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1240

Endometrial Receptivity: the Role of Diagnostic Testing in Reproductive Success

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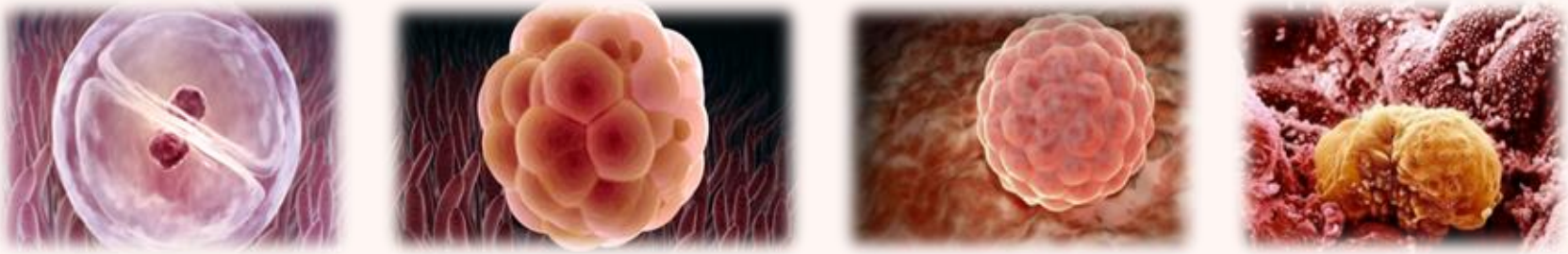
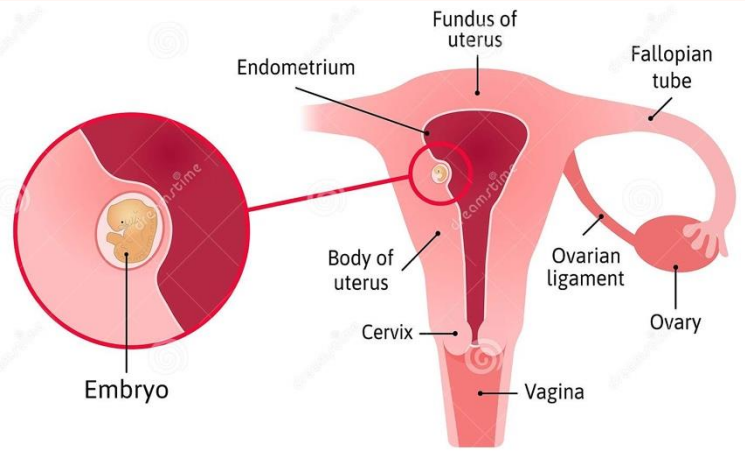
  University of Siena
- *Medically Assisted Reproduction Unit*
  Siena University Hospital



EMBRYO IMPLANTATION



IMPLANTATION

Definition

It is the process by which the BLASTOCYST penetrates the superficial compact layer of the endometrium.

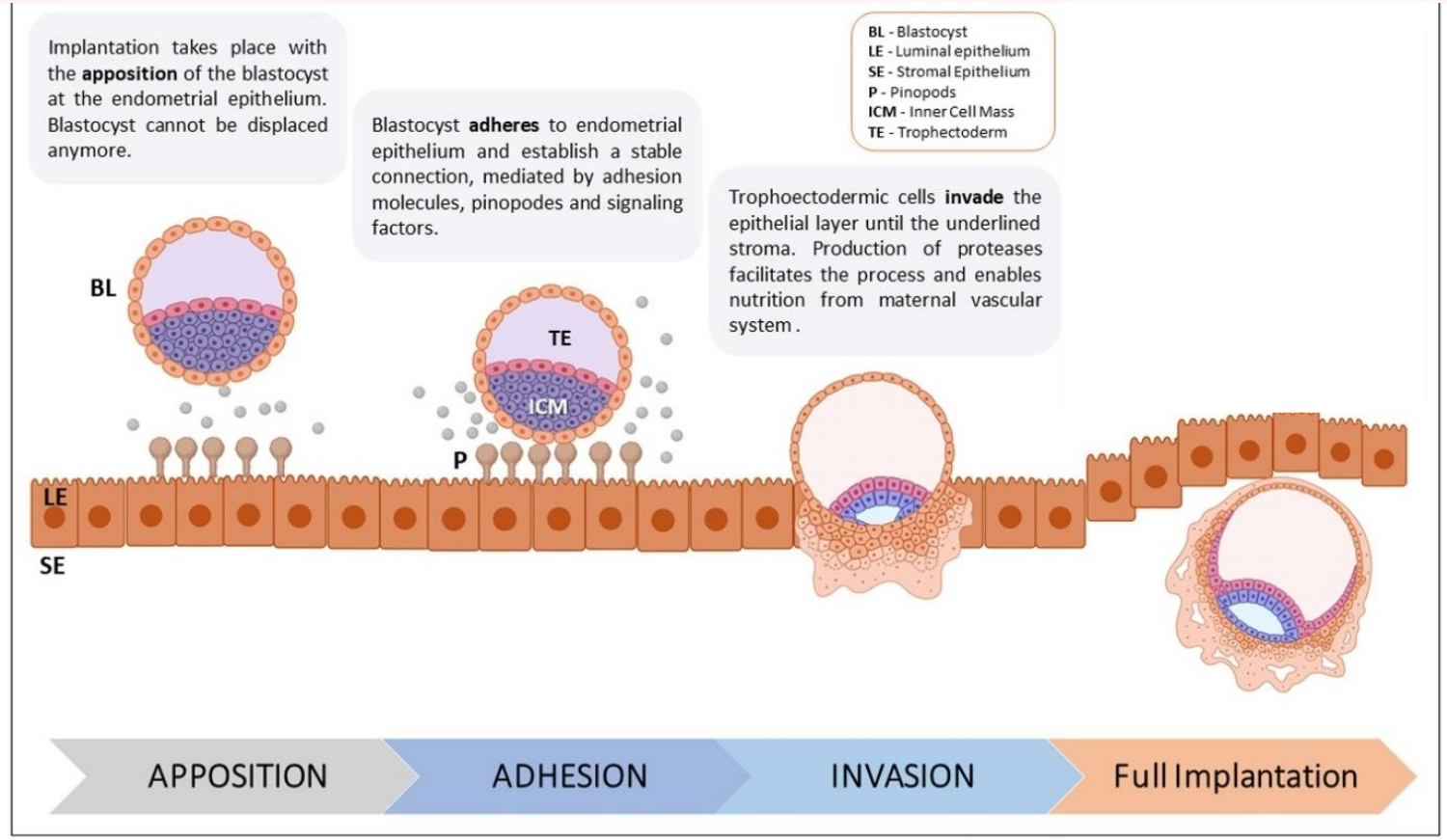
Site

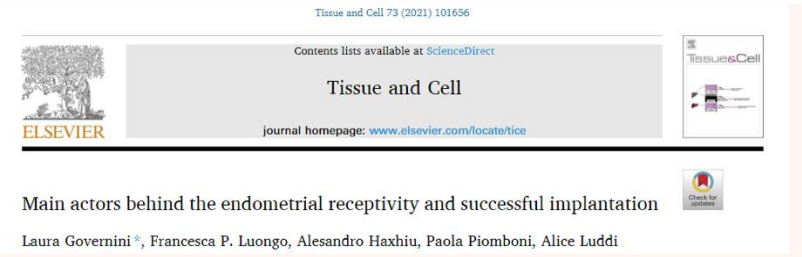
The normal site of implantation is the posterior wall of uterus near the fundus.

Time

It begins about the 6th day after fertilization, it is completed by the 11th or 12th day.

IMPLANTATION'S EVENTS




Tissue and Cell 73 (2021) 101656

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

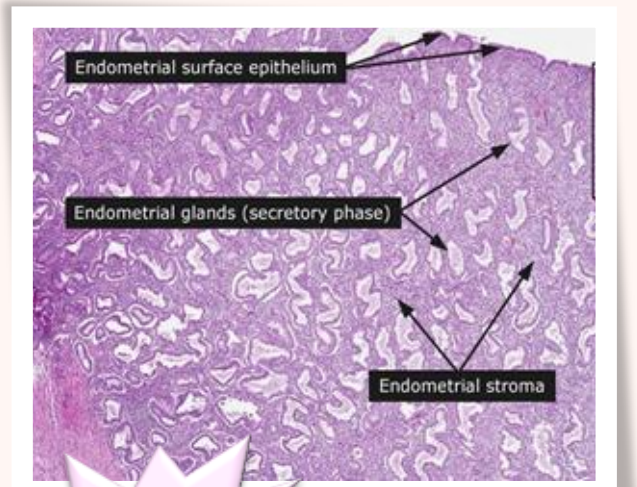
ELSEVIER

Main actors behind the endometrial receptivity and successful implantation

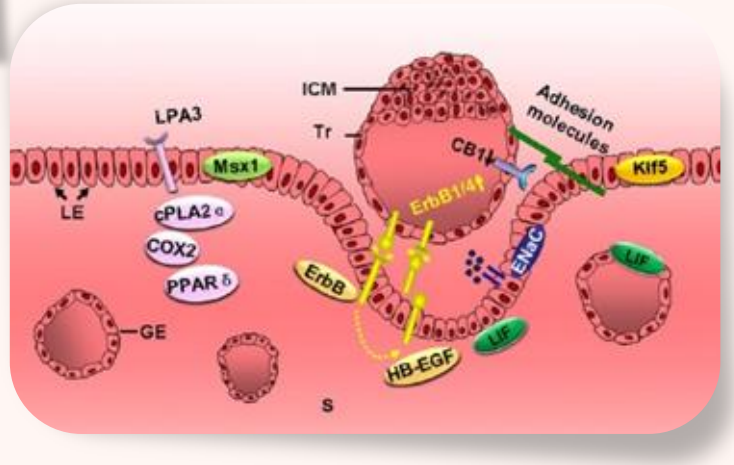
Laura Governini*, Francesca P. Luongo, Alesandro Haxhiu, Paola Piomboni, Alice Luddi

The Actors of Human Implantation

The **ENDOMETRIUM**



ENDOMETRIAL RECEPTIVITY



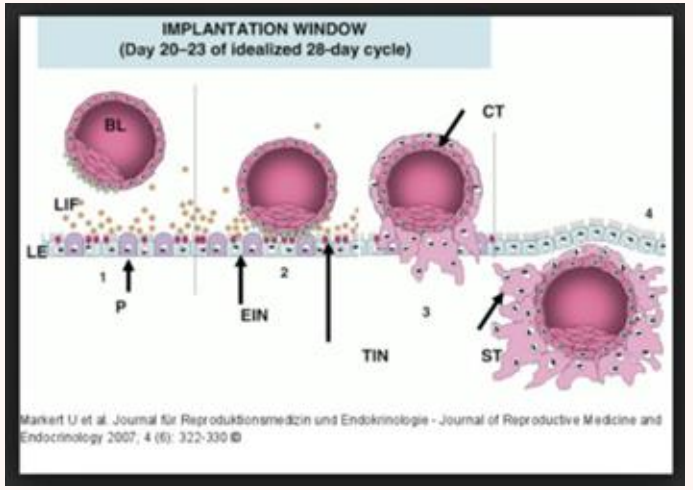
The **BLASTOCYST**

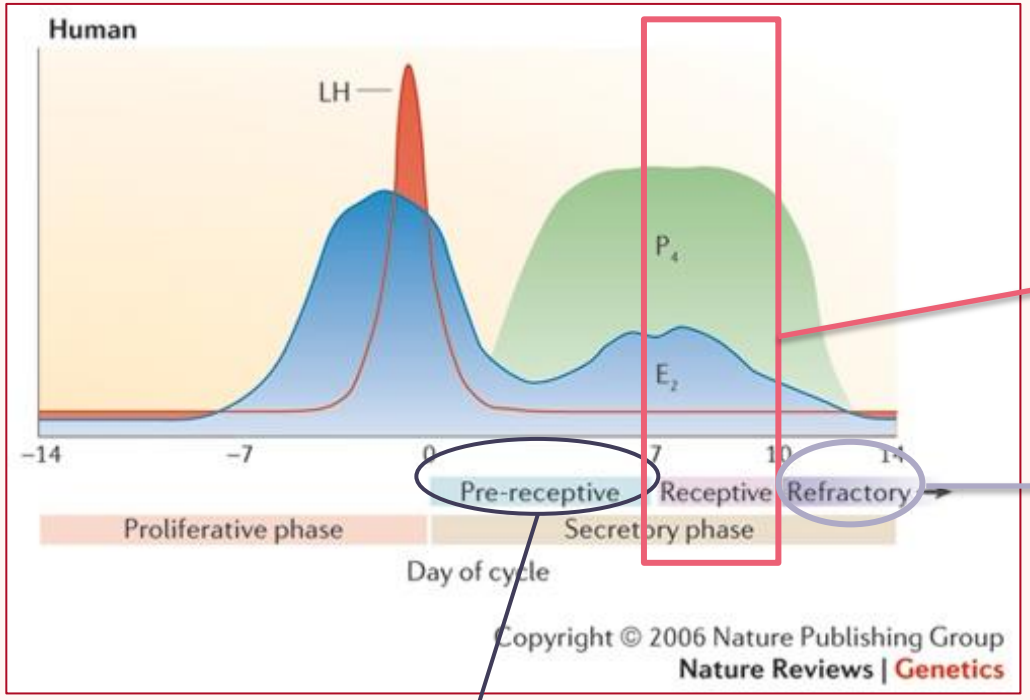
EMBRYONIC COMPETENCE

The crosstalk between the ENDOMETRIUM and the BLASTOCYST

Endometrial Receptivity

The uterus is receptive to blastocyst implantation during a spatiotemporally restricted "window" (Window of Implantation, WOI) when the uterine environment is favorable for the blastocyst.





The uterus becomes receptive during the **Mid-Secretory Phase**, which spans 6–10 days after ovulation

The **Non-Receptive Phase** comprises the rest of the secretory phase.

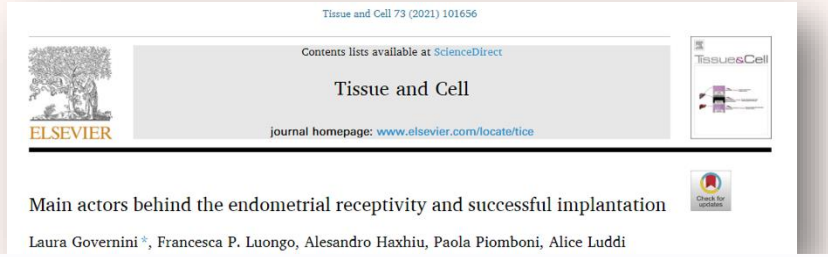
During the **Secretory Phase**, the uterus is considered prereceptive for the first 5-6 days following ovulation (day 0).

MOLECULES ASSOCIATED WITH ENDOMETRIAL RECEPTIVITY

THE **PRE-RECEPTIVE:** PRESENCE OF ANTIADHESIVE FACTORS (THE GLYCOCALYX, A POLARIZED EPITHELIUM AND LATERAL JUNCTIONS).

TO BECOME **RECEPTIVE:** EPITHELIAL AND BLASTOCYST-SECRETED ENZYMES MODIFY THE GLYCOCALYX; PINOPODES APPEAR ON THE SURFACE OF THE LUMINAL EPITHELIUM.

DECIDUALIZATION: ENDOMETRIAL STROMAL FIBROBLATS SPONTANEOUSLY DIFFERENTIATE TO SECRETORY '*EPITHELIOID*' CELLS. THEY WILL PROMOTE THE INVASION OF FETAL TROPHOBLAST.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Main actors behind the endometrial receptivity and successful implantation

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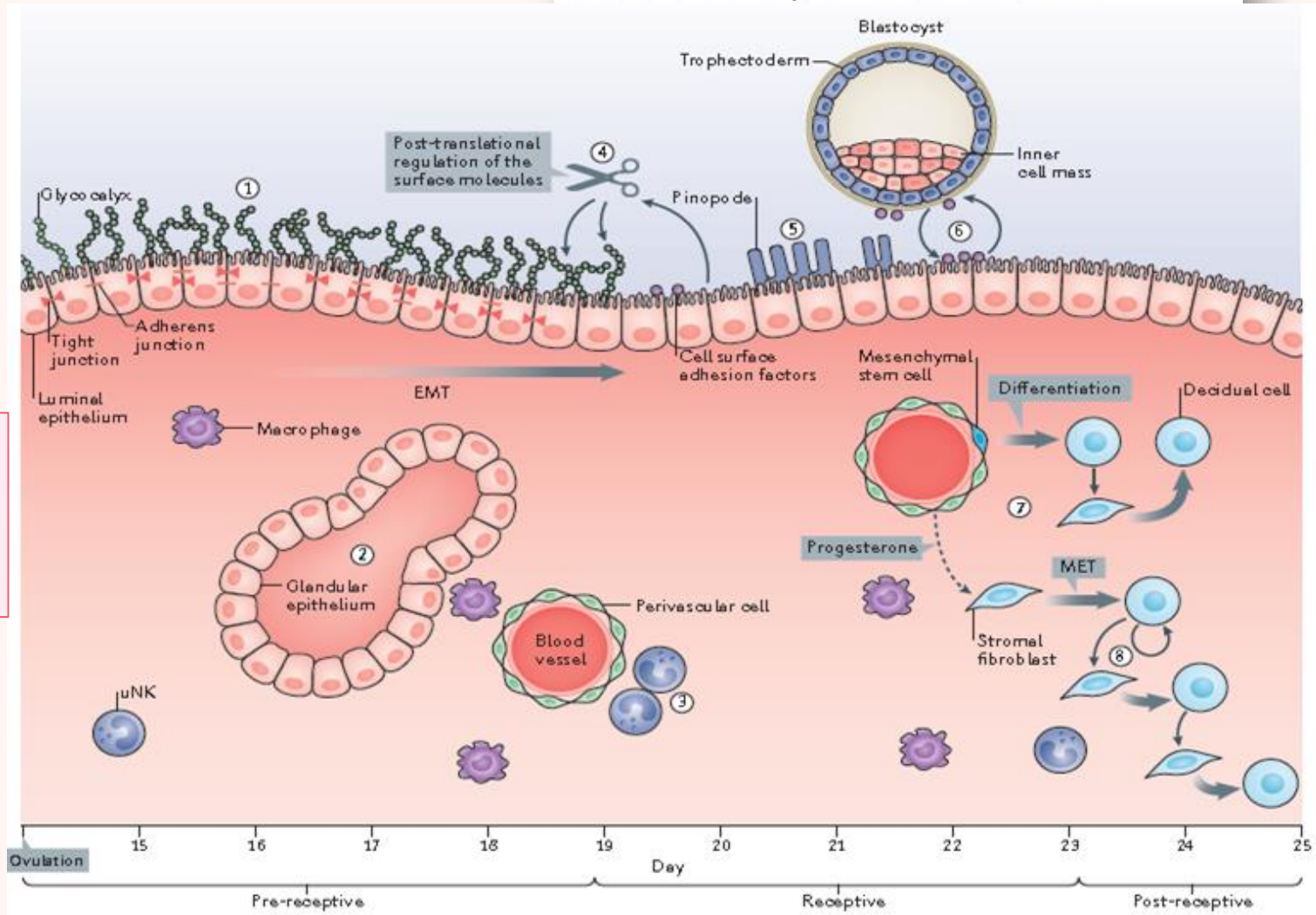
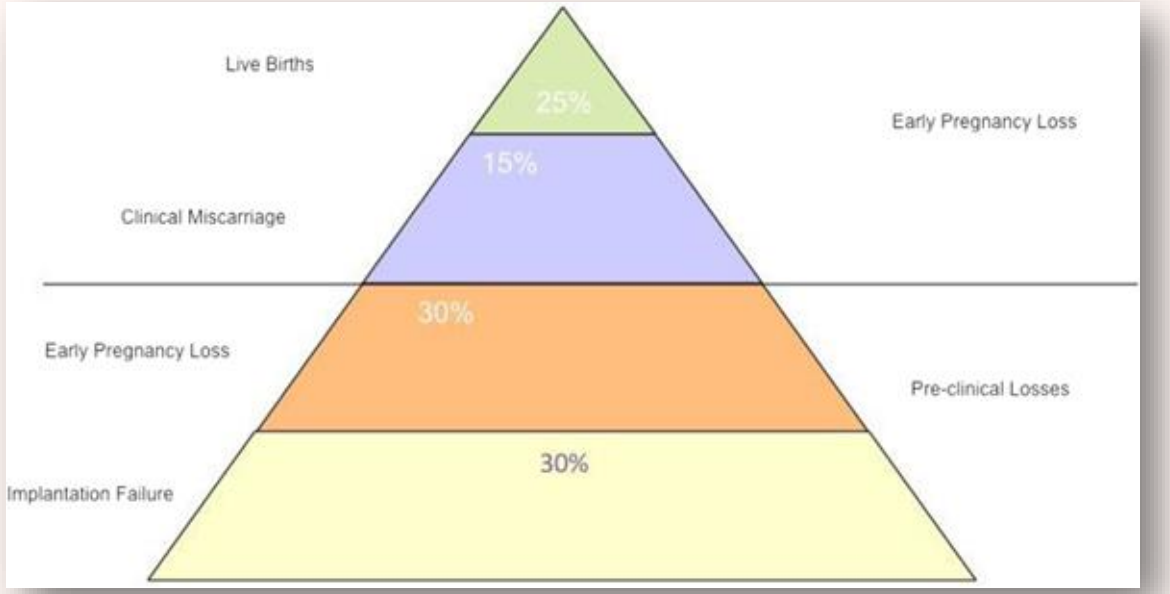


