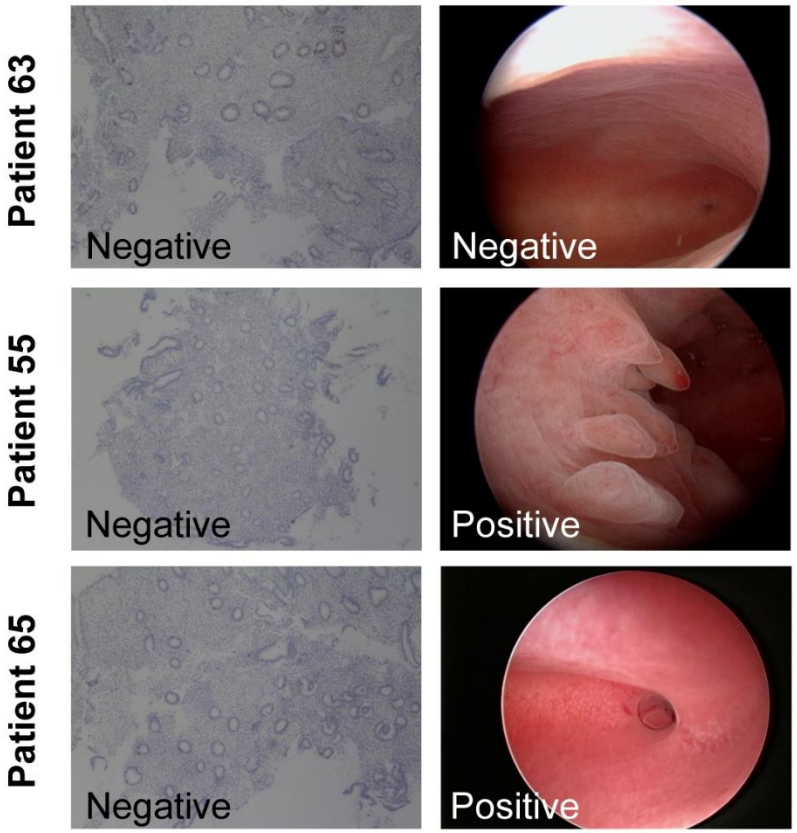
Concordant chronic endometritis results in patients/samples analysed by the four methods compared in this study (three classical methods and the RT-PCR method)

(A)

	Histology/CD138	Hysteroscopy	Microbial culture	RT-PCR
Patient 10			Negative	Negative
Patient 8			*S. agalactiae*	*Streptococcus* spp. *G. vaginalis*

Discordant chronic endometritis results in patients/samples analysed by the four methods compared in this study. Black arrows show CD138 positive cells.

(B)



Microbial culture	RT-PCR
E. coli *Ureaplasma*	*Streptococcus* spp.
Negative	Negative
S. agalactiae	*Streptococcus* spp.

RESULTS: MOLECULAR MICROBIOLOGY vs MICROBIAL CULTURE

Microbiota profile of endometrial samples by 16S rRNA gene sequencing

Patient	Microbial culture	RT-PCR	16s rRNA sequencing (genera percentage)							
			Lactobacillus	*Enterococcus*	*Staphylococcus*	*Streptococcus*	*Mycoplasma*	*Enterobacteria*	*Gardnerella*	*Ureaplasma*
8	*Streptococcus agalactiae*	*Streptococcus spp., Gardnerella vaginalis*	10.2	2.0	2.6	28.2	0.0	2.6	1.0	0.00
10	Negative	Negative	99.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	*Escherichia coli*	*Gardnerella vaginalis, Escherichia coli*	61.0	0.3	1.4	0.6	0.0	1.4	1.1	0.0
17	*Enterococcus faecalis, Ureaplasma*	Negative	94.4	0.0	0.1	0.1	0.0	0.3	0.4	0.0
18	*Streptococcus agalactiae*	*Streptococcus spp.*	0.4	0.0	0.0	77.0	0.0	0.0	0.0	0.0
19	*Escherichia coli*	*Streptococcus spp.*	61.5	0.1	2.7	0.9	0.1	4.0	2.7	0.0
24	*Ureaplasma*	*Klebsiella pneumoniae*	ND							
26	*Enterococcus faecium*	Negative	98.8	0.0	0.1	0.2	0.0	0.0	0.0	0.0
30	*Enterococcus faecalis, Streptococcus mitis*	*Enterococcus spp.*	93.2	0.2	0.0	0.0	0.0	0.5	0.0	0.0
31	*Klebsiella pneumoniae*	*Streptococcus spp.*	7.4	0.0	7.4	1.8	0.2	11.1	0.0	0.0
35	*Staphylococcus aureus*	Negative	83.1	0.0	0.1	0.6	0.0	0.0	7.0	0.0

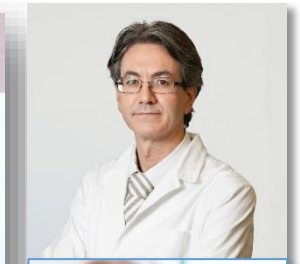
ND: Not determined

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.

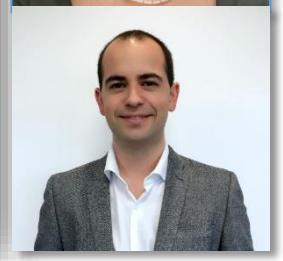
Our ERA Team

María Ruiz
Eva Gómez
José Miravet
Carlos Marín
Jessica Nieto
Ana Pozo
Miriam García
Lucía Martínez
Juan Soria

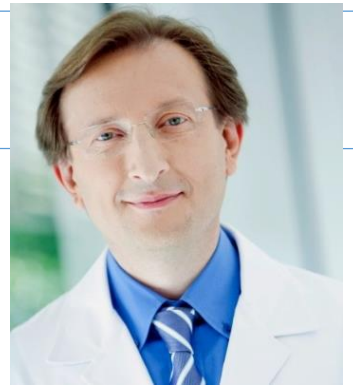


David Blesa

Diana Valbuena



Carlos Gómez



Prof. Carlos Simon's Lab

RESEARCH DEPARTMENT

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

COLLABORATORS

Pilar Alamá, IVI Valencia, Spain
Renee Reijo Pera, Montana University, USA
Steve Quake, Stanford University, USA
Ruth Lathi, Stanford University USA
Sergio Cabanillas, IVI Valencia, Spain
Susan Fisher, UCSF, USA
Ayman Al-Hendy, Georgia Regents University, USA
Vittorio Sebastiano, Stanford University, USA

FINANCIAL SUPPORT

Unión Europea
Fondo Europeo de Desarrollo Regional
"Una manera de hacer Europa"
eurostars™
MARIE CURIE ACTIONS
INCLIVA Instituto de Investigación Sanitaria
VNIVERSITAT DE VALÈNCIA
Stanford University
BCM Baylor College of Medicine
GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
Instituto de Salud Carlos III

Our **IGENOMIX** Team

Spain (Headquarters)

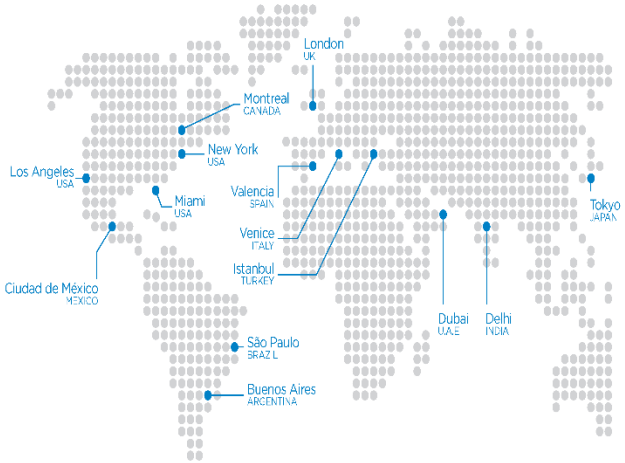
Scientists
AL-ASMAR, NASSER
ALONSO VALERO, ROBERTO
AMADOZ NAVARRO, ALICIA
BAÚ, DAVIDE
BLESA JARQUE, DAVID
BOVER CATALA, ANA
CAMPOS GALINDO, INMACULADA
CERVERO SANZ, ANA CRISTINA
CLEMENTE CISCAR, MONICA
DE LA FUENTE LUCENA, EMILIO
DIEZ JUAN, ANTONIO
GARCIA HERRERO, SANDRA
GARCIA PASCUAL, M. CARMEN
GIL SANCHIS, CLAUDIA
GOMEZ DE LA CRUZ, CARLOS ALFONSO
GOMEZ SANCHEZ, EVA MARIA
HERNANDEZ DE DIEGO, RAFAEL
HERVAS LORENTE, ARANTXA
JIMENEZ ALMAZAN, JORGE
RODRIGUEZ, JULIO CESAR
MARTINEZ CONEJERO, JOSE ANTONIO
MARIN LOPEZ DE CARVAJAL, LUCIA
MARIN VALLEJO, CARLOS
MATEU BRULL, EMILIA
MILAN SANCHEZ, MIGUEL
MIR PARDO, PERE
MIRAVET VALENCIANO, JOSE ALBERTO
MORENO GIMENO, INMACULADA
NAVARRO GAYA, ROSER
NAVARRO SANCHEZ, LUIS
PEINADO CERVERA, MARIA VANESSA
RINCON BERTOLIN, ALEJANDRO
RODRIGO VIVO, LORENA
RUBIO LLUESA, CARMEN
RUIZ ALONSO, MARIA
SANCHEZ PIRIS, MARIA ISABEL
SANTA MORENO, LAURA
SANTAMARIA COSTA, JAVIER
SANZ SALVADOR, LUCIA
SIMON VALLES, CARLOS
VALBUENA PERILLA, DIANA