PLAUSIBLE CHARTING OF ADVERSE RIPPLE EFFECTS IN HUMAN PREGNANCY



Fox et al., Fertility and Sterility, 2016

FAILURE OF ANY OF THESE STEPS DETERMINES NO PREGNANCY

PATHOLOGIES BEHIND IMPLANTATION FAILURE

i. **Endometrial diseases**
ii. **Thyroid Dysfunction**
iii. **Genetic Causes**
iv. **Immune Problems**
v. **Blood Clotting**
vi. **Autoimmunity**
vii……

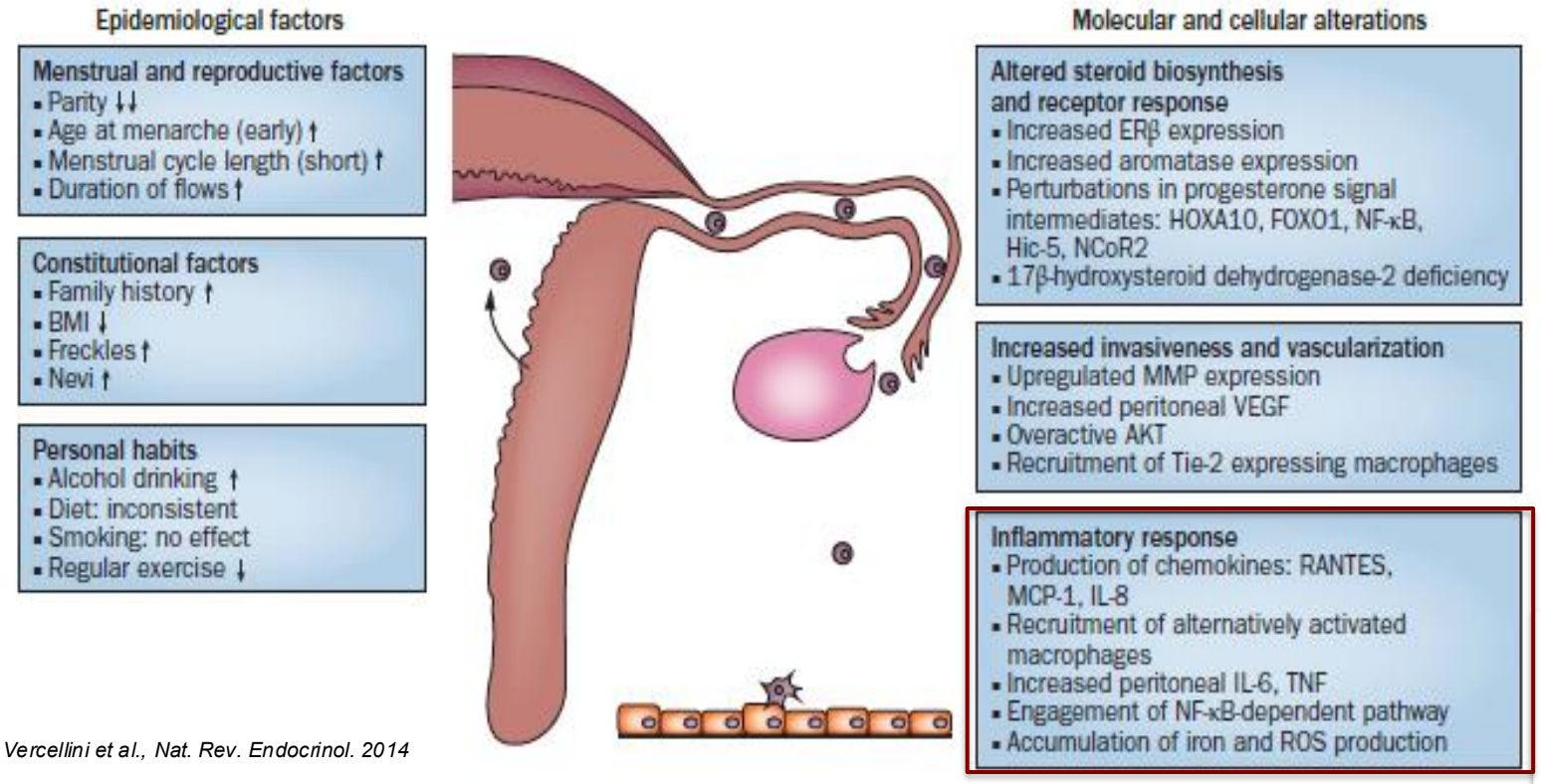
LIFESTYLES BEHIND IMPLANTATION FAILURE

i. **Endocrine disruptors**
ii. **Obesity**
iii. **Alchool consumption**
iv. **Cigarette Smoking**
v. …

CONTROLLED OVARIAN HYPERSTIMULATION

THE EFFECT OF ENDOMETRIAL DISORDERS ON RECEPTIVITY: *ENDOMETRIOSIS*

Def: ***Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity, predominantly, but not exclusively, in the pelvic compartment. It is an estrogen-dependent chronic inflammatory condition that affects women in their reproductive period and is associated with pelvic pain and infertility.***



Vercellini et al., Nat. Rev. Endocrinol. 2014

Endometriosis-associated INFERTILITY

Interference has been hypothesized with the complex mechanisms of:

- ✓ **ovulation**
- ✓ **oocyte pick-up by the fallopian tubes**
- ✓ **spermatozoa function**
- ✓ **fertilization process**
- ✓ **tubal transport of the embryos**
- ✓ **EMBRYO IMPLANTATION**

INFLAMMATION *is a typical feature of endometriosis, as* ***the presence of ectopic tissue in the peritoneal cavity is associated with overproduction of prostaglandins, cytokines and chemokines.***

biomolecules

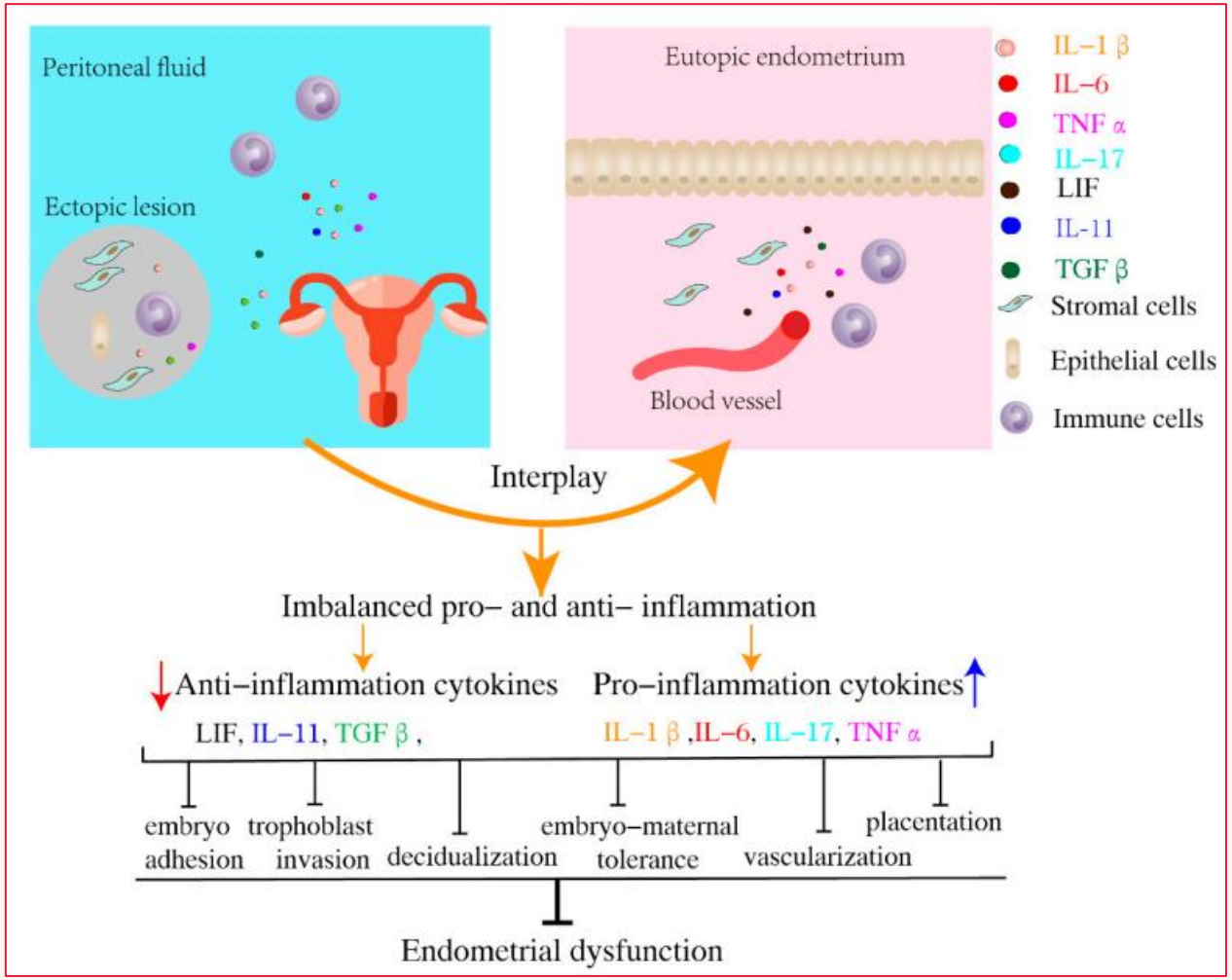
MDPI

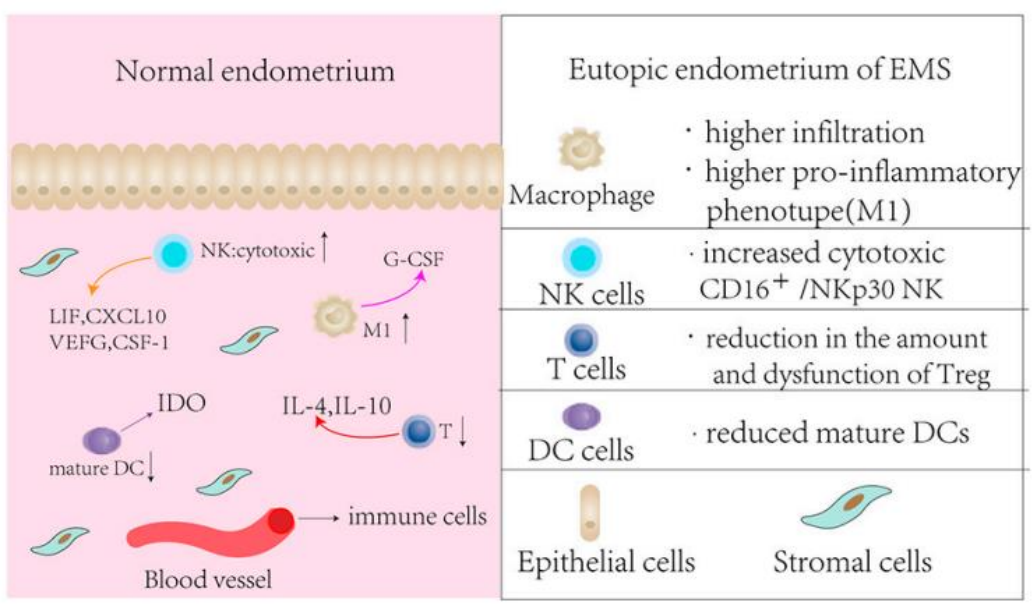
Review

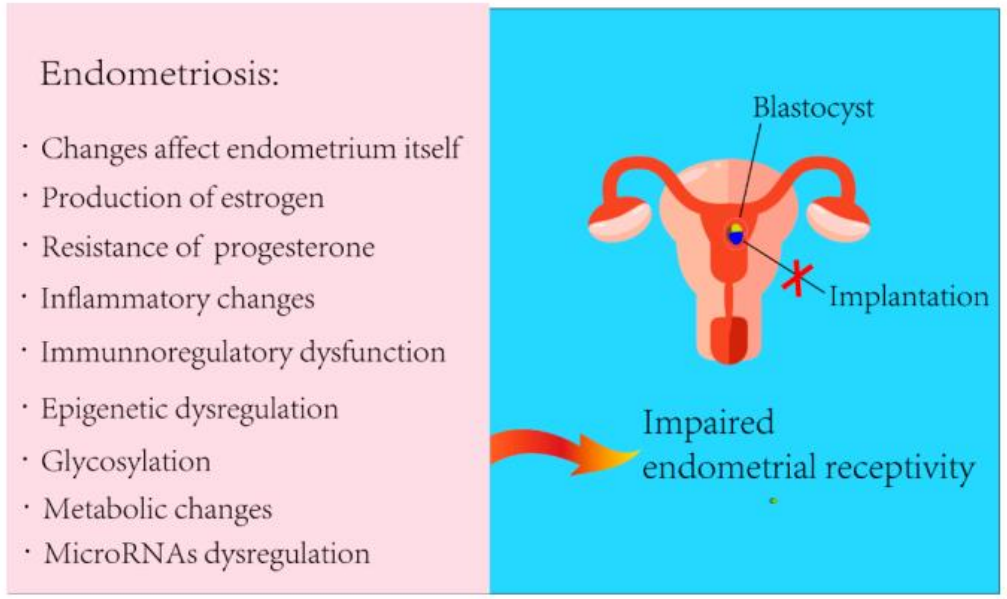
Towards a Better Understanding of Endometriosis-Related Infertility: A Review on How Endometriosis Affects Endometrial Receptivity

Jing Shan [1], Da-Jin Li [1,2,*] and Xiao-Qiu Wang [1,*]

2023







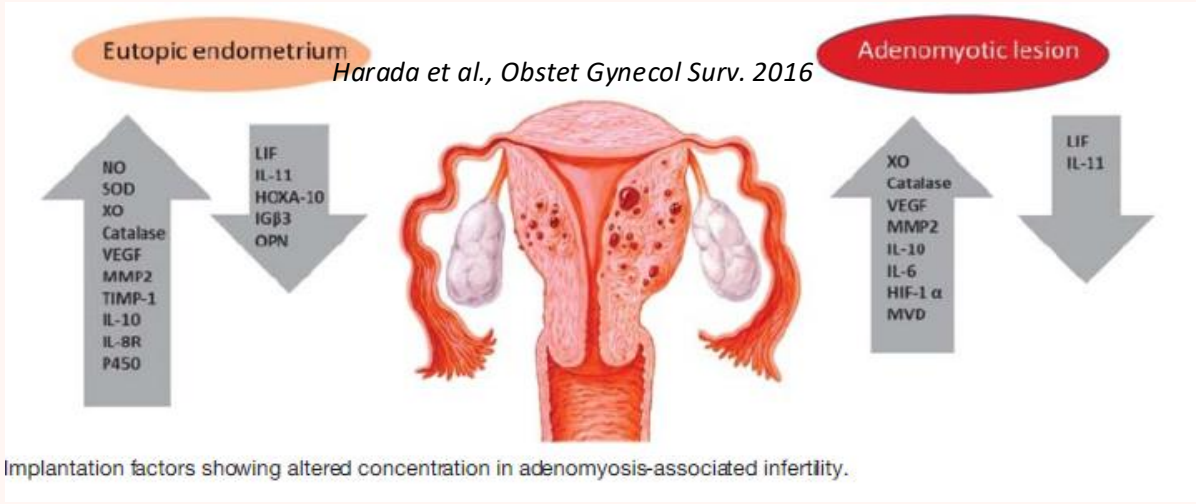
ADENOMYOSIS: WHAT ABOUT THE ENDOMETRIAL RECEPTIVITY?

Adenomyosis is a benign condition of the uterus **characterized by the presence of endometrial glands and stroma located deep within the myometrium and is associated with smooth muscle hyperplasia**. It is a common disorder in the fourth and fifth decades of life.

LACK OF EXPRESSION OF ADHESION MOLECULES

REDUCED EXPRESSION OF IMPLANTATION MARKERS

ALTERED FUNCTION OF GENE FOR EMBRYONIC DEVELOPMENT.



Harada et al., Obstet Gynecol Surv. 2016

Implantation factors showing altered concentration in adenomyosis-associated infertility.

J Hum Reprod Sci. 2018 Oct-Dec; 11(4): 353–358.
doi: 10.4103/jhrs.JHRS_52_18: 10.4103/jhrs.JHRS_52_18
PMCID: PMC6333039
PMID: 30787520

Window of Implantation is Significantly Displaced in Patients with Adenomyosis with Previous Implantation Failure as Determined by Endometrial Receptivity Assay

Nalini Mahajan,[1] Simrandeep Kaur,[2] and Maria Ruiz Alonso[3]

Taiwanese Journal of Obstetrics & Gynecology 57 (2018) 625–626
Contents lists available at ScienceDirect
Taiwanese Journal of Obstetrics & Gynecology
journal homepage: www.tjog-online.com

Editorial

Endometrial receptivity and adenomyosis

Peng-Hui Wang*

Structural abnormality of the endometrium (increased endometrial stroma vascularization and thickness)

Altered expression of:

- ✓Enzymes (cytochrome P450, aromatase activity),
- ✓Cytokines (IL-6, IL-8, IL-10, IL-II, IL-8R, CXCR1, CXCR2),
- ✓Growth factors (VEGF, TGF),
- ✓Adhesion molecules (integrins, MUC1, selectin)
- ✓Matrix metalloproteinases (MMP2 and MMP9)

THE EFFECT OF ENDOCRINE DISRUPTORS ON IMPLANTATION

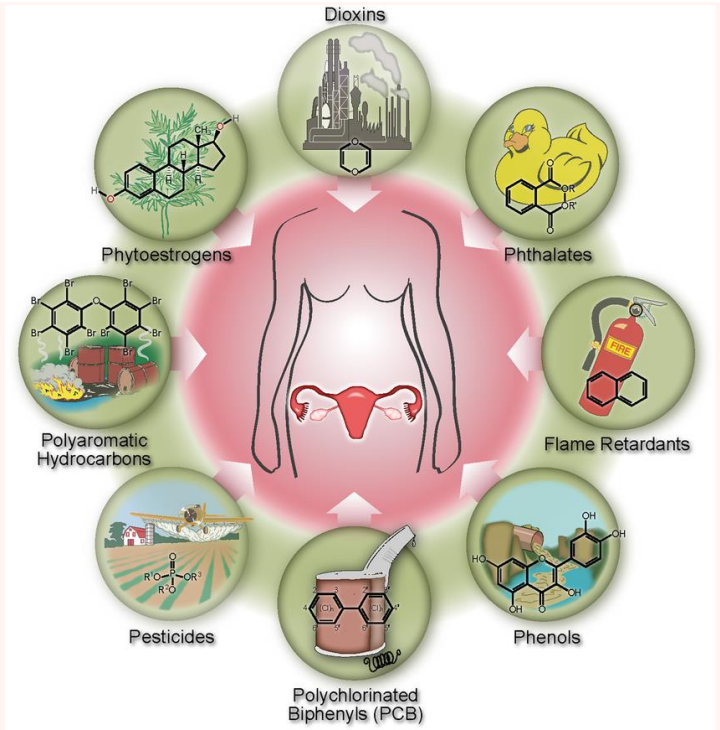
International Journal of *Environmental Research and Public Health*

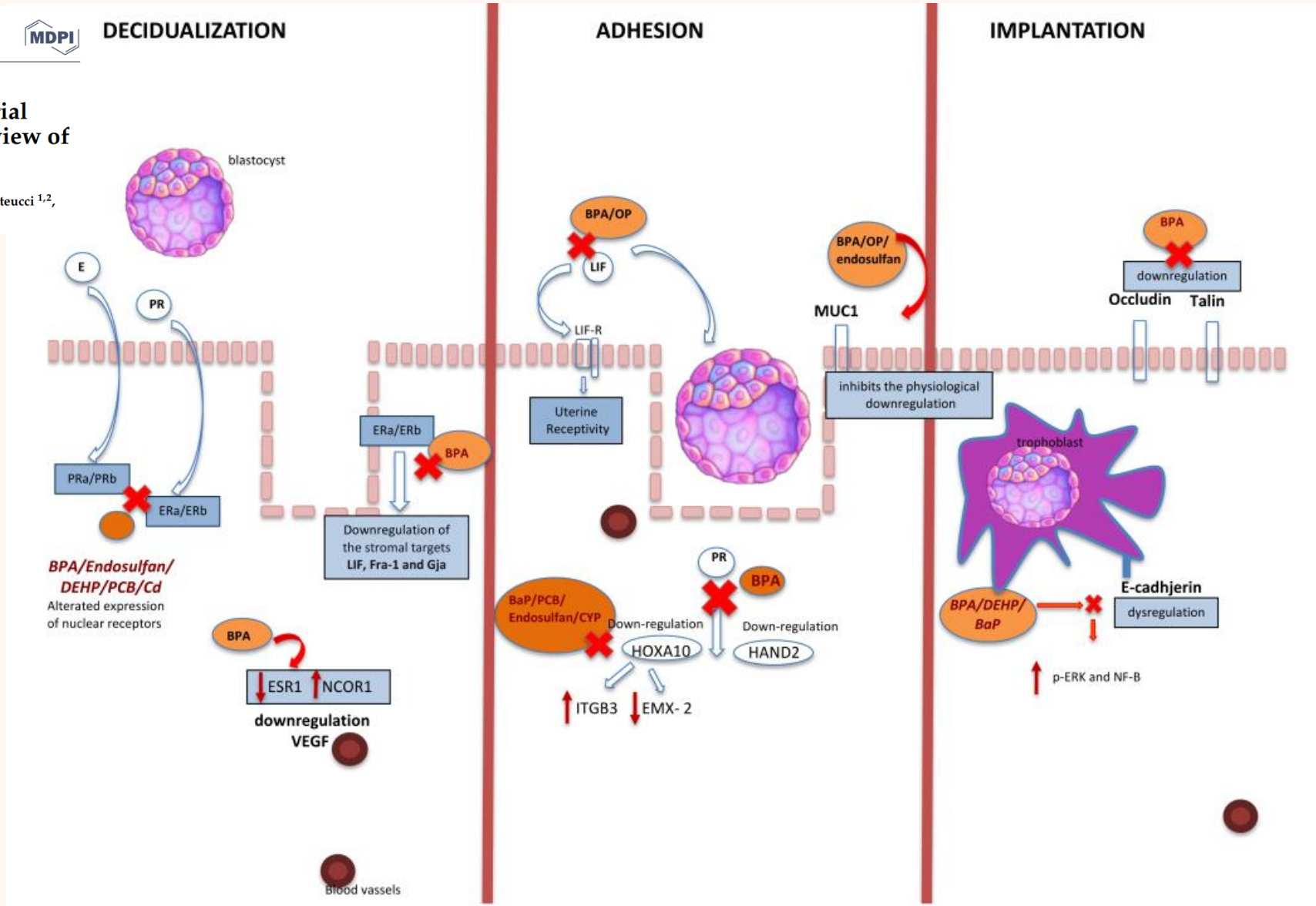
MDPI

Systematic Review

Effects of Endocrine-Disrupting Chemicals on Endometrial Receptivity and Embryo Implantation: A Systematic Review of 34 Mouse Model Studies

Donatella Caserta [1,*], Flavia Costanzi [1], Maria Paola De Marco [1], Luisa Di Benedetto [1,2], Eleonora Matteucci [1,2], Chiara Assorgi [1,2], Maria Clara Pacilli [1], Aris Raad Besharat [1], Filippo Bellati [1] and Ilary Ruscito [1]





THE EFFECT OF CONTROLLED OVARIAN STIMULATION ON IMPLANTATION

ORIGINAL ARTICLE
Endocrine Research

Horcajadas et al., 2008

Controlled Ovarian Stimulation Induces a Functional Genomic Delay of the Endometrium with Potential Clinical Implications

CONTROLLED OVARIAN STIMULATION INDUCES MORPHOLOGICAL, BIOCHEMICAL, AND FUNCTIONAL GENOMIC MODIFICATIONS OF THE HUMAN ENDOMETRIUM DURING THE WOI

Contents lists available at ScienceDirect
Best Practice & Research Clinical Endocrinology & Metabolism
journal homepage: www.elsevier.com/locate/beem

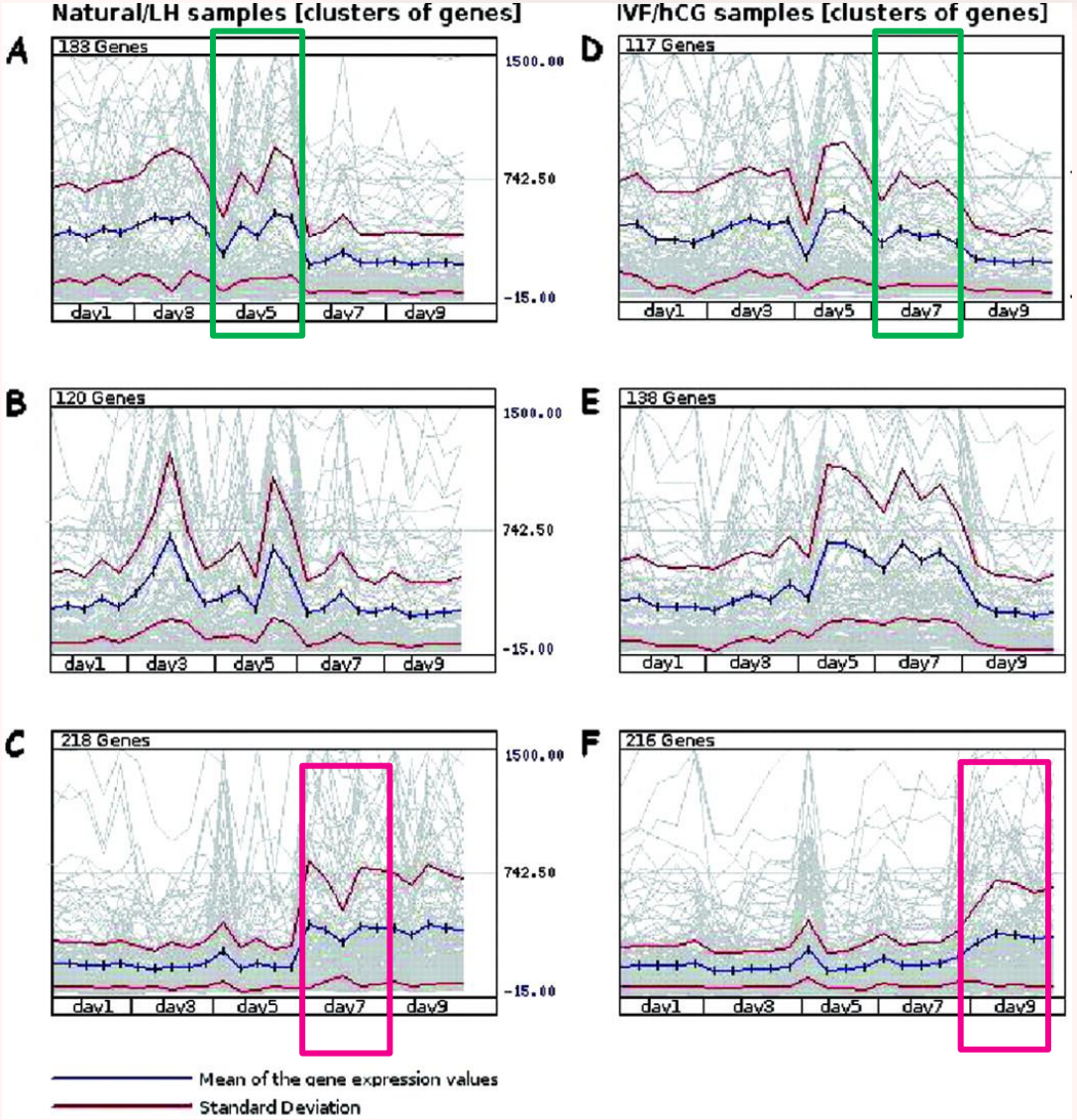
6

The endometrium during and after ovarian hyperstimulation and the role of segmentation of infertility treatment

Pedro Montoya-Botero, MD, Gynecologist [a, b],
Nikolaos P. Polyzos, MD, PhD, Professor/Clinical and Scientific Director [a, c, d, *]

Progesterone Exposure	Increased exposure to progesterone may explain early secretory transformation (1) and subsequent mid-luteal glandular maturation arrest (2)
Estradiol exposure	Elevated estradiol is associated with glandular-stromal dis-synchrony (3) and defective induction of progesterone receptors (4), with reduction of cytosolic progesterone receptors (5)
hCG exposure	hCG injection is a further possible cause for disrupted endometrial luteal phase morphology. A direct effect of hCG concerning advanced endometrial maturation and acquisition of a luteal phase phenotype has been documented in in vitro experiments (6) and hormone replacement cycles (7)
GnRH agonist exposure	GnRH agonists have anti-proliferative effects on the endometrium (8).
Summary	Everything is sustained by the finding of the large variability of endometrial patterns for similar hormone values (9) and the absence of a clear correlation between individual serum hormone measurements and endometrial dating (10)

2-d delay in the activation/repression of *two clusters composed by 218 and 133 genes,* respectively, on day hCG+7 vs LH+7. Many of these delayed genes belong to the *class window of implantation genes* affecting basic biological processes in the receptive endometrium.

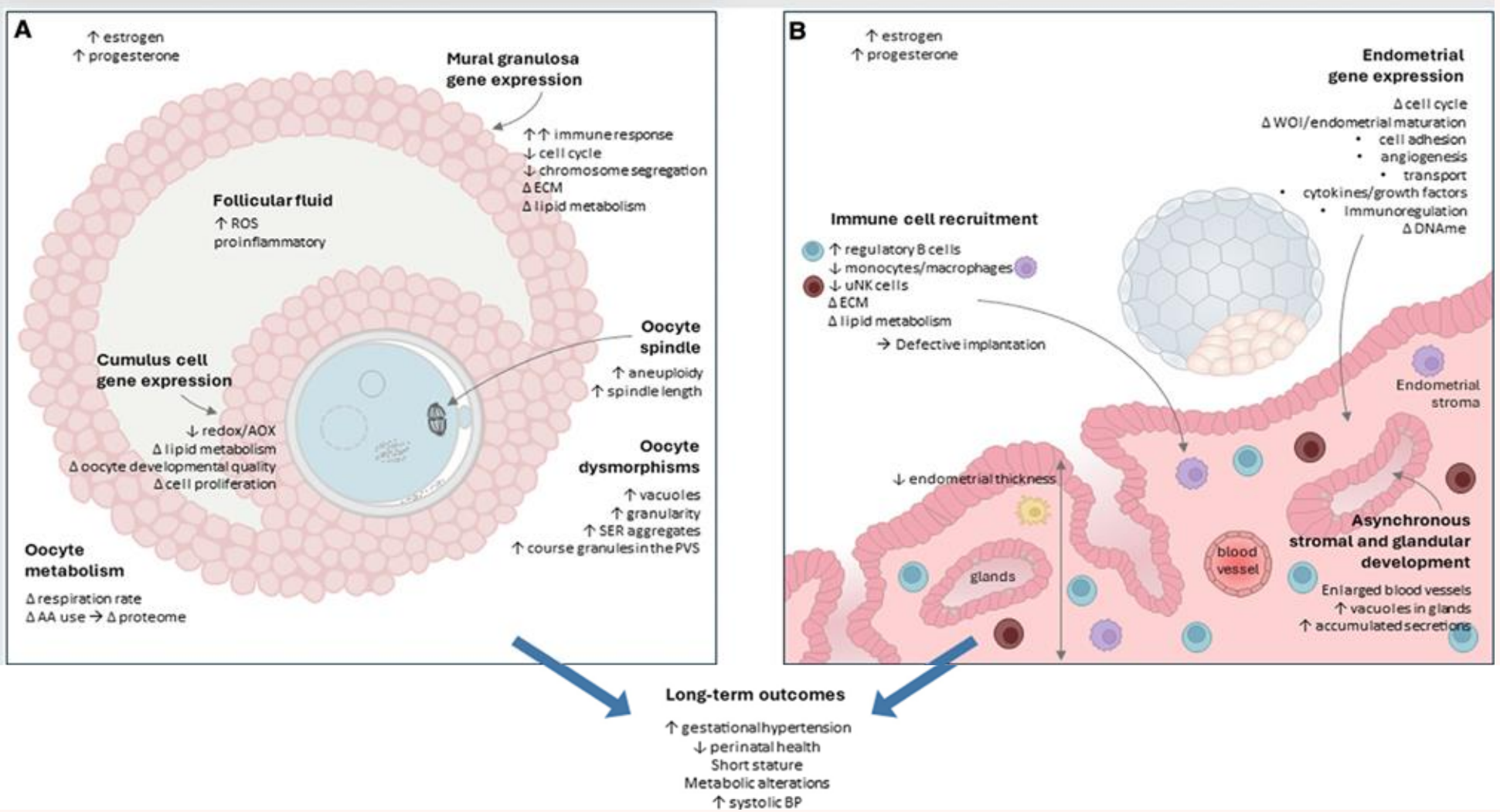


VIEWS AND REVIEWS Volume 123, Issue 1, January 2025

Ovarian stimulation protocols: impact on oocyte and endometrial quality and function

Alexandra J. Harvey, Ph.D.,[a,b] Bryn E. Willson, M.D.,[c] Eric S. Surrey, M.D.,[d] and David K. Gardner, Ph.D.[a,b]

OOCYTES, CUMULUS CELLS, AND ENDOMETRIUM ARE ALTERED BY CONVENTIONAL OVARIAN STIMULATION



Main alterations:

- **Asynchronous stromal and glandular development**
- **Reduced endometrial thickness**
- **Modulation of endometrial gene expression, particularly those involved in immunoregulation suggesting shifts in the WOI**
- **Altered recruitment, activation, and localization of immune cells.**

Best Practice & Research Clinical Endocrinology & Metabolism 33 (2019) 61–75

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Best Practice & Research Clinical Endocrinology & Metabolism

journal homepage: www.elsevier.com/locate/beem

6

The endometrium during and after ovarian hyperstimulation and the role of segmentation of infertility treatment

Pedro Montoya-Botero, MD, Gynecologist [a, b],
Nikolaos P. Polyzos, MD, PhD, Professor/Clinical and Scientific Director [a, c, d, *]

Practice points

- The freeze-all' strategy, cryopreservation of the entire cohort of embryos and transfer in a subsequent synthetic cycle has been the key towards an OHSS-free clinic
- There is evidence that the supraphysiologic levels of estradiol and progesterone during COH could lead to morphologic and biochemical modifications, and consequently impair endometrial receptivity
- Recent studies have demonstrated that uterine peristaltic wave frequency at oocyte retrieval and two days later were significantly higher in stimulated cycles when compared to natural cycles.
- High responder patients are the group of patients that benefit the most from a freeze-all strategy.
- It is still unclear if normo-responding patients benefit regarding pregnancy outcome from a freeze-all strategy as compared to traditional IVF.
- Segmentation should not be routinely recommended in low responding patients unless other practical or clinical reasons point toward this direction, i.e., progesterone elevation during COH.

Research agenda

- Studies on the impact of hormonal changes during ovarian stimulation on the genetic composition of an embryo.
- Gene expression profiles on the impact of vitrification.
- Cost-effectiveness of elective FET, when compared to fresh ET based on the recent RCTs in different ovarian response categories, could elucidate the value of segmentation for policy decision making.

EVALUATION OF ENDOMETRIAL RECEPTIVITY:

STATE OF ART

✓ *PROGESTERONE LEVEL*

✓ *ULTRASOUND TECHNIQUE*

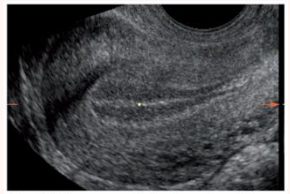
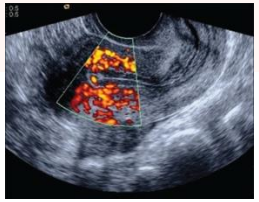
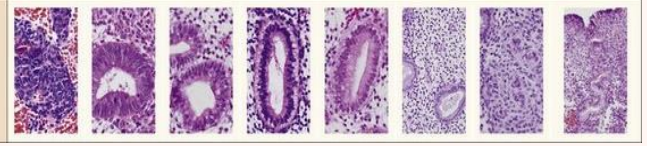


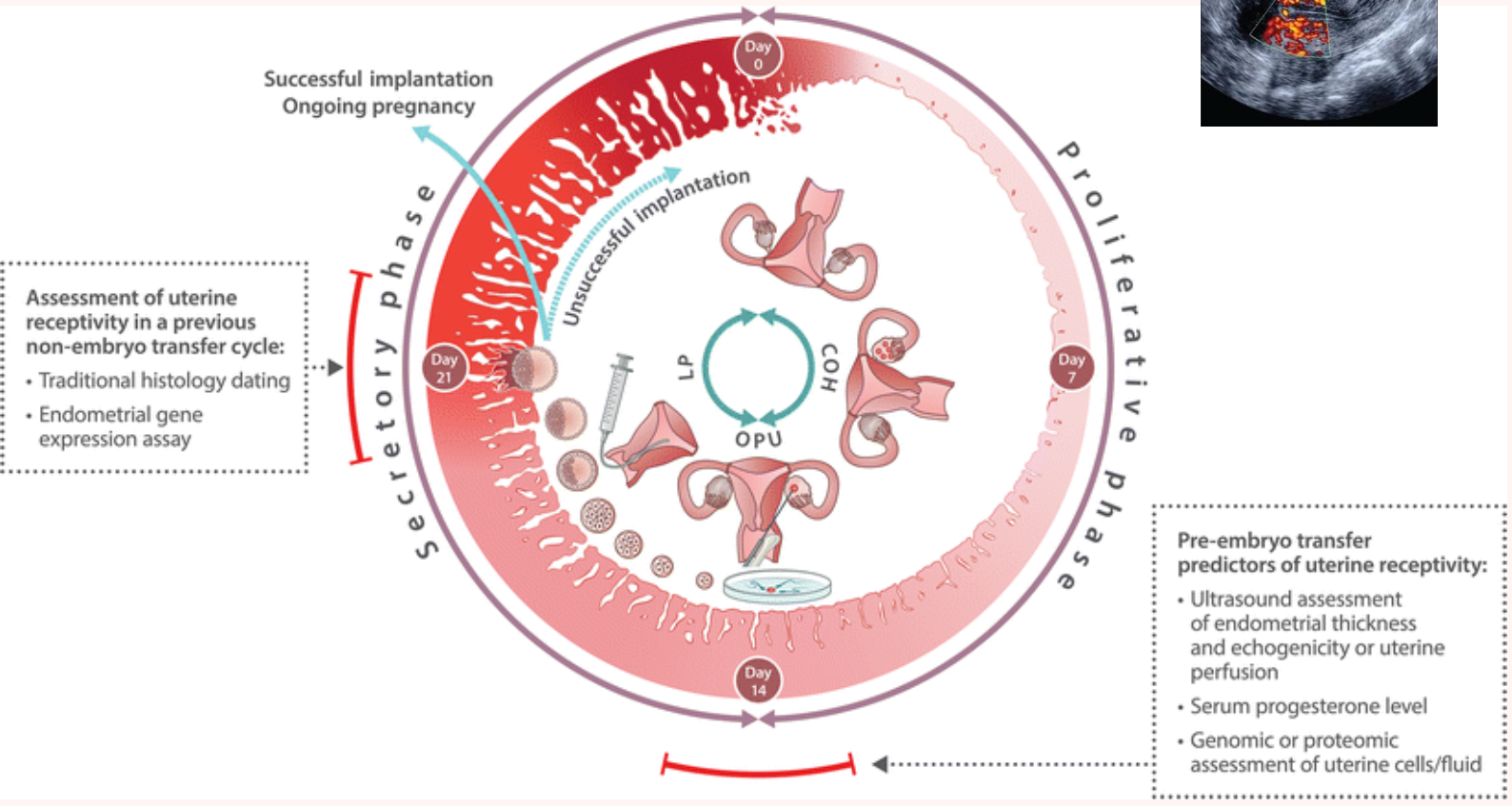
Figure 11. Trilaminar appearance of endometrium consistent with late follicular phase.

✓ *DOPPLER TECHNIQUE*



✓ *ISTHOLOGIC TECHNIQUE*

Endometrial phase		Menstrual	Proliferative	Secretory				
				'Interval'	Early	Mid	Late	
Day, of 28 day cycle (ovulation on D14)		1–3	4–15	~16	17	18	19–21	22–24	25–28
Appearances								



INNOVATIVE STRATEGIES TO MEASURE ENDOMETRIAL RECEPTIVITY

- ERA test
- Be Ready
- ER Map
- ReceptivaDX
- ALICE
- EMMA
- EndomeTRIO

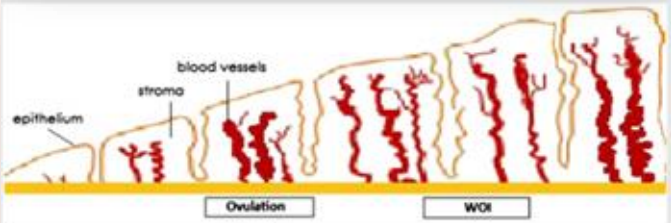
A ENDOMETRIAL RECEPTIVITY ARRAY: THE ERA TEST

THE ENDOMETRIUM

Profiling the gene signature of endometrial receptivity: clinical results 2013

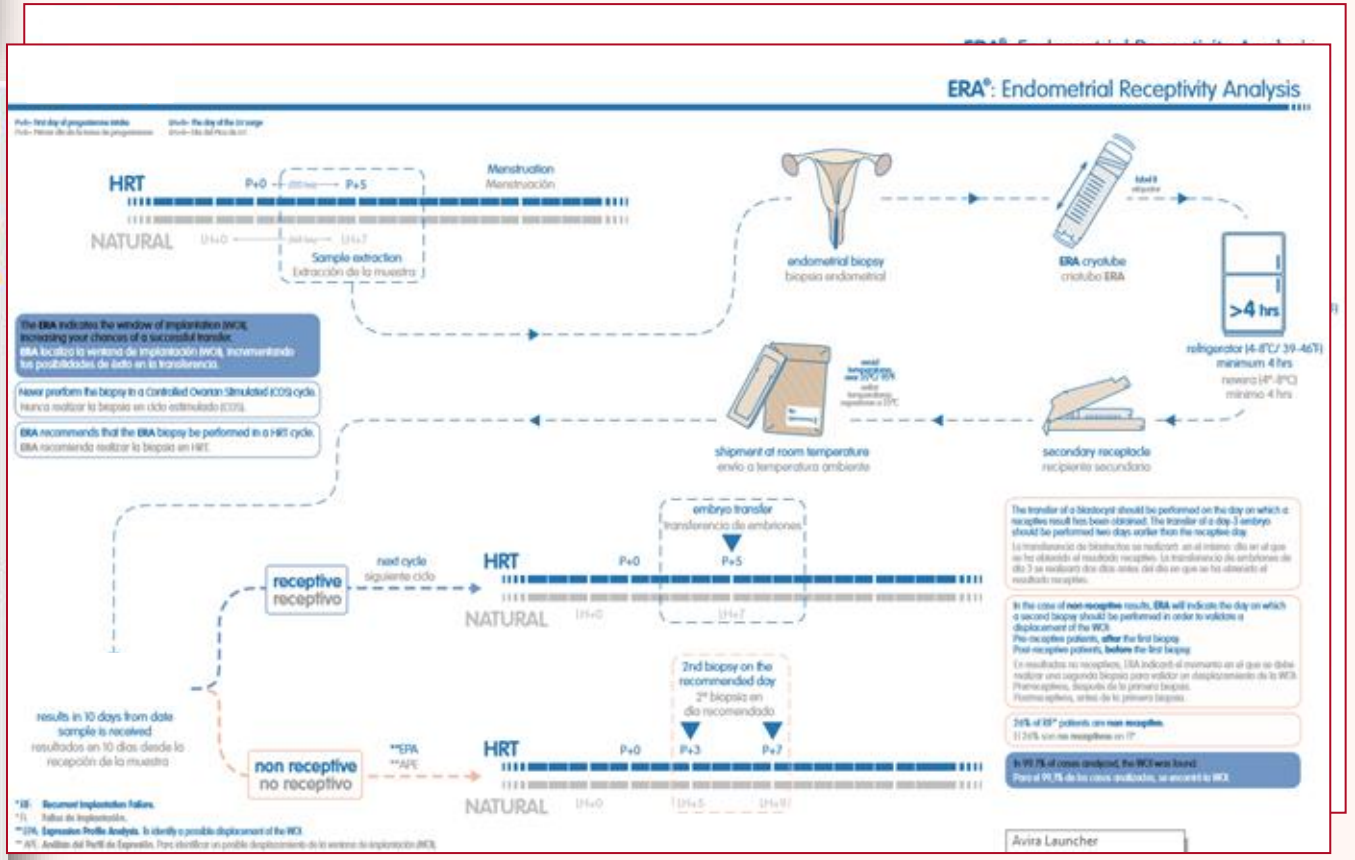
Tamara Garrido-Gómez, Ph.D.,[a] María Ruiz-Alonso,[b] David Blesa, Ph.D.,[a,b] Patricia Díaz-Gimeno, Ph.D.,[a,c] Felipe Vilella, Ph.D.,[a] and Carlos Simón, M.D., Ph.D.[a,b]

The ERA is a genetic test that evaluates the expression of genes to estimate whether the endometrial lining is properly developed to accept an embryo.



Proliferative	Pre-Receptive	Receptive	Post-Receptive
			

Proliferative functions	Secretory functions		
	Early-secretory	*Mid-secretory*	*Late-secretory*
Cellular proliferation	Metabolism	Metabolism	Extracellular matrix degradation
Cellular differentiation	Transport	Glandular secretion	Inflammatory response
Extracellular matrix remodeling	Proliferation inhibition	Cell differentiation	Apoptosis
Angiogenesis and vasculogenesis	Mitosis inhibition	Cell communication	
DNA synthesis		Innate immune response	
Adhesion		Response to stress	
Ion channels		Response to wounding	
		Adhesion	
		Proteolysis regulation	



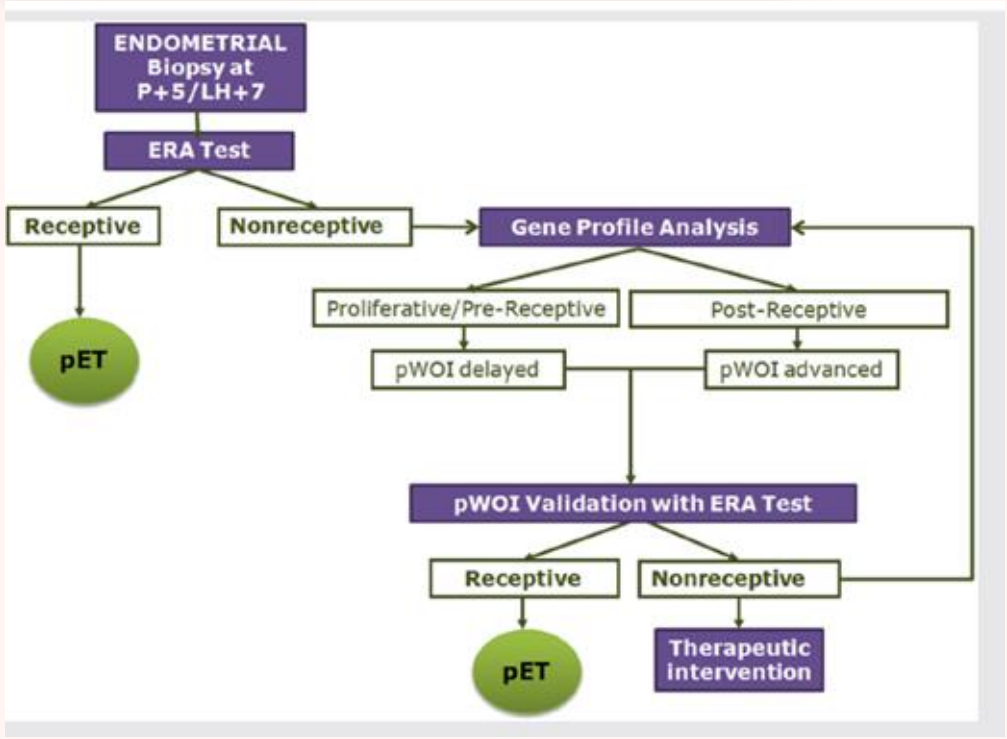
PERSONALIZED EMBRYO TRANSFER (pET)

Human Reproduction, Vol.29, No.6 pp. 1244–1247, 2014
Advanced Access publication on April 15, 2014 doi:10.1093/humrep/deu070

human reproduction CASE REPORT *Infertility*

What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study

M. Ruiz-Alonso[1], N. Galindo[2], A. Pellicer[3], and C. Simón[1,3,4,*]



ID	Age	IVF cycles pre-ERA	OD cycles pre-ERA	WOI at ET	ERA result WOI at ET	N° of cycles pre-ERA with ET	Mean of embryos per transfer [range]	IR (%)	Clinical pregnancy	Ongoing pregnancy rate
1	35	0	3	P+5	PRE-R	3	2 [2]	0/6 (0)	No	No
2	32	0	1	P+4	PRE-R	1	2 [2]	0/2 (0)	No	No
3	42	2	3	P+4	PRE-R	2	2 [2]	1/4 (25)	Biochemical	No
4	39	0	1	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
5	38	0	3	P+4	PRE-R	3	2 [2]	1/6 (17)	Clinical abortion	No
6	43	0	1	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
7	43	2	3	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
8	46	0	2	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
9	46	0	8	P+5	PRE-R	3	1.3 [1-2]	0/4(0)	No	No
10	35	0	2	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
11	47	0	1	P+5	POST-R	1	2 [2]	0/2 (0)	No	No
12	36	0	2	P+5	PRE-R	2	2 [2]	1/4 (25)	Clinical abortion	No
13	41	0	4	P+5	PRE-R	3	1 [1]	1/3 (33)	Biochemical	No
14	40	0	6	P+5	PRE-R	6	1.8 [1-2]	2/11 (18)	Clinical abortion	No
15	40	0	2	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
16	40	0	2	P+5	PRE-R	2	2 [2]	1/4 (25)	Clinical abortion	No
17	49	0	1	P+5	PRE-R	1	1 [1]	0/1 (0)	No	No
Total patients										
17	40.7	0.2 ±0.7	2.6 ±1.9		100% NR	2.1 ±1.3	1.8 [1-2]	11% (7/65)	19% (7/36)	0% (0/7)

ID	Age	IVF cycles pre-ERA	OD cycles pre-ERA	pWOI at pET	ERA result pWOI at pET	N° of cycles post-ERA with pET	Mean of embryos per transfer [range]	IR (%)	Clinical pregnancy	Ongoing pregnancy rate
1	35	0	3	P+6	R	2	1.5 [1-2]	1/3(33)	Yes	Babyat home
2	32	0	1	P+7	R	1	2 [2]	1/2(50)	Biochemical	No
3	42	2	3	P+6	R	2	2 [2]	2/4 (50)	Yes	Babyat home
4	39	0	1	P+7	R	1	2 [2]	0/2 (0)	No	No
5	38	0	3	P+7	R	1	1 [1]	0/1 (0)	No	No
								1/2(50)	Yes	Yes
								2/2(100)	Yes	Babyat home
								1/2(50)	Yes	Yes
							[1-2]	0/2 (0)	No	No
								0/2 (0)	No	No
11	47	0	1	P+7	R	1	1.5 [1-2]	1/1 (100)	Yes	Babyat home
12	36	0	2	P+4.5	R	1	2 [2]	1/2(50)	Biochemical	No
13	41	0	4	P+5.5	R	1	1 [1]	1/1 (100)	Yes	Yes
14	40	0	6	P+7	R	2	2 [2]	1/4 (25)	Yes	Yes
15	40	0	2	P+7	R	1	2 [2]	1/2(50)	Yes	Yes
16	40	0	2	P+7	R	1	2 [2]	1/2(50)	Biochemical	No
17	49	0	1	P+7	R	1	1 [1]	0/1 (0)	No	No
Total patients										
17	40.7	0.2 ±0.7	2.6 ±1.9		100% R	1.2 ±0.4	1.8 [1-2]	40% (14/35)	60% (12/20)	75% (9/12)

IR: 11% (7/65) vs 40% (14/35)
Clinical Pregnancy: 19%(7/36) vs 60% (12/20)
Ongoing pregnancy: 0% (0/7) vs 75% (9/12)

Journal of Assisted Reproduction and Genetics (2018) 35:929–930
https://doi.org/10.1007/s10815-018-1125-5

LETTER

Intra-patient variability in the endometrial receptivity assay (ERA) test

Kristy Cho[1] · SeangLin Tan[2,3] · William Buckett[2] · Michael H. Dahan[2,3]

Table 1 Summary of ERA results

ERA	Interval since last assay (days)	Interval since first assay (days)	Duration of P_4 supplementation (hours) when biopsy was performed	Endometrial receptivity classification	ERA recommendation	ERA recommendation for subsequent timing of biopsy/transfer (hours of P_4)
#1	N/A	N/A	106	Pre-receptive	Repeat endometrial biopsy	154 ± 3
#2	42	42	194	Post-receptive	Repeat endometrial biopsy	170 ± 3
#3	38	80	170	Post-receptive	Perform embryo transfer	146 ± 3
#4	33	113	148	Receptive	Perform embryo transfer	148 ± 3

N/A not applicable, P_4 progesterone

"Although this study is based on a single patient, the lack of consistency is intriguing. Hopefully, this report may lead to more researchers without a direct financial benefit to publish their experience with the ERA test."

Journal of Assisted Reproduction and Genetics (2018) 35:1307–1308
https://doi.org/10.1007/s10815-018-1212-7

LETTER TO THE EDITOR

Inter-cycle consistency versus test compliance in endometrial receptivity analysis test

Tiffany Stankewicz[1] · Diana Valbuena[2] · Maria Ruiz-Alonso[2]

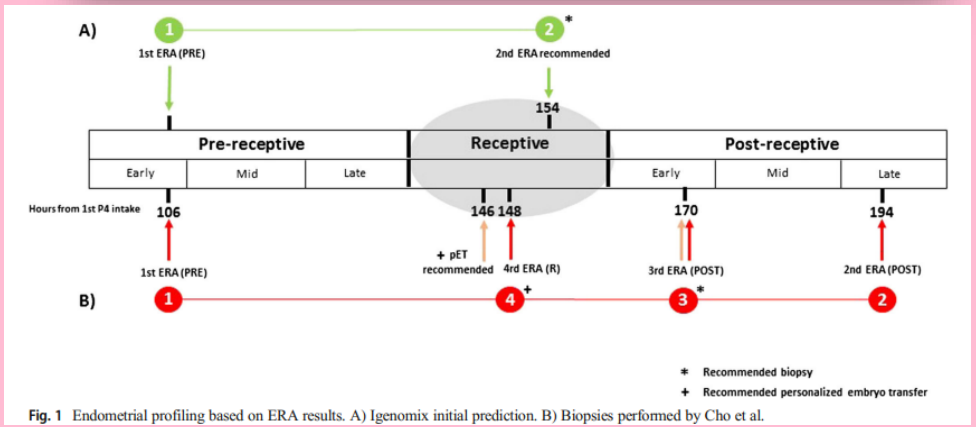


Fig. 1 Endometrial profiling based on ERA results. A) Igenomix initial prediction. B) Biopsies performed by Cho et al.

"Here, we present our case to suggest that Cho et al. have misinterpreted their own data and suggested that the ERA test is not accurate nor is it reproducible. We have pointed out a number of questionable clinical decisions made by the Cho team which led them to perform four different endometrial biopsies in one patient after receiving a recommendation for the best way forward in this case. "

Journal of Assisted Reproduction and Genetics (2018) 35:1923–1924
https://doi.org/10.1007/s10815-018-1279-1

LETTER TO THE EDITOR

Variations in the endometrial receptivity assay (ERA) may actually represent test error

Michael H. Dahan[1] · Seang Lin Tan[1]

"In conclusion, this case clearly demonstrates inconsistencies in two ERA results in the same patient performed 4 months apart, both in 2017."

ORIGINAL ARTICLES: ASSISTED REPRODUCTION

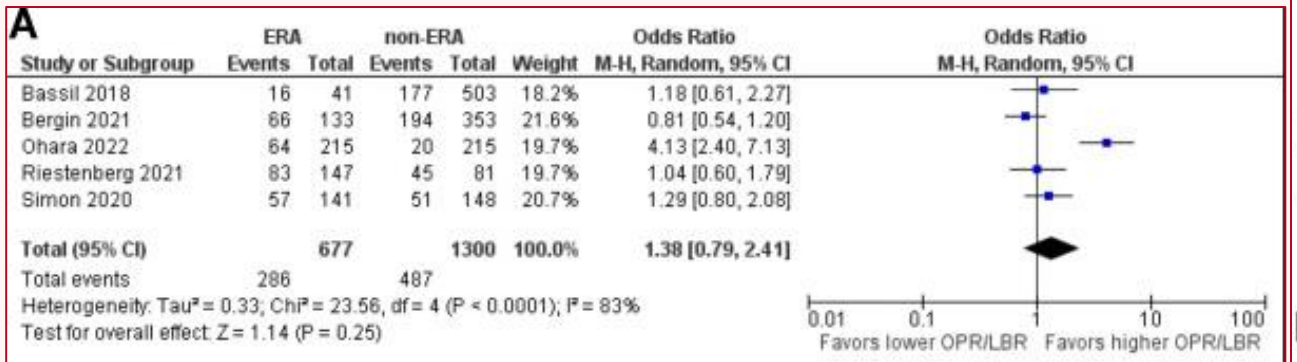
2023

Endometrial receptivity array before frozen embryo transfer cycles: a systematic review and meta-analysis

Sara E. Arian, M.D., M.S.C.I.,[a,b] Kamran Hessami, M.D.,[c] Ali Khatibi, M.D., Ph.D.,[d] Alvin K. To, M.D.,[e] Alireza A. Shamshirsaz, M.D.,[c] and William Gibbons, M.D.[a]

8 studies and over 2,000 patients, determining the optimal endometrial receptivity time using the **ERA test does not appear to improve overall pregnancy outcomes.** This may indicate that other immunological and etiological factors are involved in successful embryo implantation.

A



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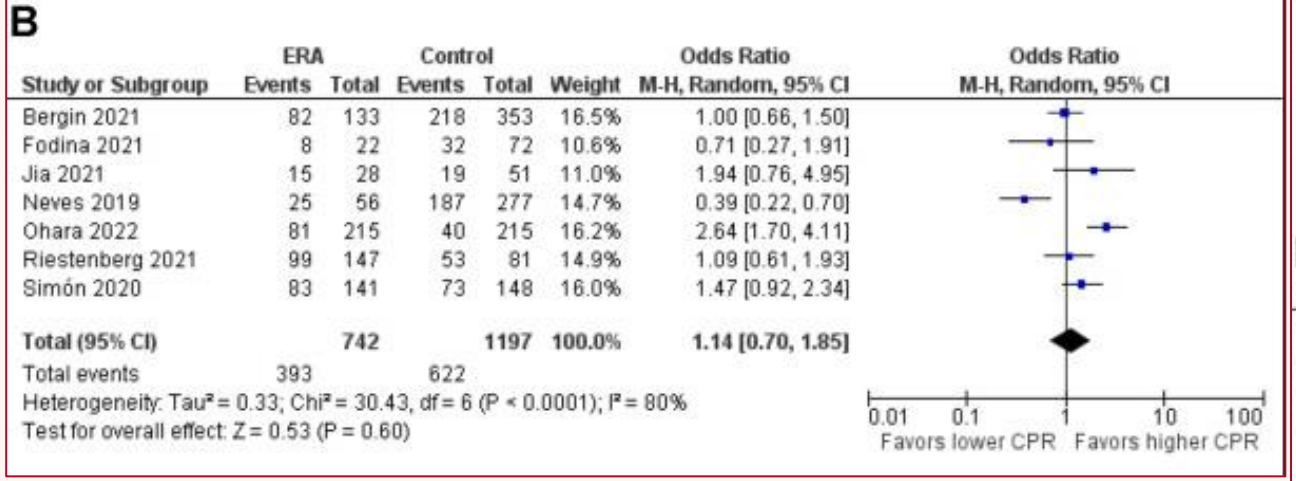

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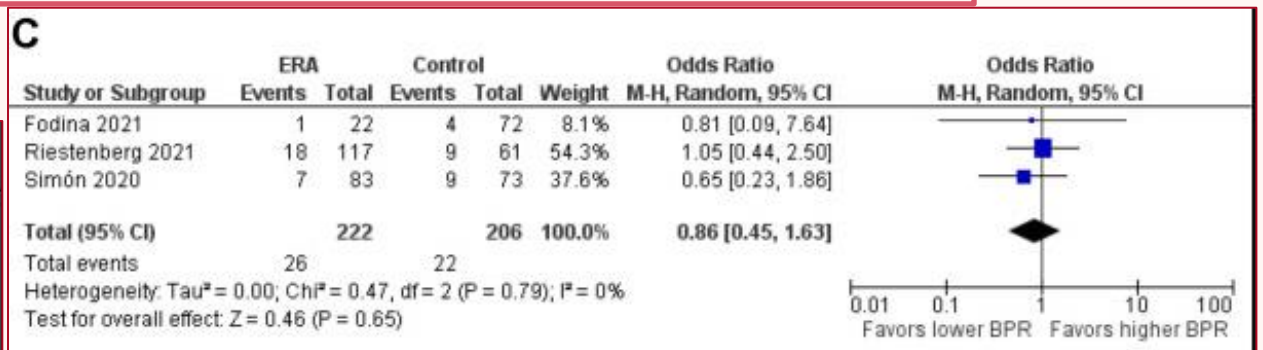

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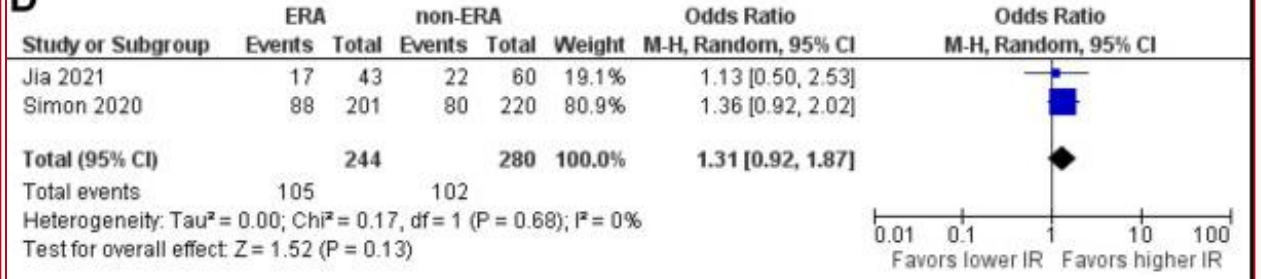

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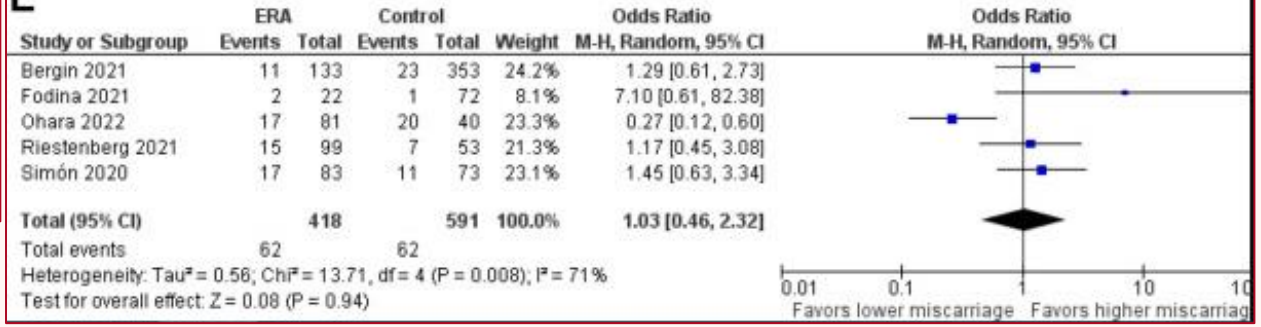
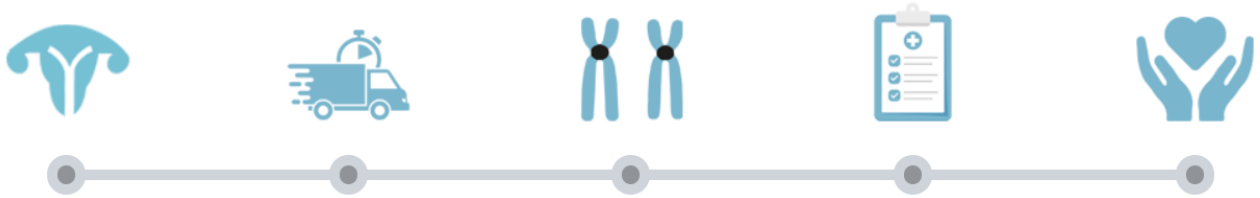


B BE READY RECEPTIVITY TEST

How does the *be*READY test work?



Endometrial sample

Sample shipping to beREADY lab

Sample processing and quality control

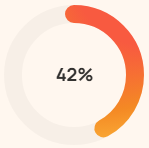
beREADY analysis and reporting

Embryo transfer at the right time

Results are available in 1-2 weeks

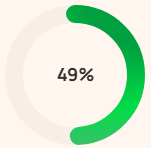
What does the *be*READY test show?

The test measures the **maturity level** of your endometrium. The information you get enables embryo transfer at the optimal time. The results may show **three possible scenarios** with probability percent. **42%** of beREADY patients have pre- or early-receptive diagnose and they need more time to mature. **About half** of the patients have an "average" maturation speed, and approximately **10%** need less time for maturation compare to "average". As you see it is difficult to make a diagnosis without testing



PRE or EARLY-RECEPTIVE

You need more time! The test shows that you have **shifted WOI**, and embryo transfer should be done later. So, the report indicates the exact timing that is optimal for you

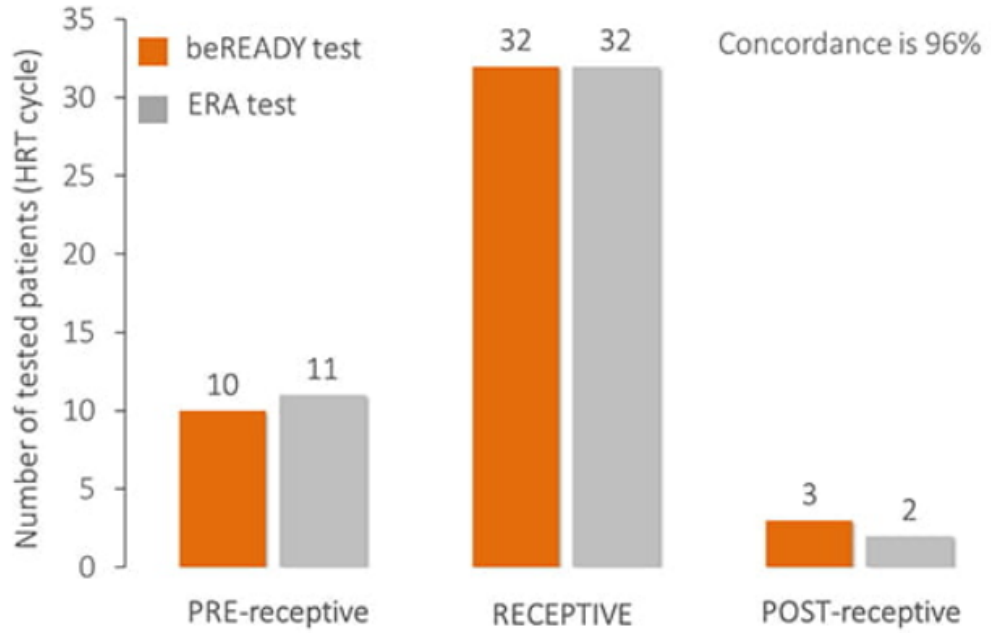


FULLY-RECEPTIVE

The test confirms that you have an average and expected WOI and there is no need to change the timing

LATE or POST-RECEPTIVE

You have rapid endometrial maturation! The test shows that you have **shifted WOI**, and embryo transfer should be done earlier. So, the report indicates the exact timing that is optimal for you



C ER MAP TEST

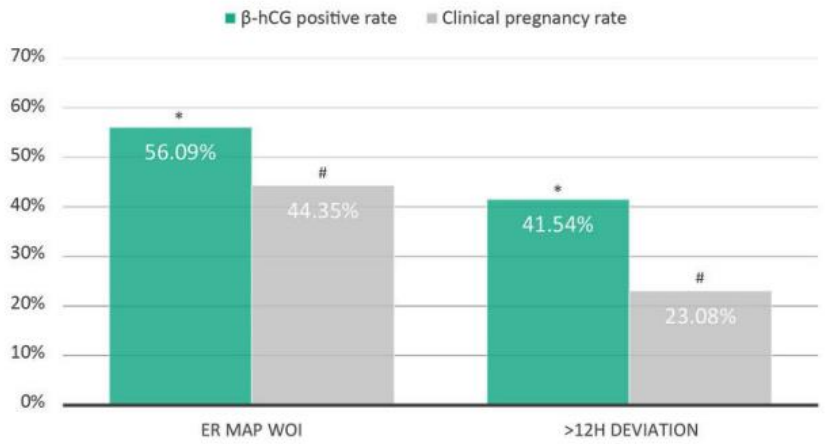


Figure 2. Reproductive outcomes of patients after the first single embryo transfer following ER Map test. Positive B-hCG rates (green bars) and clinical pregnancy rates (grey bars) comparison between the group of patients that followed ER Map progesterone pretreatment and those that deviated more than 12h from this recommendation. X^2 test, *p = 0.02, #p < 0.001 n = 746 sET (ER Map WOI N = 681, > 12h deviation n = 65).

Human Reproduction, Vol.33, No.2 pp. 220–228, 2018
Advanced Access publication on January 5, 2018 doi:10.1093/humrep/dex370

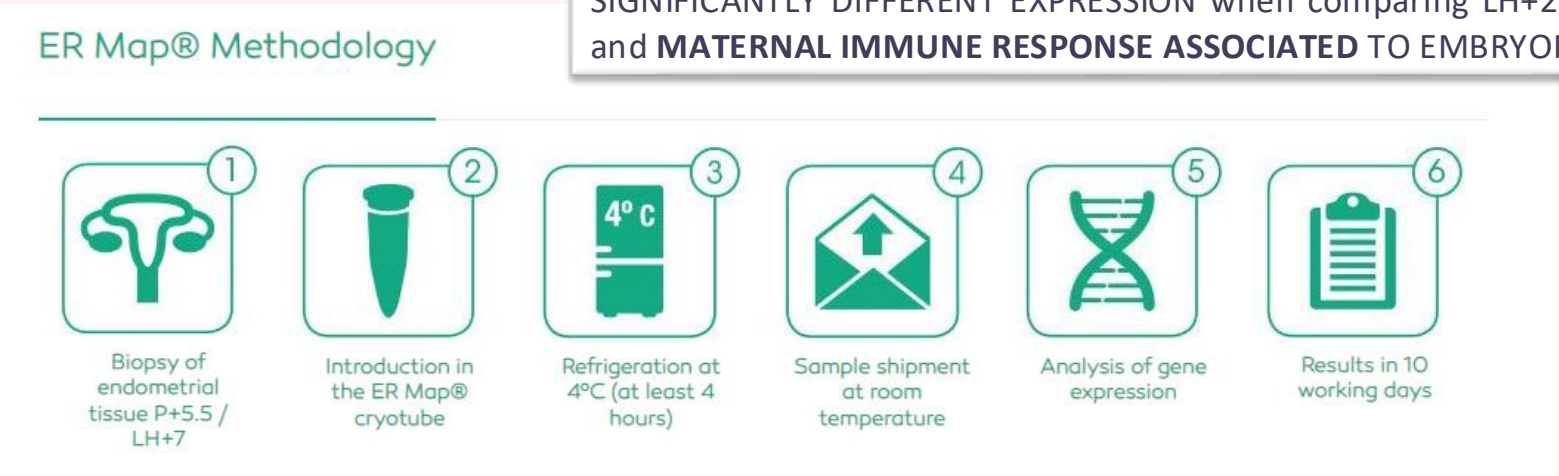
human reproduction ORIGINAL ARTICLE *Infertility*

Development of a new comprehensive and reliable endometrial receptivity map (ER Map/ER Grade) based on RT-qPCR gene expression analysis

M. Enciso[1,*,†], J.P. Carrascosa[2,†], J. Sarasa[1], P.A. Martínez-Ortiz[3], S. Munné[4], J.A. Horcajadas[2,*], and J. Aizpurua[3]

ER MAP TEST CAN PREDICT ENDOMETRIAL RECEPTIVITY STATUS BY **RT-qPCR** USING A **NEW PANEL** OF **184 GENES**

96 fertile women and 120 assisted reproduction treatment (ART) patients: 85 out of 184 selected genes SHOWED A SIGNIFICANTLY DIFFERENT EXPRESSION when comparing LH+2 and LH+7 RELATED TO **ENDOMETRIAL PROLIFERATION** and **MATERNAL IMMUNE RESPONSE ASSOCIATED** TO EMBRYONIC IMPLANTATION



D RECEPTIVADX TEST

ReceptivaDx
Endometrial Biopsy Analysis

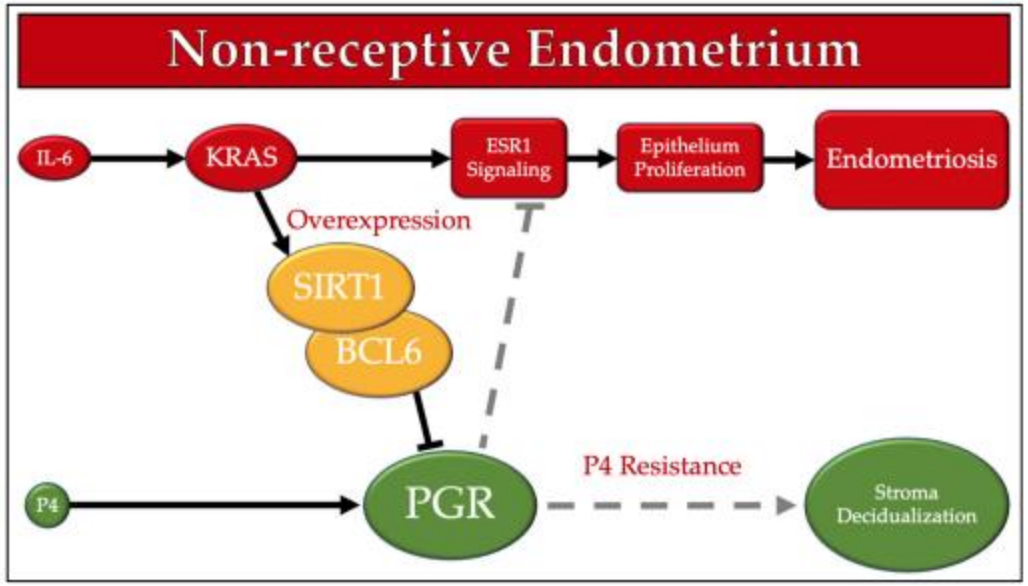
current issues in molecular biology

MDPI

Article

Evaluation of BCL6 and SIRT1 as Non-Invasive Diagnostic Markers of Endometriosis

Alison M. Sansone [1], Brooke V. Hisrich [1], R. Brandt Young [1], William F. Abel [1], Zachary Bowens [2], Bailey B. Blair [1], Avery T. Funkhouser [1], David P. Schammel [3], Lisa J. Green [2], Bruce A. Lessey [4] and Anna V. Blenda [1,*]



What is ReceptivaDx™?

ReceptivaDx measures BCL6, an inflammatory marker not normally present during the window of implantation.

BCL6 identifies uterine lining dysfunction and progesterone resistance, two key factors that may impact the ability for women to become pregnant and maintain pregnancy.

Women who test positive and are appropriately treated can expect a 60% chance of achieving pregnancy in their next transfer attempt.

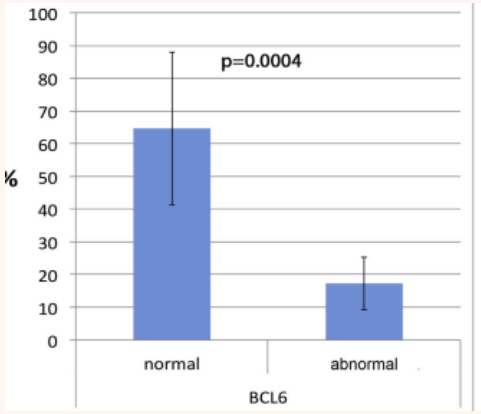
A negative result offers reassurance that the uterine lining is not likely a factor in the patient's infertility.

ReceptivaDx™ Predicts Chances for a Successful Transfer and Successful Pregnancy

WOMEN TESTING <u>POSITIVE</u> FOR RECEPTIVADX:
- Less than 18% Pregnancy rate in next transfer attempt
- Less than 11% Success rate resulting in live birth

WOMEN TESTING <u>NEGATIVE</u> FOR RECEPTIVADX:
- More than 70% Pregnancy rate in next transfer attempt
- More than 59% Success rate resulting in live birth

% pregnant women according to BCL6 expression:



ERT TEST: **endometrial biomarker test** based on the progesterone response

Received: 18 October 2024 | Revised: 8 March 2025 | Accepted: 1 April 2025 | Published online: 13 April 2025
DOI: 10.1002/ijgo.70157

CLINICAL ARTICLE
Gynecology

Novel endometrial receptivity test increases clinical pregnancy and live birth rates in patients with recurrent implantation failure: Secondary analysis of a prospective clinical trial

Yu Zheng[1] | Na Xu[1] | Biao Chen[1,2] | Jun Dai[1,2] | Jian Bai[1] | Bo Huang[1] | Lei Jin[1,2] | Xiyuan Dong[1,2] | Zhou Li[1,2]

TABLE 3 The pregnancy outcomes of patients with RIF.

	ERT	Non-ERT	*P* value
No. of patients	45	40	
Clinical pregnancy rate (*n*, %)	26 (57.78)	14 (35.00)	0.036
Live birth rate (*n*, %)	24 (53.33)	12 (30.00)	0.030
Miscarriage rate (*n*, %)	2 (7.69)	2 (14.29)	0.507
Ectopic pregnancy rate (*n*, %)	0 (0.00)	0 (0.00)	NA

Abbreviations: CP, clinical pregnancy; ERT, endometrial receptivity test; NA, not available; RIF, recurrent implantation failure.

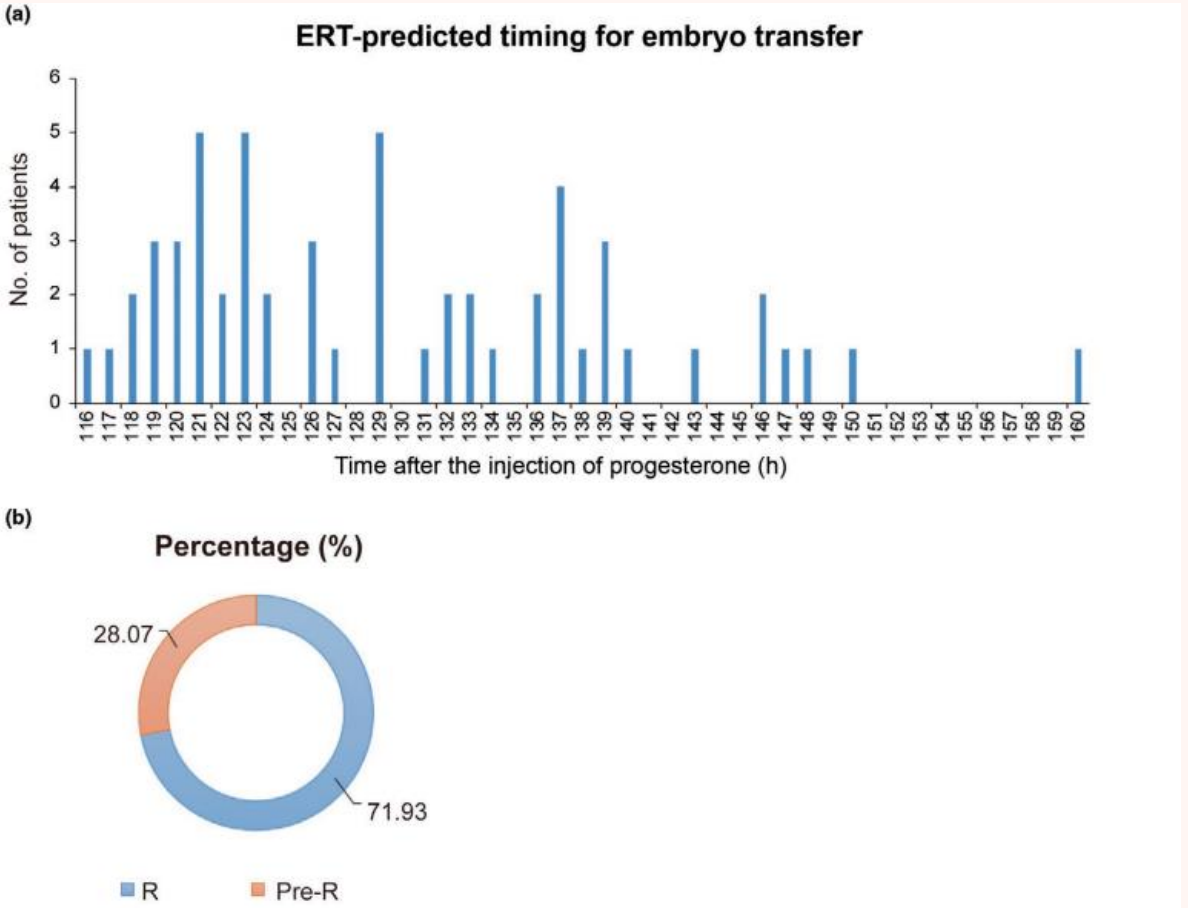
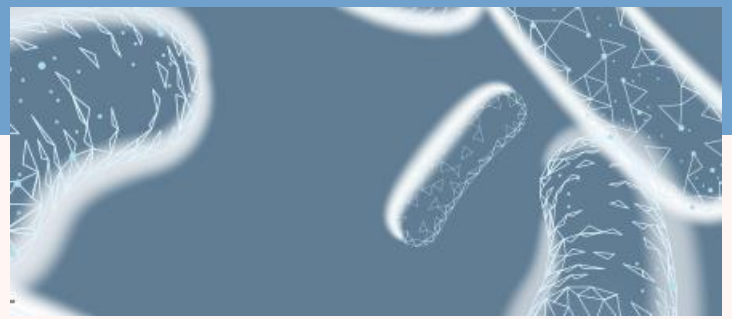


FIGURE 2 Endometrial receptivity test (ERT) results in patients with recurrent implantation failure (RIF). (a) After administration with progesterone, a predicted timing for embryo transfer was provided by ERT. The blue columns represent the number of patients; (b) red, percentage of pre-receptivity (pre-R); blue, percentage of receptivity (R).

It can be considered an **improvement over the ERA test** because it **focuses on fewer, more specific biomarkers, making the analysis faster, more streamlined, and easier to interpret**

Combined Endometrial Receptivity Test: Emma & Alice



EMMA

Endometrial Microbiome Metagenomic Analysis

Endometrial health is essential for a successful pregnancy.

A healthy endometrium needs the optimal amount of healthy bacteria.

EMMA evaluates your endometrial microbiome and provides information about the balance of your endometrial flora, improving your pregnancy prospects.

Patients with a pathogen-free endometrial microbiome are more likely to have successful outcomes such as live birth*.

Evaluation of the endometrial microbiome prior to transfer can offer an opportunity to further advance diagnosis and treatment strategies, and improve clinical outcomes*.

ALICE

Analysis of Infectious Chronic Endometritis

Chronic endometritis is one of the causes of infertility. This disease causes endometrial inflammation, with no visible symptoms in most cases.

Traditional diagnostic methods cannot accurately identify the infectious bacteria, and broad spectrum antibiotics are often prescribed.

ALICE detects the most frequent bacteria causing chronic endometritis allowing for the prescription of specific antibiotics and probiotics for successful treatment.

Chronic endometritis affects up to 30% of infertile patients. In cases of repeat implantation failure or recurrent pregnancy loss, this can rise to 66%.**

Identification and treatment of pathogens associated with chronic endometritis can significantly improve clinical outcomes such as implantation and live birth.

EMMA: Endometrial Microbiome Metagenomic Analysis
ALICE: Analysis Of Infectious Chronic Endometritis

Human Reproduction, Vol.31, No.4 pp. 795–803, 2016
Advanced Access publication on February 23, 2016 doi:10.1093/humrep/dew026

human reproduction — ORIGINAL ARTICLE *Infertility*

Abnormal vaginal microbiota may be associated with poor reproductive outcomes: a prospective study in IVF patients

T. Haahr[1], J.S. Jensen[2], L. Thomsen[1], L. Duus[3], K. Rygaard[4], and P. Humaidan[1,*]

[1]Faculty of Health, Aarhus University and the Fertility Clinic, Skive Regional Hospital, Skive, Denmark [2]Statens Serum Institute, Copenhagen, Denmark [3]Department of Clinical Microbiology, Regional Hospital Central and West Jutland, Denmark [4]Trianglen Fertility Clinic, Copenhagen, Denmark

*Correspondence address. The Fertility Clinic, Skive Regional Hospital, Faculty of Health, Aarhus University, Resenvej 25, 7800 Skive, Denmark. Tel: +4523815991; E-mail: peter.humaidan@midt.rm.dk

ALTERED GENITAL TRACT AND ENDOMETRIAL MICROBIOTA MAY NEGATIVELY AFFECT IMPLANTATION AND ART SUCCESS.

ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

Patient information	Sample information	Clinic information
Unique pat id:	Date received:	Clinic:
Patient name:	Report date/time:	Clinician:
Patient DOB:	Sample type:	
Allergy to antibiotics:	Cycle type:	
	Cycle day:	
	No. Biopsy:	
	Date of biopsy:	

RESULTS OF EMMA TEST:

LACTOBACILLUS		
BACTERIA	RESULT	OPTIMAL RESULT
Lactobacillus spp	Detected	At least one species detected
Lactobacillus crispatus	Not detected	
Lactobacillus gasseri	Not detected	
Lactobacillus iners	Detected	
Lactobacillus jensenii	Not detected	

BACTERIA OF THE REPRODUCTIVE TRACT			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
Actinomyces israelii	Not detected	N/A	Absent
Atopobium vaginae	Not detected	N/A	(0 - 3.58)
Bacteroides fragilis	Not detected	N/A	(0 - 3.57)
Bifidobacterium spp †	Not detected	N/A	(0 - 4.22)
Clostridium sordellii	Not detected	N/A	Absent
Fusobacterium nucleatum	Not detected	N/A	Absent
Gardnerella vaginalis	Detected	3.81*	(0 - 3.80)
Haemophilus ducreyi	Not detected	N/A	Absent
Mycobacterium tuberculosis	Not detected	N/A	Absent
Mobiluncus spp	Not detected	N/A	(0 - 3.57)
Peptostreptococcus anaerobius	Not detected	N/A	(0 - 3.57)
Porphyromonas asaccharolytica	Not detected	N/A	(0 - 3.57)
Prevotella bivia	Not detected	N/A	(0 - 3.57)
Prevotella disiens	Not detected	N/A	(0 - 3.57)
Sneathia spp	Not detected	N/A	(0 - 3.57)
Treponema pallidum	Not detected	N/A	Absent

RESULTS OF ALICE TEST:

BACTERIA RELATED TO CHRONIC ENDOMETRITIS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
Chlamydia trachomatis	Not detected	N/A	Absent
Enterococcus faecalis	Not detected	N/A	(0 - 3.57)
Escherichia coli	Not detected	N/A	(0 - 3.57)
Klebsiella pneumoniae	Not detected	N/A	(0 - 3.57)
Mycoplasma genitalium	Not detected	N/A	(0 - 3.57)
Mycoplasma hominis	Not detected	N/A	(0 - 3.57)
Neisseria gonorrhoeae	Not detected	N/A	Absent
Staphylococcus aureus	Not detected	N/A	(0 - 3.57)
Streptococcus agalactiae group B/ Streptococcus viridans	Not detected	N/A	(0 - 3.57)
Ureaplasma urealyticum	Not detected	N/A	(0 - 3.57)

†Bifidobacterium spp, when detected without other bacteria, could be displaced from its niche using probiotics.
*Values out of reference range.

RECOMMENDATION

The suggested treatment for the bacteria which DNA has been detected would be Metronidazole 500 mg/12h for 7 days orally, according to the standard Microbiology Guides (4,5). Subsequently, to recolonize the reproductive tract, it is suggested to use probiotics composed exclusively of Lactobacillus strains (preferably vaginal) following the manufacturer's instructions regarding dose and duration.

The analysis of a new biopsy is also recommended after treatment. The new sample must be taken following the standard test protocol.

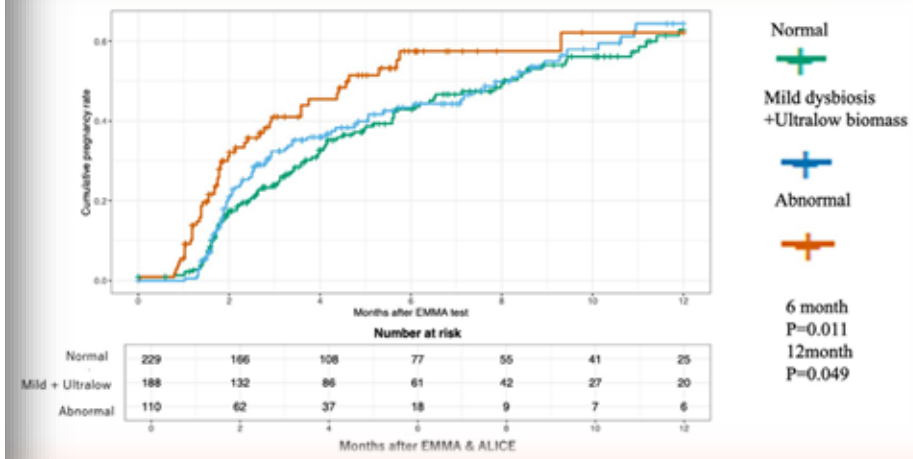
Received: 12 September 2024 | Accepted: 16 January 2025
DOI: 10.1002/rmb2.12634

ORIGINAL ARTICLE

Reproductive Medicine and Biology — WILEY

"Shortening time to pregnancy in infertile women by personalizing treatment of microbial imbalance through Emma & Alice: A multicenter prospective study"

Nanako Iwami[1] | Shinnosuke Komiya[2] | Yoshimasa Asada[3,4] | Kenichi Tatsumi[5] | Toshihiro Habara[6] | Takeshi Kuramoto[7] | Moritoshi Seki[8] | Hiroaki Yoshida[9] | Kazuhiro Takeuchi[10] | Masahide Shiotani[11] | Tetsunori Mukaida[12] | Yasushi Odawara[13] | Yasuyuki Mio[14] | Hirobumi Kamiya[1]



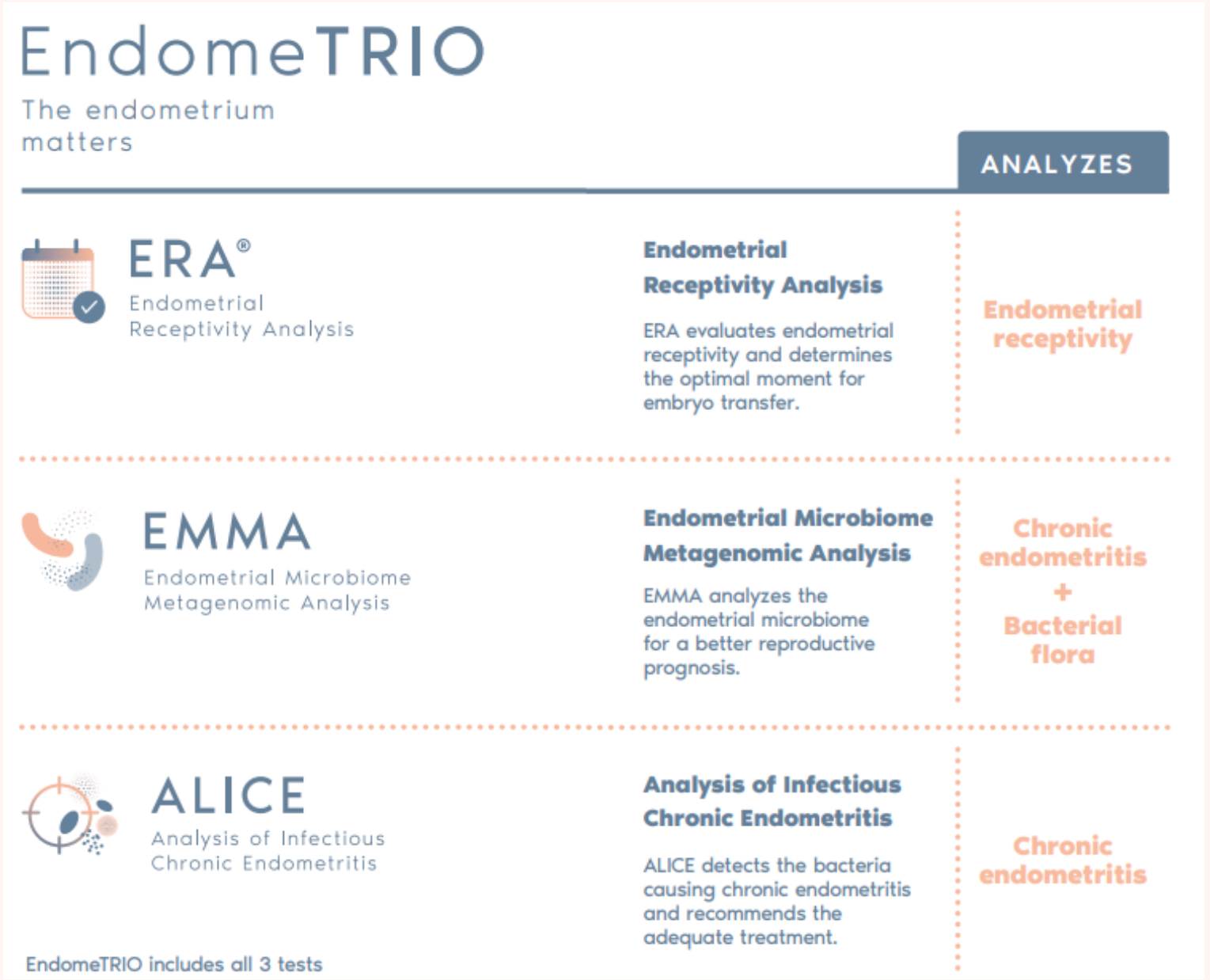
LIMITS

- Does **not** assess antibiotic resistance.
- Normal microbiota thresholds (e.g., % Lactobacillus) are still under investigation.
- Not a direct test of **endometrial receptivity** (ERA is used for that).

A multicenter cohort (527 women with RIF/RPL) showed higher ongoing pregnancy rates after microbiome-guided therapy based on EMMA/ALICE results.

- ***Identifies microbial imbalance that can impair implantation.***
- ***Facilitates tailored treatments improving uterine environment.***

A ENDOMETRIO: *EMMA & ALICE & ERA*



THE BIOPSY IS PERFORMED AT A SPECIFIC MOMENT IN THE CYCLE (**OFTEN 5 DAYS AFTER STARTING PROGESTERONE ADMINISTRATION IN A REPLACEMENT CYCLE**), SIMILAR TO WHAT HAPPENS WITH THE ERA TEST ALONE.

- **HOLISTIC APPROACH**: PROVIDES A MORE COMPREHENSIVE DIAGNOSIS COMPARED TO EVALUATING ONLY THE WOI GENE EXPRESSION.
- **TARGETED TREATMENT**: THE RESULTS GUIDE SPECIFIC THERAPY FOR THE DAY OF THE TRANSFER AND FOR TREATING ANY INFECTIONS/DYSBIOSIS.

Clinical Opinion August 2023 AJOG Global Reports ajog.org

Endometrial receptivity tests in reproduction: a SWOT analysis

Juan A. Garcia-Velasco, MD, PhD; Joaquín Llácer, MD, PhD; Antonio Requena, MD, PhD; Miguel Ángel Checa, MD, PhD; José Bellver, MD, PhD; Ernesto Bosch, MD, PhD; Juan José Espinós, MD, PhD; Francisco Fabregues, MD, PhD; Ana Isabel Ortega, PharmD, PhD; Juan Fontes, MD, PhD; On behalf of the Spanish Infertility SWOT Group

STRENGTHS
WOI Personalization
Physiological plausibility
Accuracy of current technology (NGS)
Could improve the cPR
Provide more information about the tissue than P4 plasma levels
Contributes to discriminate situations of endometrial pathology and allows to perform other studies (CE / Dysbiosis)

OPPORTUNITIES
Increase the knowledge of new phenotypes
Enable the development of non-invasive tests
Expand gene panel
Easy implementation in clinical practice
Probable benefit in a correct profile of patients
Decrease drop out rate
Learn about the interaction with other pathologies that affect implantation (dysbiosis, endometriosis, etc.)
Development of adequate guidelines that specify the indication of ERt.

WEAKNESS
Low reproducibility inter- and intra-cycle
Commercial interest / Bias conflict of interest
Inconsistencies about their clinical benefit in different groups of patients
Worse PR
Partial assessment of IF/ Not report functionality.
Methodological heterogeneity
No correlation with P4
Invasive test
Costs and time-consuming
Force to Freeze All
Overdiagnosis – overtreatment

THREATS
Marketing prior to validation
Development of other tests
Studies +/- 1 day of P4 do not alter the results
Failure to address other causes of IF
Comorbidities can distort test results
False expectations to the patient
Learn the interaction with other pathologies that affect implantation (Microbiota/CE)
Ethical difficulty to validate these studies
Demand from patients and professionals
Regulation of diagnostic tests with limited evidence

There are still no definite indications on whether its inclusion in the study of the infertile couple or the study of patients with repeated implantation failure is essential.

OXFORD human reproduction

Human Reproduction, 2023, 38(11), 2062–2104
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ESHRE Pages

Good practice recommendations on add-ons in reproductive medicine†

ESHRE Add-ons working group: K. Lundin[1], J.G. Bentzen[2], G. Bozdag[3], T. Ebner[4], J. Harper[5], N. Le Clef[6], A. Moffett[7], S. Norcross[8], N.P. Polyzos[9], S. Rautakallio-Hokkanen[10], I. Sfontouris[11], K. Sermon[12], N. Vermeulen[6], and A. Pinborg[2,*]

Good practice recommendations on add-ons in reproductive medicine

ESHRE aimed to develop clinically relevant and evidence-based recommendations focusing on the safety and efficacy of add-ons currently used in fertility procedures. The multidisciplinary working group formulated 42 recommendations across three sections:

- diagnosis and diagnostic tests,
- laboratory tests and interventions and
- clinical management.

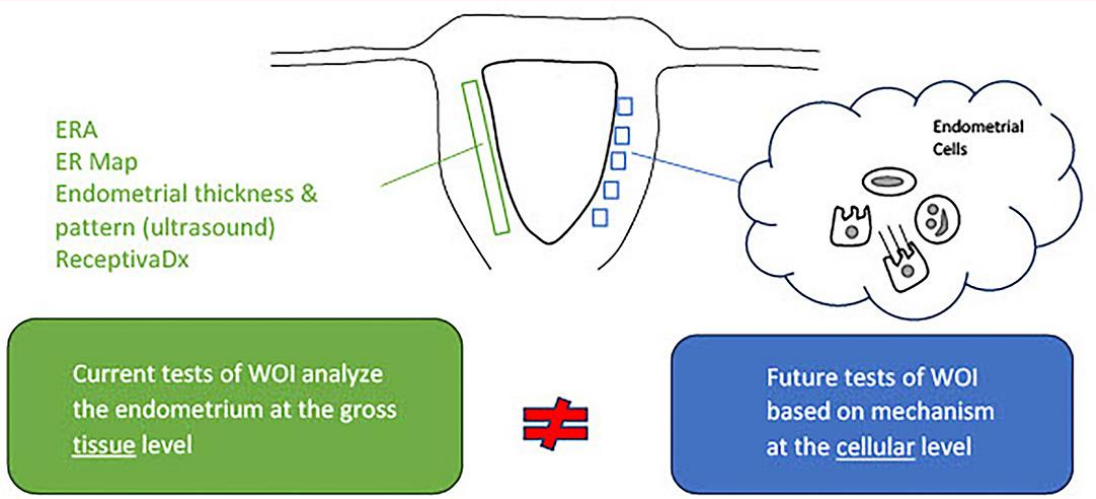
These recommendations offer valuable direction for healthcare professionals who are responsible for the care of patients undergoing ART treatment for infertility. Their purpose is to promote safe and effective ART treatment, enabling patients to make informed decisions based on realistic expectations. The good practice recommendations aim to ensure that patients are fully informed about the various treatment options available to them and the likelihood of any additional treatment or test to improve the chance of achieving a live birth.

Table 2. Overview of all recommendations on diagnosis and diagnostic tests with their level of evidence, benefit versus harm and other considerations that contributed to their formulation.

Intervention		Benefits versus harms (efficacy versus safety)	Level of evidence for efficacy (LBR/CPR)[1]	Level of evidence for safety[1]	Considerations	Recommendation
Screening hysteroscopy		Unselected patients: no benefit on LBR RIF: might be beneficial effect on LBR No evidence of an effect on miscarriage rate Complications are minimal	⊕⊕◯◯	⊕⊕◯◯	/	Screening hysteroscopy is **currently not recommended for routine clinical use**. Screening hysteroscopy **can be considered** in patients with recurrent implantation failure.
Endometrial receptivity tests		No effect on LBR, inconclusive effect on cLBR No data on safety, biopsy procedure can be painful	⊕⊕◯◯	No data	Clinical and methodological heterogeneity in patient populations (number of previously failed cycles), reported comparisons and unit of analysis (per couple or per cycle)	The presently available endometrial receptivity tests are **not recommended**.
Immunology tests and treatments	Immunology tests	Benefit on LBR or miscarriage rate is unclear due to lack of understanding of the mechanisms Harms: misinformation	⊕◯◯◯	No data	No rationale for these tests, no standardization	Peripheral blood tests for immune parameters and uNK-cell testing are **not recommended**. KIR and HLA genotyping is **currently not recommended for routine clinical use**.
	Immunology treatments	Benefit on LBR and miscarriage rate are unclear Significant safety concerns	⊕⊕◯◯	No data	No rationale for these treatments, no standardization	Immunomodulating treatments, such as Intralipid, IVIG, rh-LIF, PBMCs, and anti-TNF, are **not recommended**.

[1] Quality of Evidence Grades: ⊕⊕⊕⊕, body of evidence is of high quality (at least evidence from RCTs); ⊕⊕⊕◯, body of evidence is of moderate quality (evidence from RCTs or a number of observational studies showing a similar large effect); ⊕⊕◯◯, body of evidence is of low quality (mainly observational data); ⊕◯◯◯, body of evidence is of very low quality (few observational data).
cLBR: cumulative live birth rate; IVIG: intravenous immunoglobulin infusion; rhLIF: recombinant human leukaemia inhibitory factor; PBMC: peripheral blood mononuclear cell; PGT-A: preimplantation genetic testing for aneuploidy; RIF: repeated implantation failure; RCT: randomized controlled trial; KIR: killer-cell immunoglobulin-like receptor; uNK: uterine natural killer cells; TNF: tumour necrosis factor; PBMC: peripheral blood mononuclear cell.

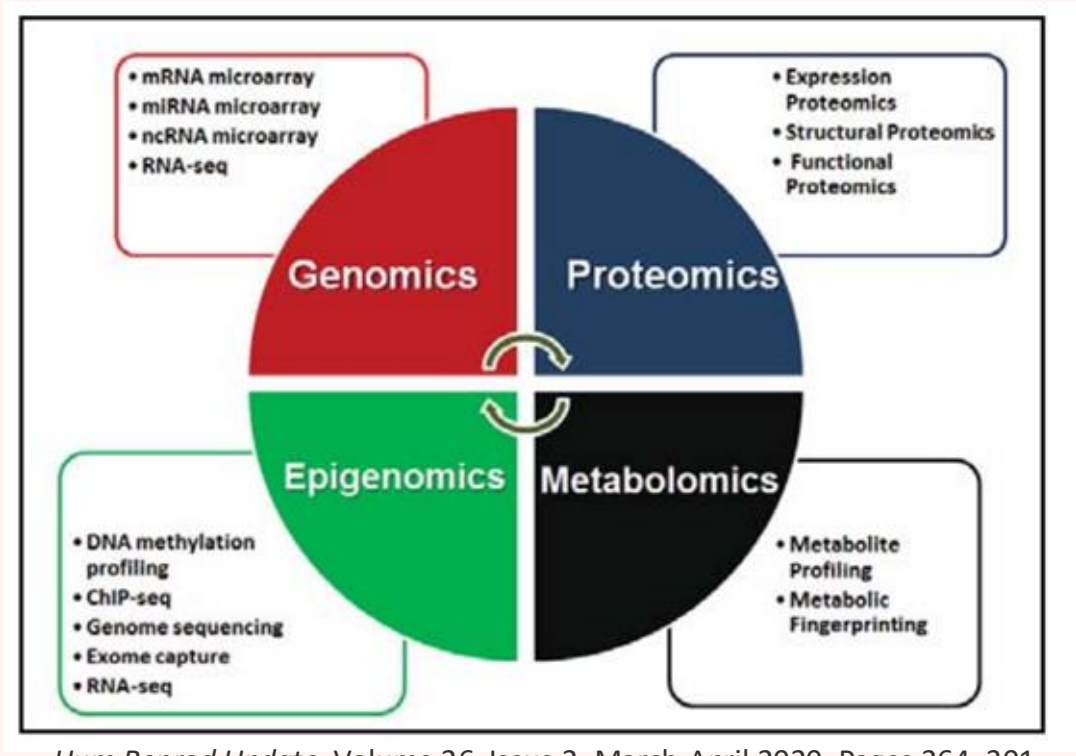
https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Addons



FUTURE PERSPECTIVES IN MEASURING ENDOMETRIAL RECEPTIVITY & EMBRYO IMPLANTATION

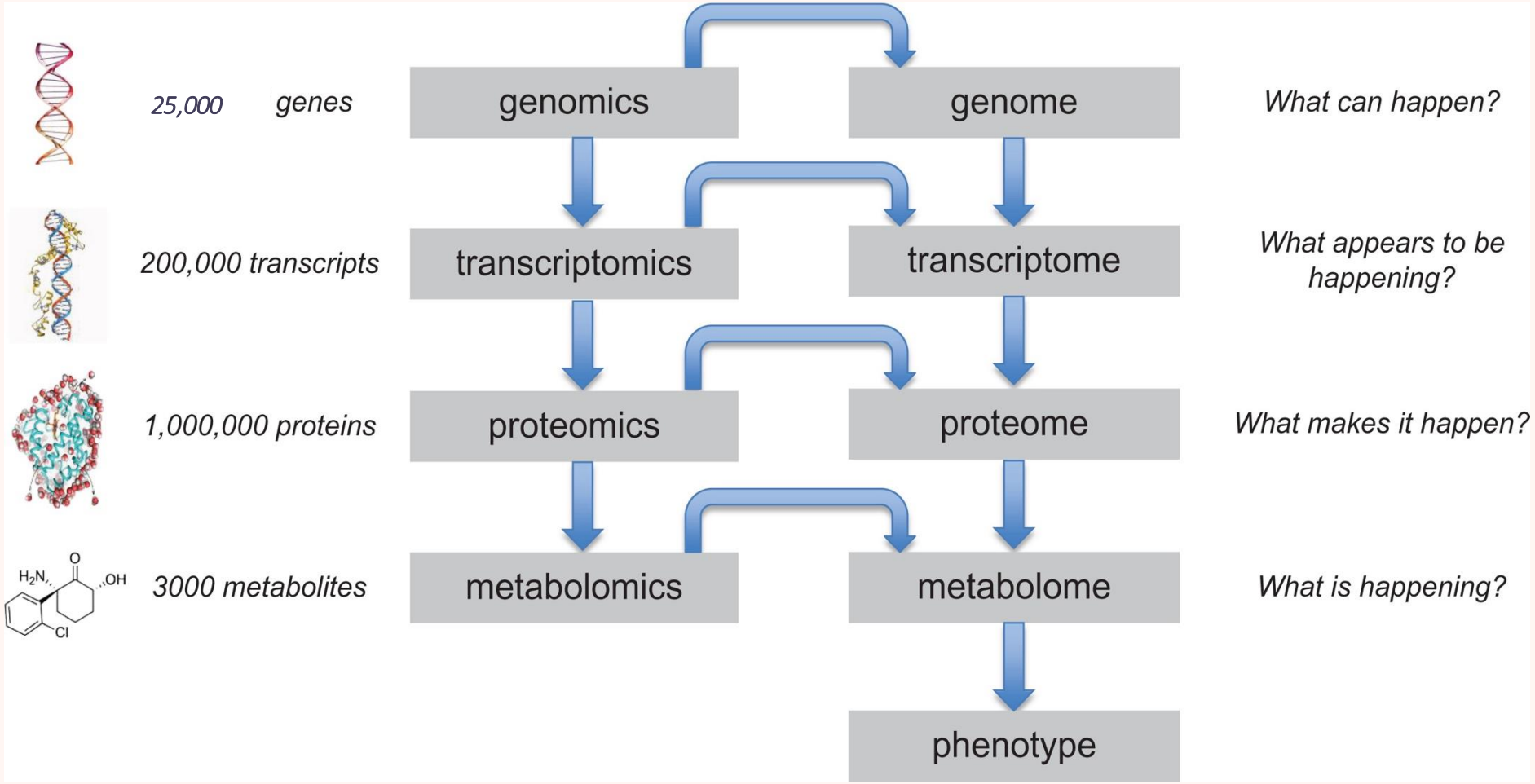
1. OMICS
2. Extracellular vesicles
3. 3D in vitro model

From omics to systems biology in deciphering the complexity of biological systems.



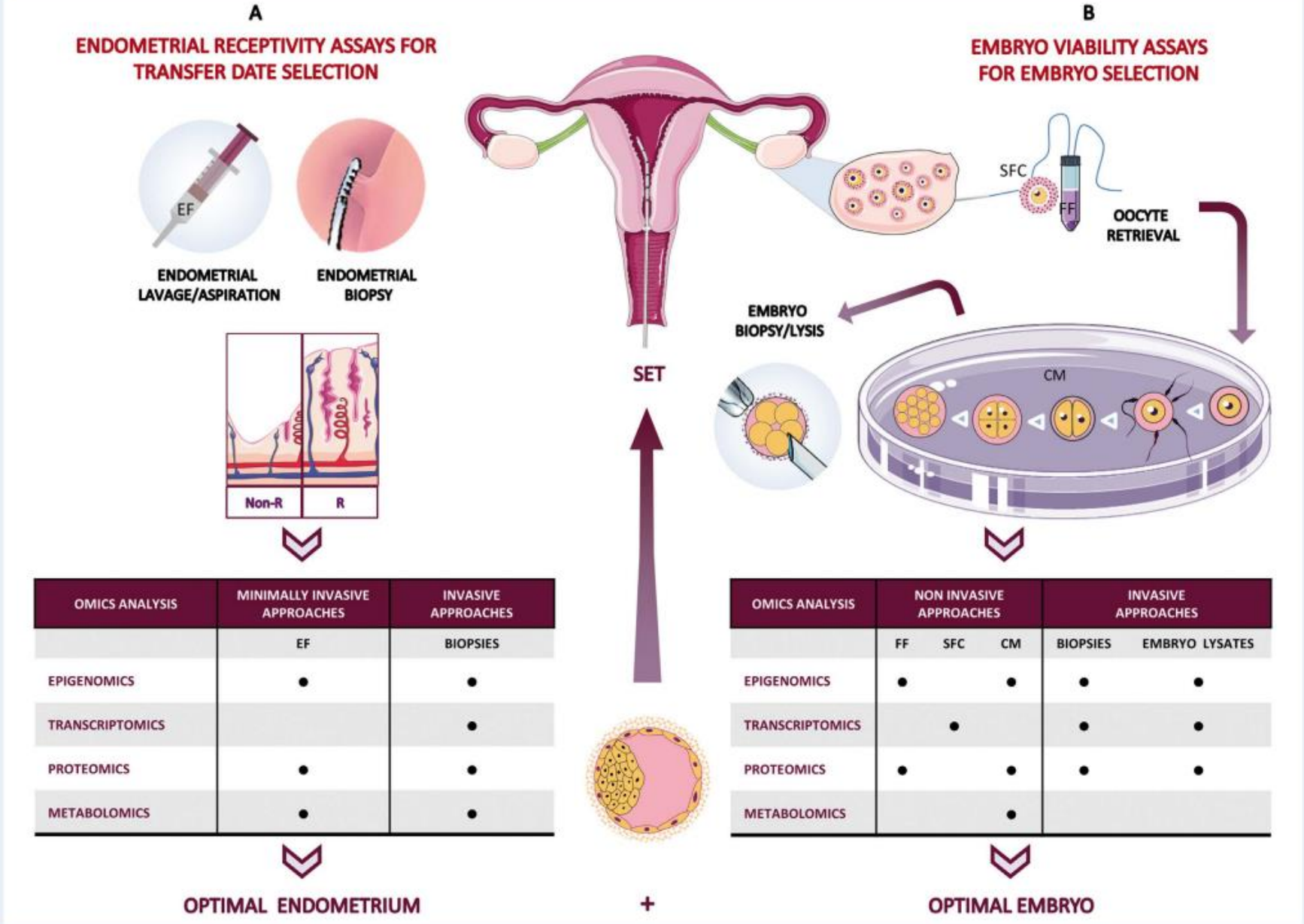
Hum Reprod Update, Volume 26, Issue 2, March-April 2020, Pages 264–301

THE ‘OMICS’ CASCADE DESCRIBES THE FLOW OF BIOLOGICAL INFORMATION IN AN ORGANISM.

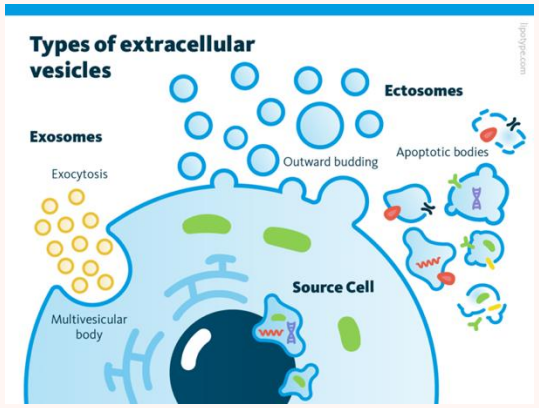


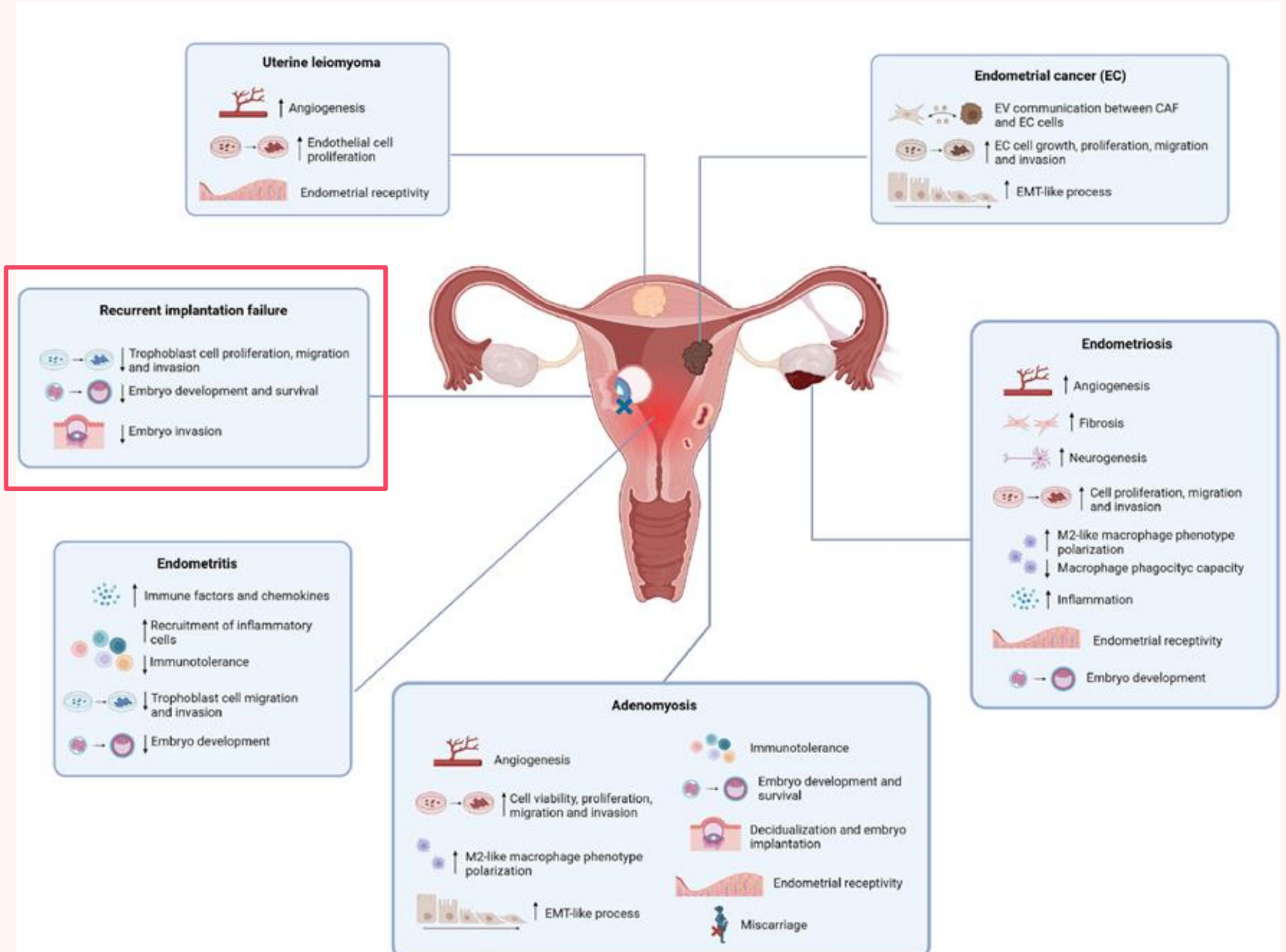
Hum Reprod Update, Volume 23, Issue 6, November-December 2017, Pages 723–736

- ✓ *ENDOMETRIAL BIOPSIES OR ENDOMETRIAL FLUID help identify the MOLECULAR SIGNATURE OF ENDOMETRIAL RECEPTIVITY, allowing prediction of the optimal window for embryo transfer.*
- ✓ *OMICS TECHNOLOGIES APPLIED TO EMBRYOS (via biopsies or lysates) and to clinically discarded samples — such as follicular fluid, somatic follicular cells, or embryo culture media — reveal gene expression profiles linked to embryo viability.*
- ✓ *These invasive and non-invasive assessments can provide objective biomarkers and may be used alone or combined with traditional methods to select the best embryo for single embryo transfer (SET).*



THE ROLE OF UTERINE EVs IN PATHOLOGICAL CONDITIONS AND ASSOCIATED INFERTILITY.

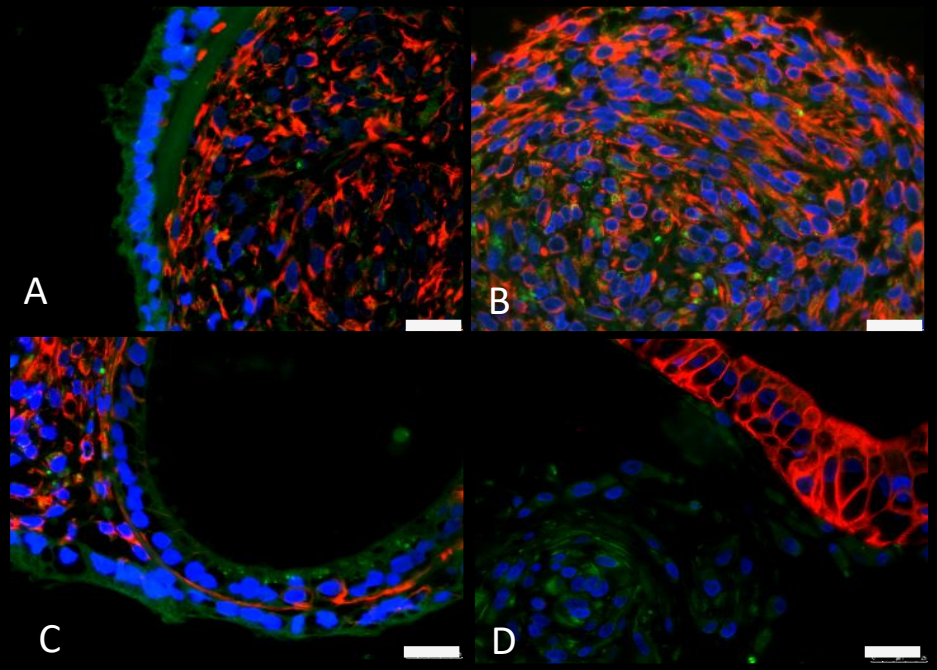




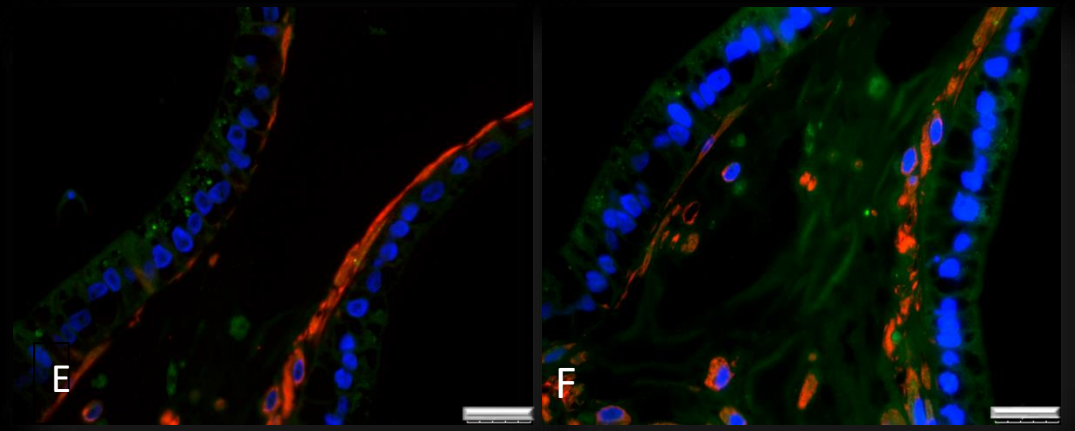
3D MODEL SUCCESSFULLY GENERATED WITH STROMAL AND EPITHELIAL PRIMARY CELLS: static conditions

Organoids Formation and characterization

Menstrual Blood



Endometrial Biopsy



(A-B-C) Epithelial (CK19) Stromal cells (Vimentin)
(D-E-F) Epithelial and Stromal Nuclei (DAPI)
Scale bar 25 µm

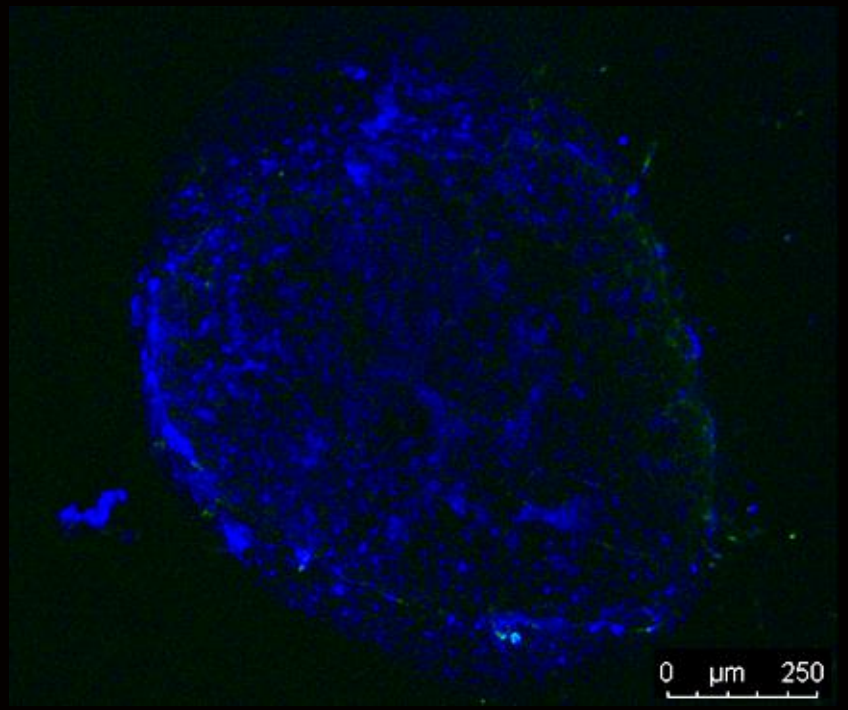
Luongo et al., Unpublished

Droplets with stromal and epithelial cells → THESC´s + EM42: dynamics conditions

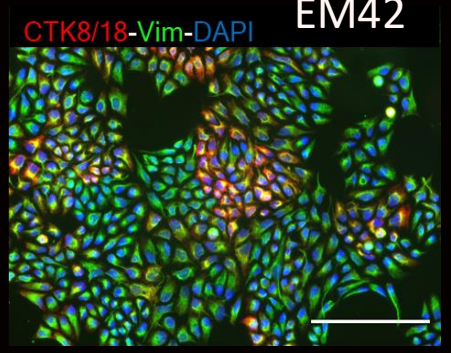
Cytokeratin 8/18
Epithelial cells

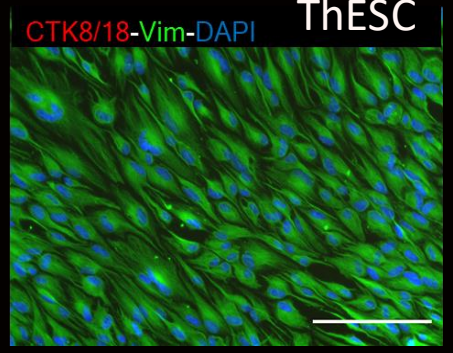
Vimentin
Stromal cells

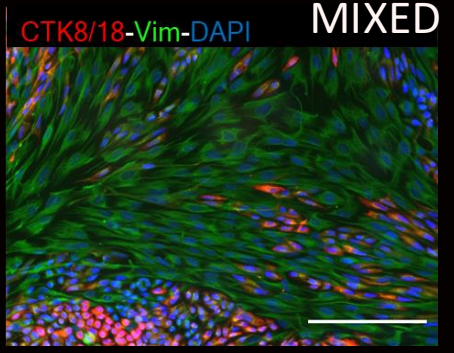
Nuclei



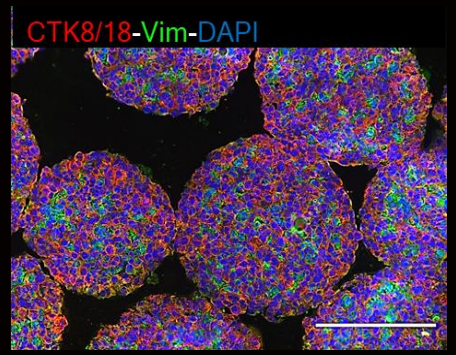
2D

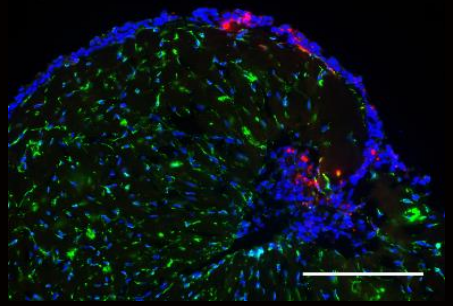


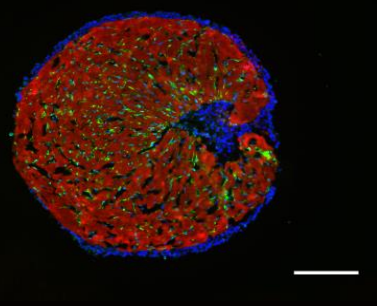




3D







Scale 200µm

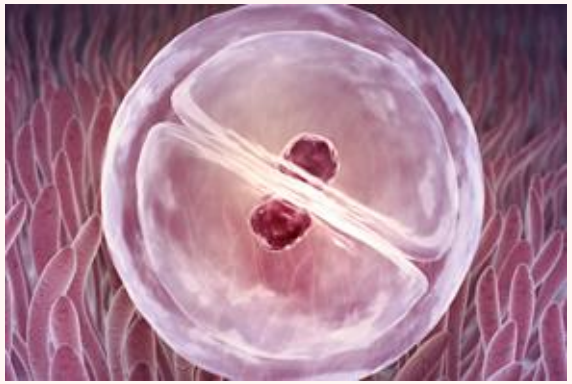
✓ There are some ready approaches to measure endometrial receptivity

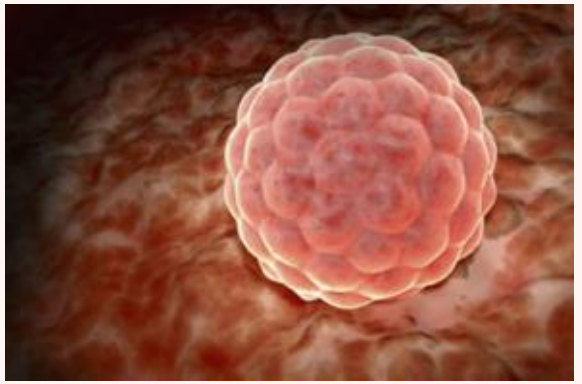
➡ **THEIR EFFECTIVENESS IS TO DATE STILL DEBATED**

✓ Others –OMICS strategies are far from the clinical practice

✓ Uterine extracellular vesicles in pathological conditions and associated infertility
✓ 3D Organoids/Assembloids in static or dynamic conditions

➡ **THEY RECAPITULATE FEATURES OF UTERINE MICROENVIRONMENT AND REPRESENT A POWERFUL TOOL FOR ENDOMETRIAL EVALUATION**





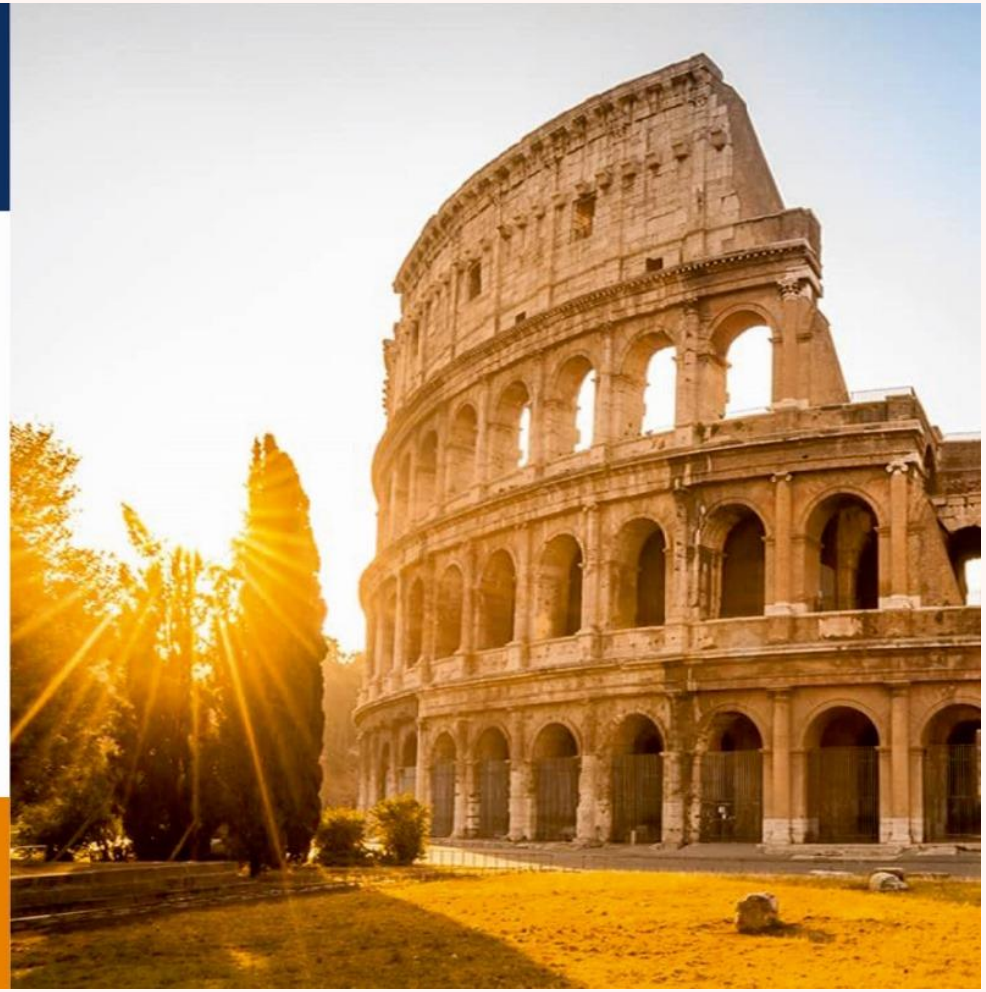
ISIVF International Society for In Vitro Fertilization

S.I.R.U. Società Italiana della Riproduzione Umana

CECOS ITALIA

23rd World Congress on In Vitro Fertilization

Rome, 6 – 8 May 2027



Save the date