Technicians
AGUILA CLARES, BEGOÑA
AYALA ALVAREZ, GUSTAVO LEONARDO
BARRERO, DESIRE
BERMELL JUNCOS, SOLEDAD
BOSCH IBAÑEZ, ALVARO
BURGOS LUJAN, INES
CENTELLES PASTOR, VICENTE
COLOMA MARCO, MARIA DOLORES
ESCOBEDO LUCEA, MILAGROS
ESCORCIA MORA, PATRICIA
FERRO BARBERO, AZARINA
GALVEZ VIEDMA, MARTA
GARCIA MORENO, MIRIAM
GOMEZ LOPEZ, MARIA
HERRERO BAENA, MARIA
IÑIGUEZ QUILES, LAURA
MADRIGAL GIMENEZ, TANIA
MARTINEZ BENITO, TANTRA
MARTINEZ ESCRIBANO, SEBASTIAN
MARTINEZ FERNANDEZ, M. ASUNCION
MARTINEZ MERINO, LUCIA
MATEOS GREGORIO, PABLO
MOLES SELMA, SARA
MORATA GARCIA, MARIA JESUS
NIETO ALFANI, JESSICA
PEREZ SORIANO, CRISTINA
PERIS PARDO, LAURA
POZO CRUZ, ANA MARIA
SANCHEZ GONZALEZ, ESTELA
SILVESTRE IVARS, MARIA
TERUEL IZQUIERDO, VANESA
VELERT CARRION, GEMMA

USA & Canada

Scientists
AKINWOLE, ADEDOYIN
CINNIOGLU, CENGIZ
DARVIN, TRISTAN
HAGHI, GHAZAL
HARTON, GARY
JAKUBOWSKA MILENA
KAYALI, REFIK
MAE HOOVER, LARISSA
PHILLIPS, KIMBER
SNEIDER, ALYSSA
STANKEWICZ, TIFFANY
YEH, CHRISTINE

Technicians
ALVAREZ, INALVIS
BAUTISTA, ABELARD
BOJI NESCU, ANCA
DUENAS, FRANCISCO
CUI, KATHY
GRIFFIN, MARISA
LAYNE, NICOLE
LOANIDIS, ALEXANDROS
NGUYEN, VI
PENA, DAYTERNA
PHAM, QUON
SANTI, ANNAI
SEN, GURKAN
SHAIBI, DEREK
TUYEN, KENNY



Mexico

Scientists
COYOTECATL, CRISTINA
POO LLANILLO, MARIA EUGENIA

Technicians
MORALES BECERRIL, KARLA JENESSES
OROZCO PANTOJA, MARITZA

Brasil

Scientists
AYALA, CAMILA
COPRERSKI, BRUNO
CUZZI, JULIANA
DA MOTA, PRISCILA
DE OLIVEIRA, PRISCILA
DO CARMO, GABRIEL
ESTEVES MORAES, CAMILA
GLORIA, THIAGO
MOARES, CAMILA
PEREIRA, KEITTY
RIBOLDI, MARCIA
UEHARA DE SOUZA, MARIANE

Argentina

Scientists
LOPEZ IGLESIAS, PILAR
RIVADENEIRA, ANDREA

UK

Scientists
MUTLU, AYLIN
NAJA, ROY
RABERI, ARAZ
THORNHILL, ALAN

Italy

Scientists
CAPALBO, ANTONIO
GIRARDI, LAURA
PATASSINI, CRISTINA
ROMANELLI, VALERIA

Technicians
GIANCANI, ADRIANO
MORETTO, MARTINA

Dubai

Scientists
CHOPRA, RUPALI

Technicians
ALNIMS, HAYA
ROY CHOWDGHURY, SHEWETA
SHARMA, SHEWETA
WARRIER EDAKUNNY, SHRUTI

India

Scientists
KHAJURIA, RAJNI
SINGH BUTTAR, BRINDERJIT

Technicians
UPADHYAY, DIVYESH

Japan

Scientists
CHANG, ANDY
HSU, HANSIU
MATSUOKA, TOSHIKI

Technicians
ARAI, CHIHIRO
ICHIKAWA, ERIKO
MIYAZAWA, MAKI

Colaborators: