The impact of endometriosis on oocyte and endometrium in ART

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Sistema Socio Sanitario Regione Lombardia

Conflicts of interest

Personal COIs:

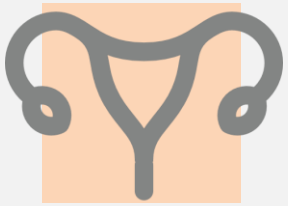
- 2019–2022: Editorial Board Involvement (*Reprod Sci*, *PloS One*, *Reprod Biol Endocrinol*)
  - Speaker consultancy fees (2019, n=1; 20210 n=2)
  - Reviewer honorarium (2021, n=1)

Institutional COIs:

- 2019-2022: Grants (competitive and non-competitive) for research activity
  - Theramex, 2019
  - Italian Ministry of Health, 2020

Content

1. Oocyte
    1. Observational/biological studies
    2. Meta-analyses on IVF outcomes
    3. Clinical studies

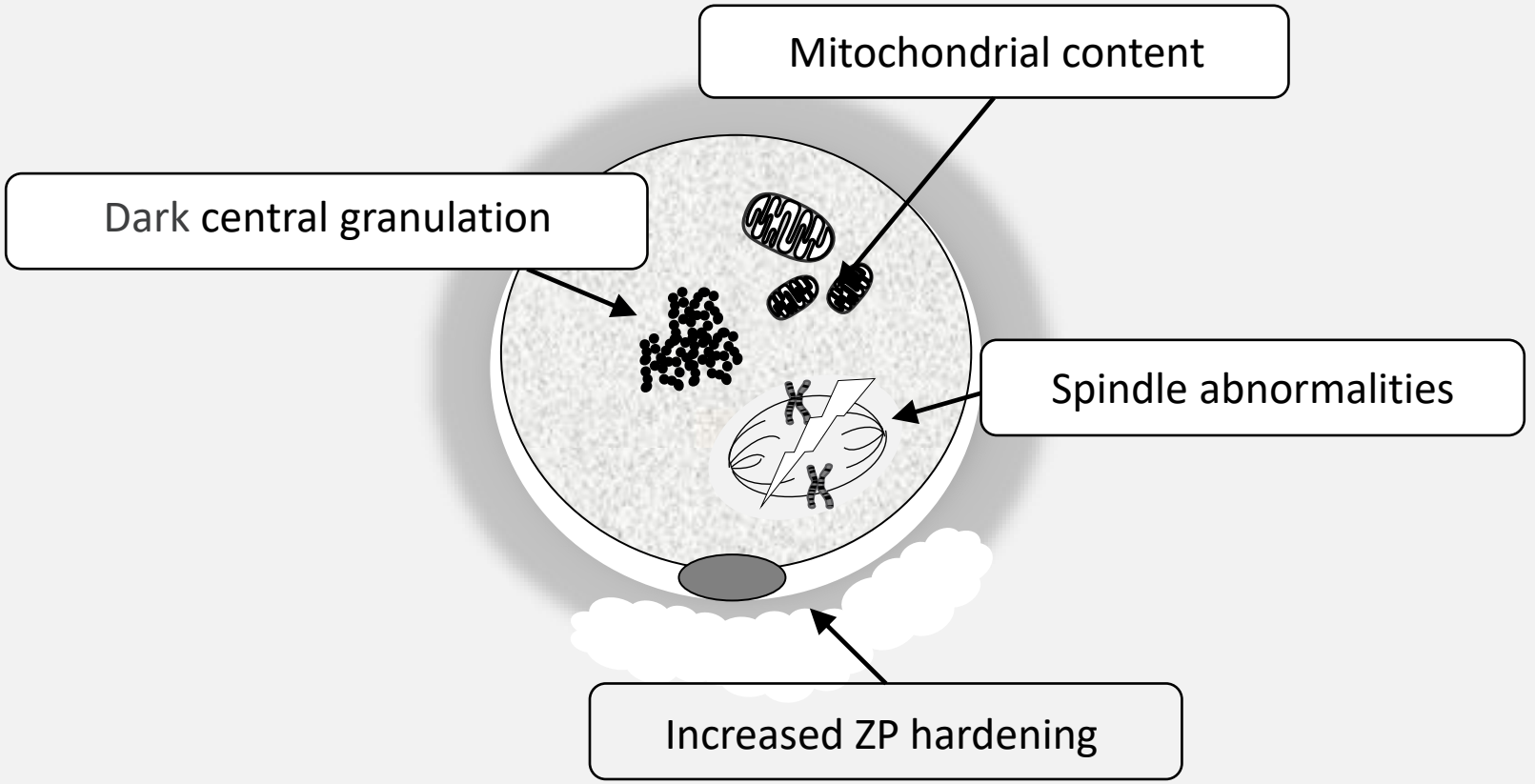


2. Endometrium
    1. Observational/biological studies
    2. Prospective study on endometrial receptivity gene signature
    3. Clinical studies on oocyte donation experience (recipient women)

Effect of endometriosis on the oocyte

1. Oocyte
    1. Observational/biological studies
    2. Meta-analyses on IVF outcomes
    3. Clinical studies

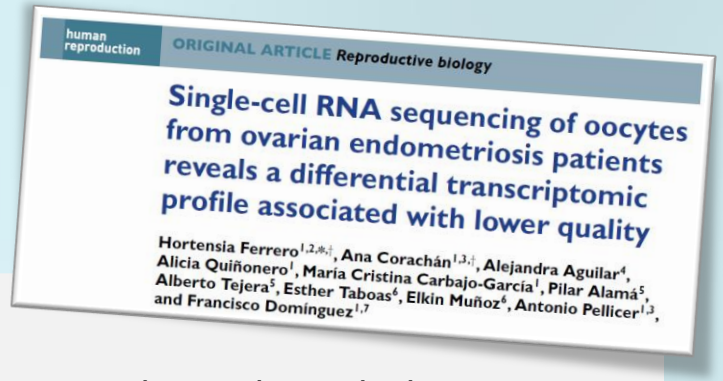
Oocyte quality: observational/biological studies



ZP, zona pellucida

Reviewed in Sanchez AM, et al. J Ovarian Res 2017;10:43

Oocyte quality: Single-cell RNA sequencing of oocytes from ovarian endometriosis patients

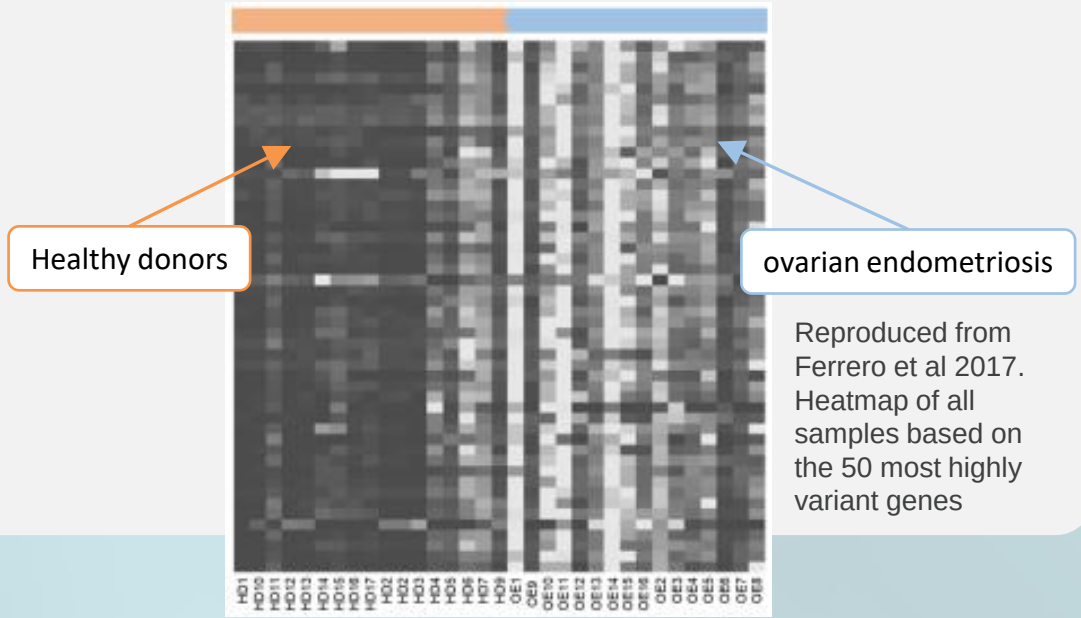

human reproduction ORIGINAL ARTICLE Reproductive biology

Single-cell RNA sequencing of oocytes from ovarian endometriosis patients reveals a differential transcriptomic profile associated with lower quality

Hortensia Ferrero[1,2,*,†], Ana Corachán[1,3,†], Alejandra Aguilar[4], Alicia Quiñonero[1], María Cristina Carbajo-García[1], Pilar Alamá[5], Alberto Tejera[5], Esther Taboas[6], Elkin Muñoz[6], Antonio Pellicer[1,3], and Francisco Domínguez[1,7]

Assigned functional group	Function name	Benjamini
Steroid metabolism	Cholesterol metabolism	0,000821492
	Lipid biosynthesis	0,001743372
	Sterol metabolism	0,001801183
	Steroid metabolism	0,002077042
	Cholesterol biosynthetic process	0,0405753
	Cholesterol biosynthesis	0,003090741
	Sterol biosynthesis	0,009368646
	Steroid biosynthesis	0,029363838
	Lipid metabolism	0,074815102
Response to oxidative stress	Response to drug	0,085548635
	Response to oxidative stress	0,039106294
	Cellular oxidant detoxification	0,057279013
	Oxirreductase	0,00736925
	Oxidation-reduction process	0,084755546
	Peroxidase	0,009368646
Cell growth regulation	Ubl conjugation	0,001047052
	Insulin-like growth factor-binding protein, IGFBP	0,007526397
	Growth factor binding	0,020426485
	Alternative initiation	0,022846071
	Apoptosis	0,05399448
	Citoskeleton	0,073967873
	Cell cycle	0,072882907
	Positive regulation of osteoblast differentiation	0,074615003
	Cell division	0,09631798
Mitochondrion	Mitochondrion	0,023868583
Others	Methylation	0,027900288
	Angiogenesis	0,054486575

- MII oocytes (n = 16) from ovarian endometriosis patients (n = 7) vs
- MII oocytes (n = 16) from healthy egg donors (n = 5)



Reproduced from Ferrero et al 2017. Heatmap of all samples based on the 50 most highly variant genes

MII, metaphase II

Ferrero H, et al. Hum Reprod 2019;34:1302–12

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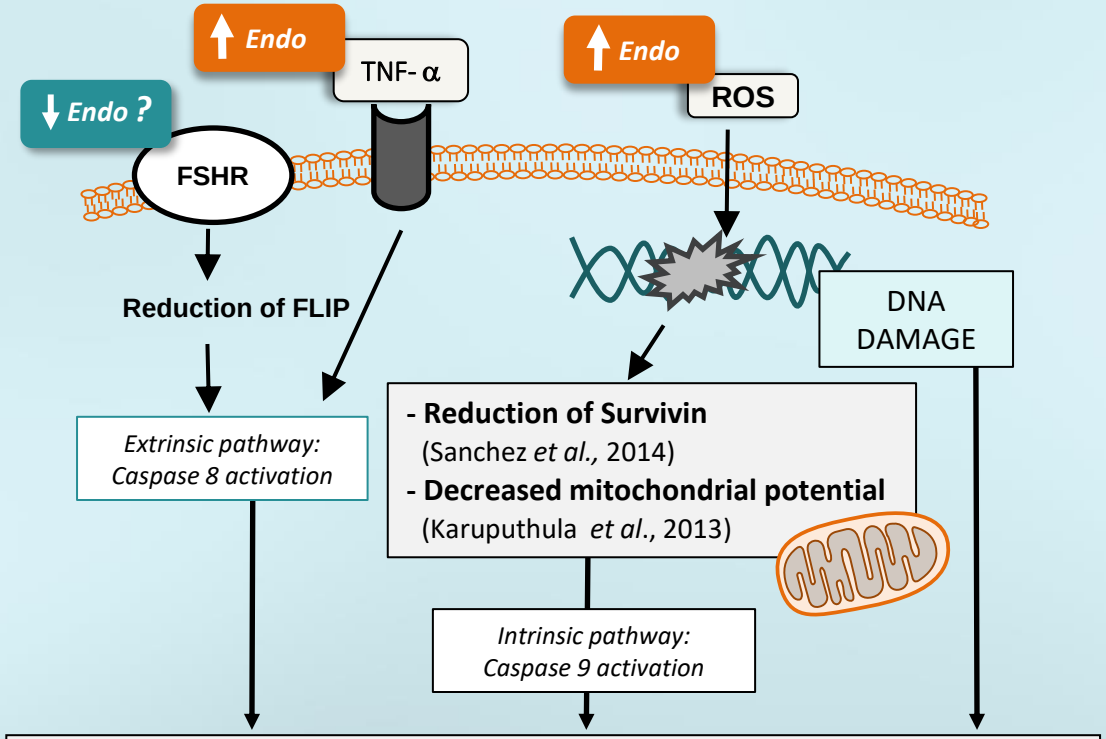
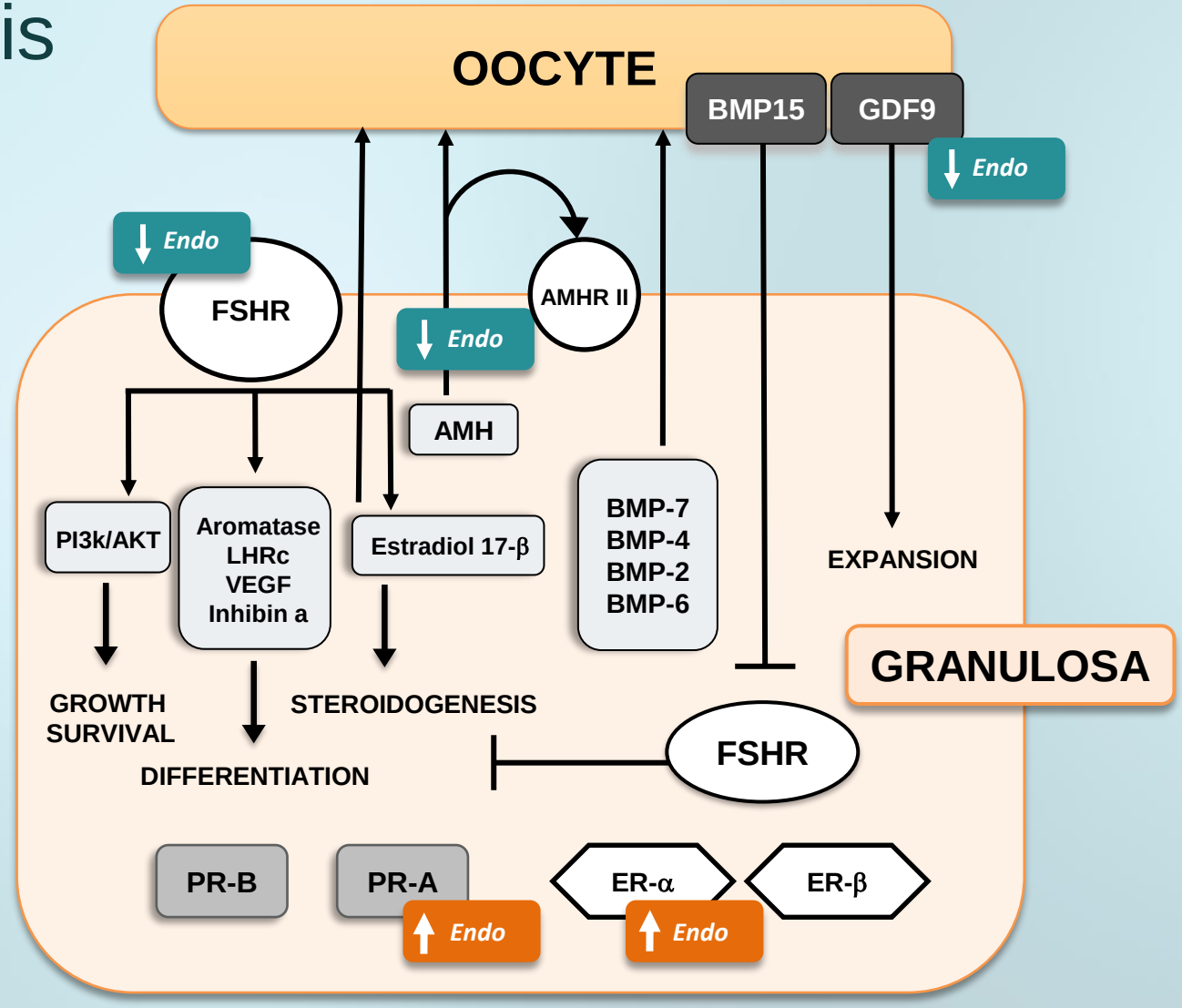
Granulosa cells in endometriosis





Reviewed in Sanchez AM, et al. J Steroid Biochem Mol Biol 2016;155(Pt A):35–46

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Meta-analyses: number of retrieved oocytes

Meta-analysis	Endometriosis groups[a]	OUTCOME: NUMBER OF OOCYTES RETRIEVED	
		Studies included (n)	Endometriosis vs controls Odds ratio (95% CI)
Barnhart, 2002	**Overall**	**22**	**0.92 (0.85-0.99)[a]**
	Stage I-II		0.56 (0.49-0.65)[a]
	Stage III-IV		0.94 (0.91-0.98)[a]
Yang, 2015	Untreated endometrioma	9	Mean difference -1.5 (−2.84 to −0.15)[a]
Rossi, 2016	**Overall**	**9**	**-1.93 (-3.67 to −0.18)[a]**
	Stage I-II	4	-0.16 (-0.85 to 0.52)
	Stage III-IV	6	-2.96 (-4.72 to -1.19)[a]
	Treated disease (surgery)	8	-2.11 (-4.04 to -0.19)[a]
	Untreated disease	2	-0.50 (-1.56 to 0.56)
	Endometrioma	3	-2.47 (-3.31 to -1.63)[a]
Alshehre, 2021	Untreated endometrioma	8	Mean difference -2.25 (3.43 to −1.06)[a]

[a]Statistically significant

Barnhart K, et al. Fertil Steril 2002;77:1148–55; Yang C, et al. Reprod Biomed Online 2015;31:9–19;
Rossi AC, Prefumo F. Arch Gynecol Obstet 2016;294:647–55; Alshehre SM, et al. Arch Gynecol Obstet 2021;303:3–16

Results from meta-analyses providing insights on the effect of endometriosis on oocyte competence

Meta-analysis	Endometriosis groups[a]	OUTCOME: MII OOCYTES RETRIEVED		OUTCOME: FERTILIZATION RATE	
		Studies included (n)	Endometriosis vs controls (95% CI)	Studies included (n)	Endometriosis vs controls (95% CI)
Barnhart, 2002	**Overall** Stage I-II Stage III-IV			**Unknown** Unknown Unknown	**OR 0.81 (0.79 to 0.83)[a]** OR 0.94 (0.93 to 0.96)[a] OR 1.54 (1.39 to 1.70)[a]
Harb, 2013	Untreated Stage I-II Untreated Stage III-IV			7 3	RR 0.93 (0.87 to 0.99)[a] RR 1.01 (0.93 to 1.10)
Yang, 2015	Untreated endometrioma	2	MD -3.61 (-4.44 to -2.78)[a]	2	OR 1.06 (0.71 to 1.60)[b]
Rossi, 2016	**Overall** Stage I-II Stage III-IV Treated (surgery) Untreated disease Endometrioma	**4** 2 3 3 1 2	**OR -1.22 (-2.38 to -0.06)[a]** OR -0.55 (-1.34 to 0.25) OR -0.83 (-1.73 to 0.08) OR -1.62 (-3.31 to 0.07) OR -0.50 (-1.59 to 0.59) OR -2.48 (-4.43 to -0.53)[a]		
Alshehre, 2021	Untreated endometrioma	4	MD -4.64 (5.65 to -3.63)[a]		

[a]Statistically significant; [b]Control group: contra-lateral healthy ovary. MD, mean difference; OR, odds ratio; RR, relative risk

Reviewed in Sanchez AM, et al. J Ovarian Res 2017;10:43. ***Refs:*** Barnhart K, et al. Fertil Steril 2002;77:1148–55; Harb HM, et al. BJOG 2013;120:1308–20; Yang C, et al. Reprod Biomed Online 2015;31:9–19; Rossi AC, Prefumo F. Arch Gynecol Obstet 2016;294:647–55; Alshehre SM, et al. Arch Gynecol Obstet 2021;303:3–16

Oocyte competence: fertilization rate in conventional IVF cycles

Table 1: Baseline characteristics of the study groups

Characteristics	Endometriosis n=157	Controls n=157	p
Age (years)	35 [32 - 37]	35 [32 - 37]	0.89
BMI (Kg/m2)	20.8 [19.5 - 22.7]	21.6 [19.5 - 24.6]	0.05
FSH (IU/ml)	6.7 [5.6 - 8.6]	6.8 [5.8 - 8.0]	0.68
AFC	12 [7 - 16]	11 [8 - 16]	0.72
Duration of infertility (years)	2.0 [1.5 - 3.5]	3.0 [3.0 - 5.0]	< 0.05
Previous deliveries	6 (4%)	10 (6%)	0.44
Previous IVF cycles	20 (13%)	30 (19%)	0.17
Indications to IVF			< 0.05
Endometriosis	157 (100%)	-	
Unexplained	-	109 (69%)	
Tubal factor	-	17 (11%)	
Ovulatory disorder	-	15 (10%)	
Reduced ovarian reserve	-	16 (10%)	
Total number of retrieved oocytes	6 [3 - 11]	6 [4 - 11]	0.90

Data are reported as median [interquartile range] or number (percentage)

Table 2. Basal characteristics of the groups considered: male partner's semen characteristics

Characteristics	Endometriosis n=157	Controls n=157	p
Indications to IVF			1.00
No male factor	149 (95%)	149 (95%)	
Mild male factor	8 (5%)	8 (5%)	
Seme characteristics			
Volume	2.7 [2.0 - 3.5]	2.9 [2.0 - 4.0]	0.33
Basal number/ml	66 [44 - 102]	58 [39 - 92]	0.08
Basal progressive motility (%)	47 [41 - 55]	48 [40 - 55]	0.98
Number/ml after gradient	10 [4 - 25]	10 [5 - 24]	0.71
Progressive motility (%) after gradient	94 [91 - 96]	95 [92 - 97]	0.19

Data are reported as median [interquartile range] or number (percentage)

Viganò P, et al. Manuscript in preparation

Oocyte competence: fertilization rate in conventional IVF cycles

Table 4: Main ART outcomes after c.IVF in patients with and without endometriosis

Characteristics	Endometriosis n=157	Controls n=157	p
Total fertilization failure rate	4 (3%)	6 (4%)	0.75
Fertilization rate	77.7 [60.0 - 100.0]	75.0 [55.6 - 90.0]	0.24
Number of cleavage stage embryos	3 [2 - 6]	3 [2 - 6]	0.79
Number of TOP embryos	2 [1 - 4]	1 [0 - 2]	< 0.05
Number of viable embryos obtained	6 (4%)	9 (6%)	0.60
Number of blastocysts	1 [0 - 2]	0 [0 - 2]	0.13
Number of TOP blastocysts	0 [0 - 2]	0 [0 - 1]	0.11
Fresh transfer performed			0.42
at cleavage stage	96 (86%)	104 (90%)	
at blastocyst stage	16 (14%)	12 (10%)	
Clinical pregnancy rate in fresh embryo transfer	42 (37%)	33 (28%)	0.21
Subsequent cryopreserved embryo transfer	67 (43%)	78 (50%)	0.26
Cumulative pregnancy rate/retrieval	86 (55%)	69 (44%)	0.07
Cumulative live births/retrieval	81 (52%)	65 (41%)	0.09

Data are reported as median [interquartile range] or number (percentage)

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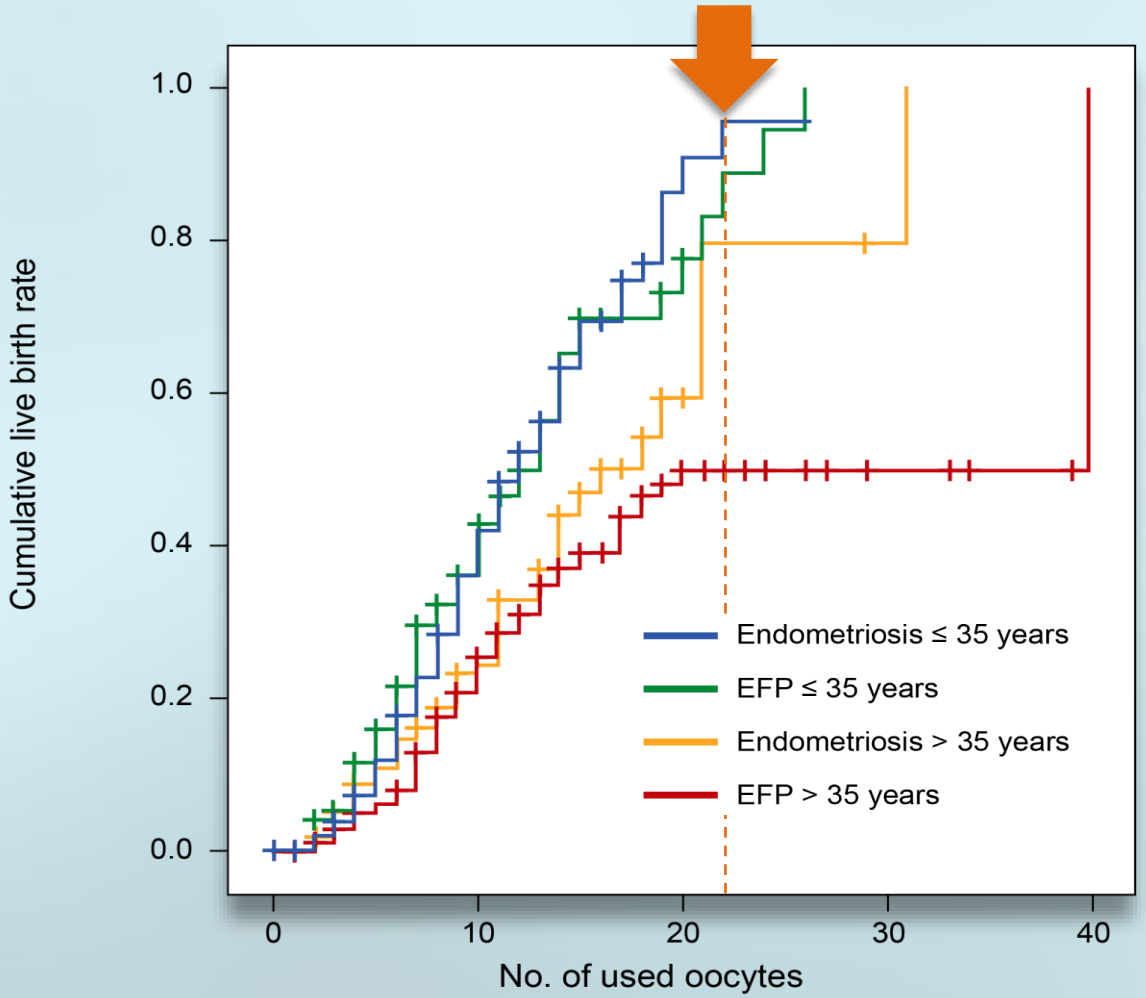
Oocyte competence: oocyte preservation cycles



Number needed to freeze: cumulative live birth rate after fertility preservation in women with endometriosis

- The outcome was better in the endometriosis group as compared to elective fertility preservation patients:
  - CLBR:
    89.5% (95%CI 80–99%) vs
    59.9% (95%CI 51–68%), respectively,
    when 22 oocytes were used
    (P<0.00001)

CLBR, cumulative live birth rate; EFP, elective fertility preservation

Cobo A, et al. Reprod Biomed Online 2021;42:725–32

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Effect of endometriosis on the endometrium

2. Endometrium
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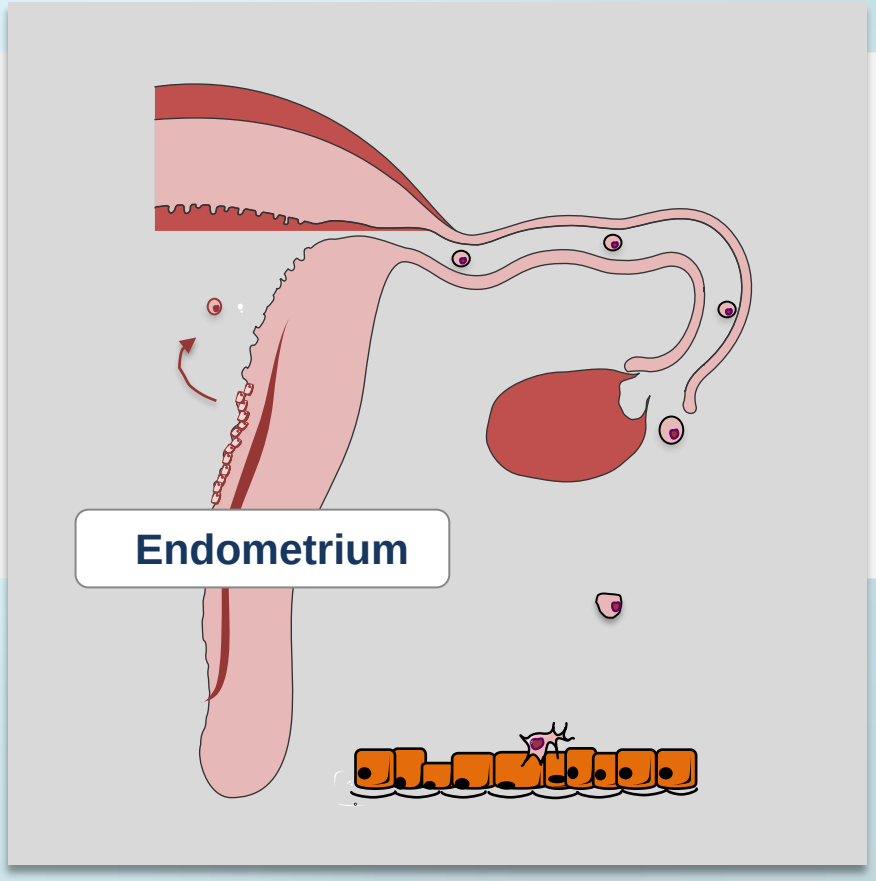
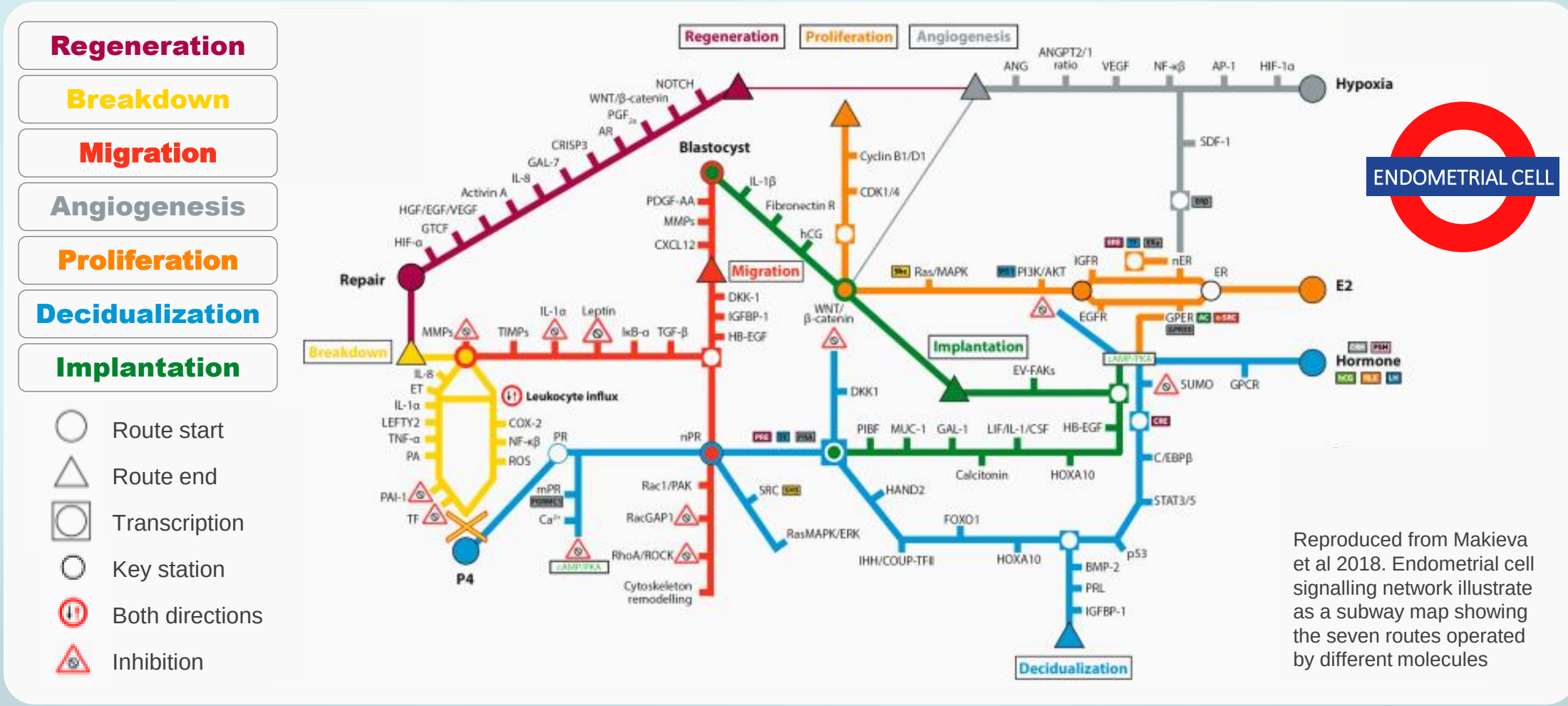


The endometrial cell signalling network



Reproduced from Makieva et al 2018. Endometrial cell signalling network illustrate as a subway map showing the seven routes operated by different molecules

Makieva S, et al. Int J Mol Sci 2018;19:2477.

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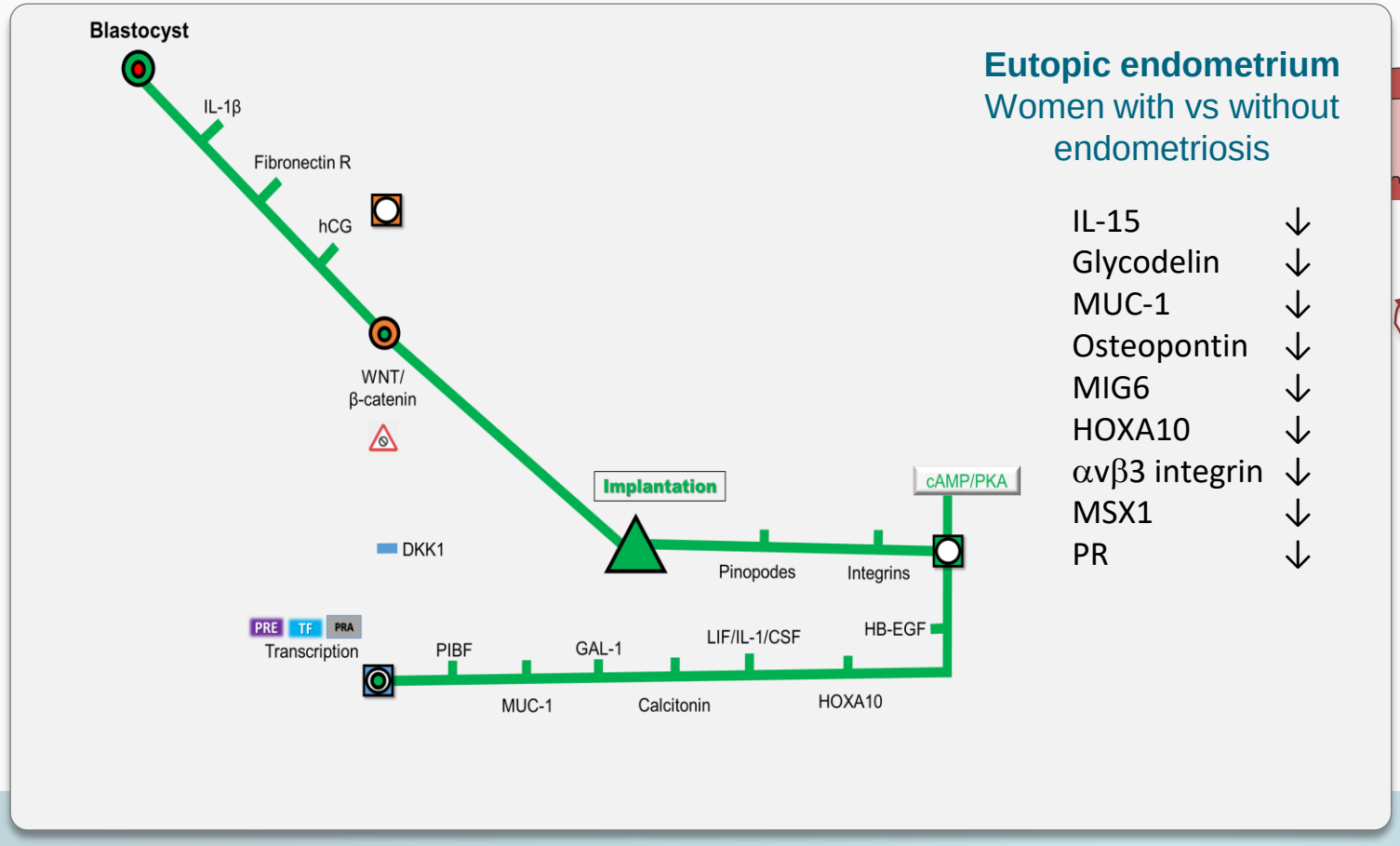
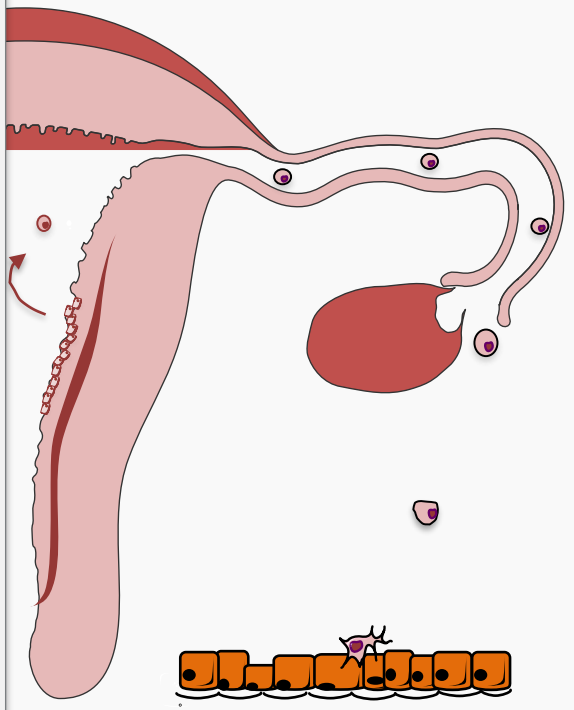
The endometrial cell signalling network: alterations in endometriosis

Regeneration | **Breakdown** | **Migration** | **Angiogenesis** | **Proliferation** | **Decidualization** | **Implantation**



Eutopic endometrium
Women with vs without endometriosis

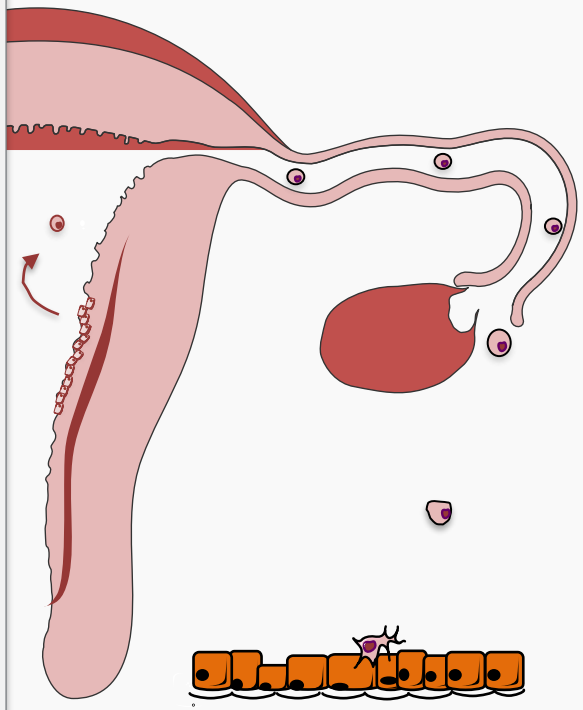
- IL-15 ↓
- Glycodelin ↓
- MUC-1 ↓
- Osteopontin ↓
- MIG6 ↓
- HOXA10 ↓
- αvβ3 integrin ↓
- MSX1 ↓
- PR ↓



Progesterone-related alterations in eutopic endometrium

Eutopic endometrium: Women with vs without endometriosis

ICAM-1	↓
IL-6	↑
MCP-1	↑
IL-37	↓
Galectin-1,-3,-9	↑
CX3CR1	↑
Fractalkine	↑
PIAS3	↓
TSG-6	↑
Semaphorin E	↑
IL-15	↓
Glycodelin	↓
FOXO1	↓
MAD2L1	↓
MUC-1	↓
Osteopontin	↓
MIG6	↓
PR	↓
NF-kBp65	↑
GPER	↑
Aromatase	↑
StAR	↑
PGE2	↑
AC002454.1	↑
CDK6	↑
MYC	↑
PDCD4	↓
p27	↑
ERK	↑
Cyclin D1	↑
Activin A	↑
GLI1	↓
MMP9	↑
MMP1	↑
IGFBP1	↓
RhoA/ROCKII	↑
TGFbeta1	↑



Progesterone resistance

cAMP?
hCG?

Decidualization in eutopic endometrium from women with endometriosis

Genes critical to implantation and decidualization: Phenotypes in female knockout mice

Deleted gene	Phenotype
Acvr1 (Alk2)	Decidualization failure
Bmp2	Decidualization failure
Bmpr2	Decidualization failure
Bsg	Implantation failure
Cdh1	Implantation failure; decidualization failure
Cebpb	Defective stromal cell proliferation; decidualization failure
Ctnnb1	Implantation failure
Dedd	Decidualization failure
Dlgap5 (Hurp)	Implantation failure; defective stromal cell proliferation; decidualization failure
Errfi1	Implantation failure
Fkbp4 (Fkbp52)	Implantation failure; decidualization failure
Foxa2	Implantation failure; decidualization failure
Gja1 (Cx43)	Decidualization failure
Hand2	Implantation failure
Hbegf	Implantation failure
Hmx3	Implantation failure
Hoxa10	Implantation failure; defective stromal cell proliferation; decidualization failure
Hoxa11	Implantation failure; decidualization failure
Ihh	Implantation failure
IL-11R	Decidualization failure

Deleted gene	Phenotype
Il6st (Gp130)	Implantation failure
Klf5	Implantation failure; decidualization failure
Lif	Implantation failure
Msx1/Msx2	Implantation failure
Ncoa2 (Src2)	Decidualization failure
Nodal	Decidualization failure
Notch1	Decidualization failure
Nr2f2 (COUP-TFII)	Defective stromal cell proliferation; decidualization failure
Nr3C3 (PGR-A)	Decidualization failure
Nr5a2	Decidualization failure
Phb2 (REA)	Decidualization failure
Prlr	Implantation failure
Pten	Decidual regression failure
Ptgs2 (Cox-2)	Implantation failure; decidualization failure
Ptx3	Implantation failure; decidualization failure
Smo	Decidualization failure
Sphk1/Sphk2	Decidualization failure
Src (c-Src)	Decidualization failure
Trp53 (p53)	Implantation failure; decidual senescence
Wnt4	Implantation failure; decidualization failure
Wnt7a	Implantation failure

20/41

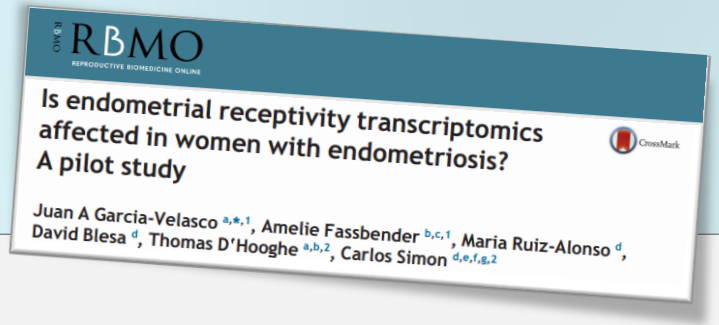
Gellersen B, Brosens JJ. Endocr Rev 2014;35:851–905

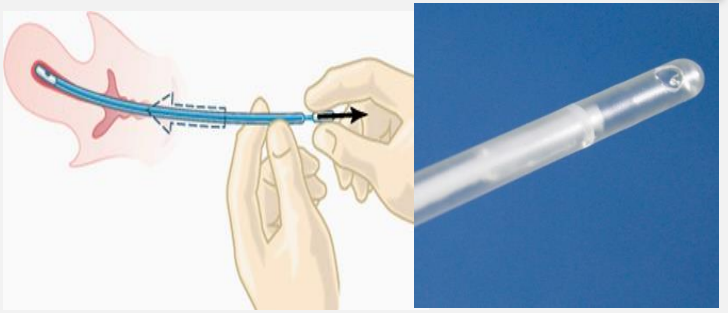
Impact of endometriosis on the ERA test

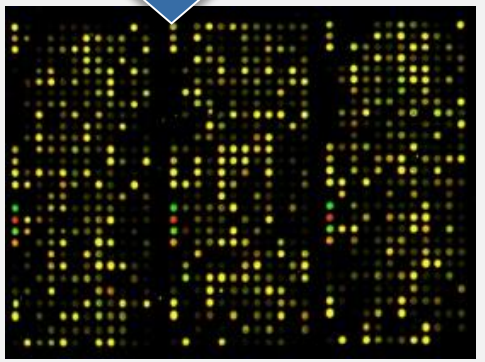
Prospective study on endometrial receptivity gene signature

- Genomic diagnostic tool based on the transcriptomic profile on an endometrial biopsy
- Examines 238 genes implicated in the receptive endometrium
- Reveals timing of WOI for personalized embryo transfer

Epidemiological characteristics of the study population		
	Endometriosis (n=17)	Control (n=5)
Age (years, mean ± SD)	31.3 ± 2.3	33.2 ± 1.3
Stages		
Minimum (I)	I = 7	
Mild (II)	II = 3	
Moderate (III)	III = 4	
Severe (IV)	IV = 3	
Infertility		
Primary	P = 12	P = 1
Secondary	S = 4	S = 4







None of the 238 genes present in the ERA array was significantly different between women with endometriosis and controls

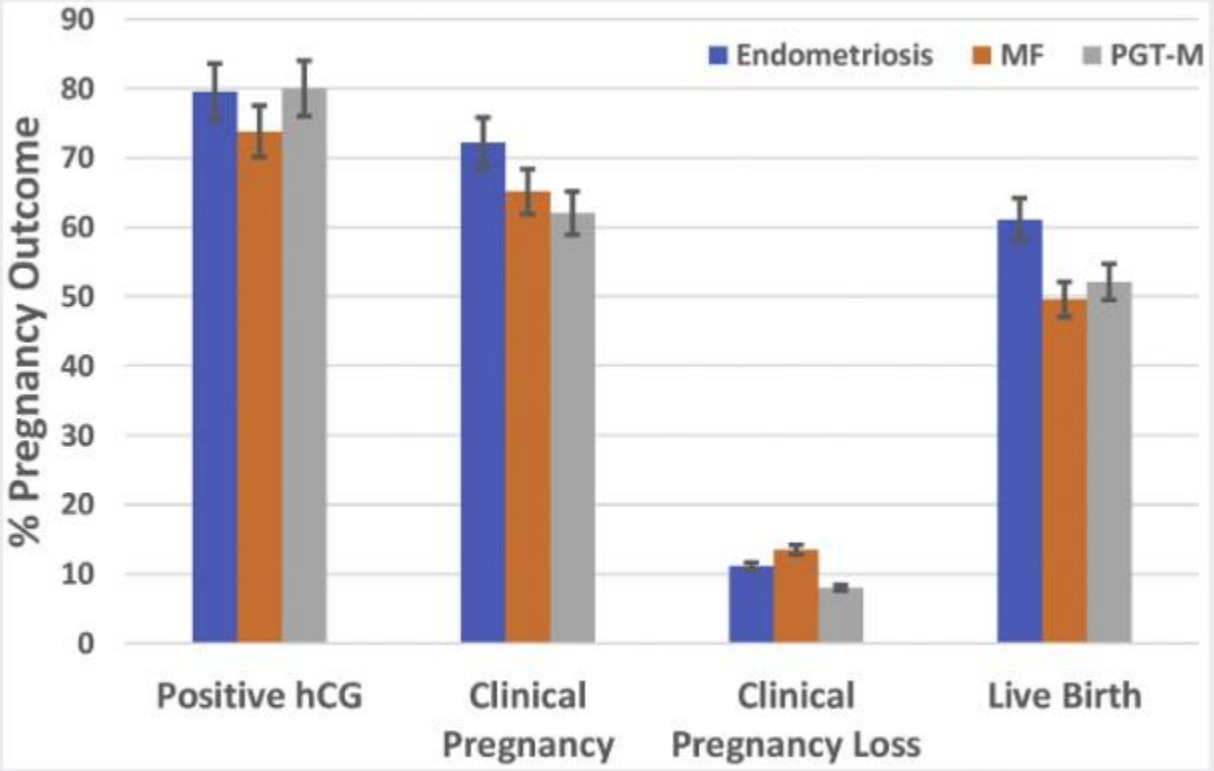
Sensitivity: 99.8%

Specificity: 88.6%

ERA, endometrial receptivity array; SD, standard deviation; WOI, window of implantation

Garcia-Velasco JA, et al. Reprod Biomed Online 2015;31:647–54

Impact of endometriosis on frozen cycles



Pregnancy outcomes in patients with endometriosis compared with those of patients in treatment for male factor infertility and noninfertile patients undergoing preimplantation genetic testing for monogenic disorders (PGT-M).

Bishop. Endometriosis affects in euploid transfers. Fertil Steril 2020.

Bishop, et al. Fertil Steril 2021

Endometrial quality: oocyte donation experience (recipients)

Study	Setting	Main findings
Sung et al.[1]	Retrospective study: Recipients with endometriosis (group I: subdivided into mild and moderate-severe endometriosis) were compared to recipients without endometriosis (group II)	PRs and IRs were comparable between group I and group II.
Budak et al.[2]	Retrospective study: Ovarian stimulation and oocyte retrieval in donors. Embryo transfer performed in recipients after endometrial preparation	Similar cumulative PRs were observed regardless of recipient age, indication for oocyte donation (endometriosis)
Diaz et al.[3]	Splitting oocytes from the same donor between recipients with and without stage III/IV endometriosis (prospective matched case-control study)	PRs, IRs, MRs and LBRs not affected by recipients' endometriosis status
Bodri et al.[4]	Retrospective matched case-control study of cycles with discordant outcomes	No different in indications (i.e. proportion of endometriosis patients)
Prapas et al.[5]	Prospective comparative study including a population of menopausal recipients with and without endometriosis sharing sibling oocytes coming from the same donor	PRs and IRs were significantly lower in the endometriosis group compared to the control group respectively

PR, pregnancy rate; IR, implantation rate; MR, miscarriage rate; LBR, live-birth rate.

1. Sung L, et al. J Assist Reprod Genet 1997;14:152–56; 2. Budak E, et al. Fertil Steril 2007;88:342–49; 3. Díaz I, et al. Fertil Steril 2000;74:31–34;
4. Bodri D, et al. Fertil Steril 2007;88:1548–53; 5. Prapas Y, et al. Reprod Biomed Online 2012;25:543–48

Endometrial quality: oocyte donation experience

Study	Setting	Main findings
Sung et al.[1]	Retrospective study: Recipients with endometriosis (group I: subdivided into mild and moderate-severe endometriosis) were compared to recipients without endometriosis (group II)	n = 932 endometriosis patients n = 7,178 non-endometriosis patients
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Conclusions

1. **Oocyte quantity:** Good evidence for a reduced number of retrieved oocytes in meta-analyses
2. **Oocyte competence and endometrial quality:** Controversial evidence between basic and clinical studies

The poor agreement between basic and clinical evidence hints for an in-depth rethinking on:

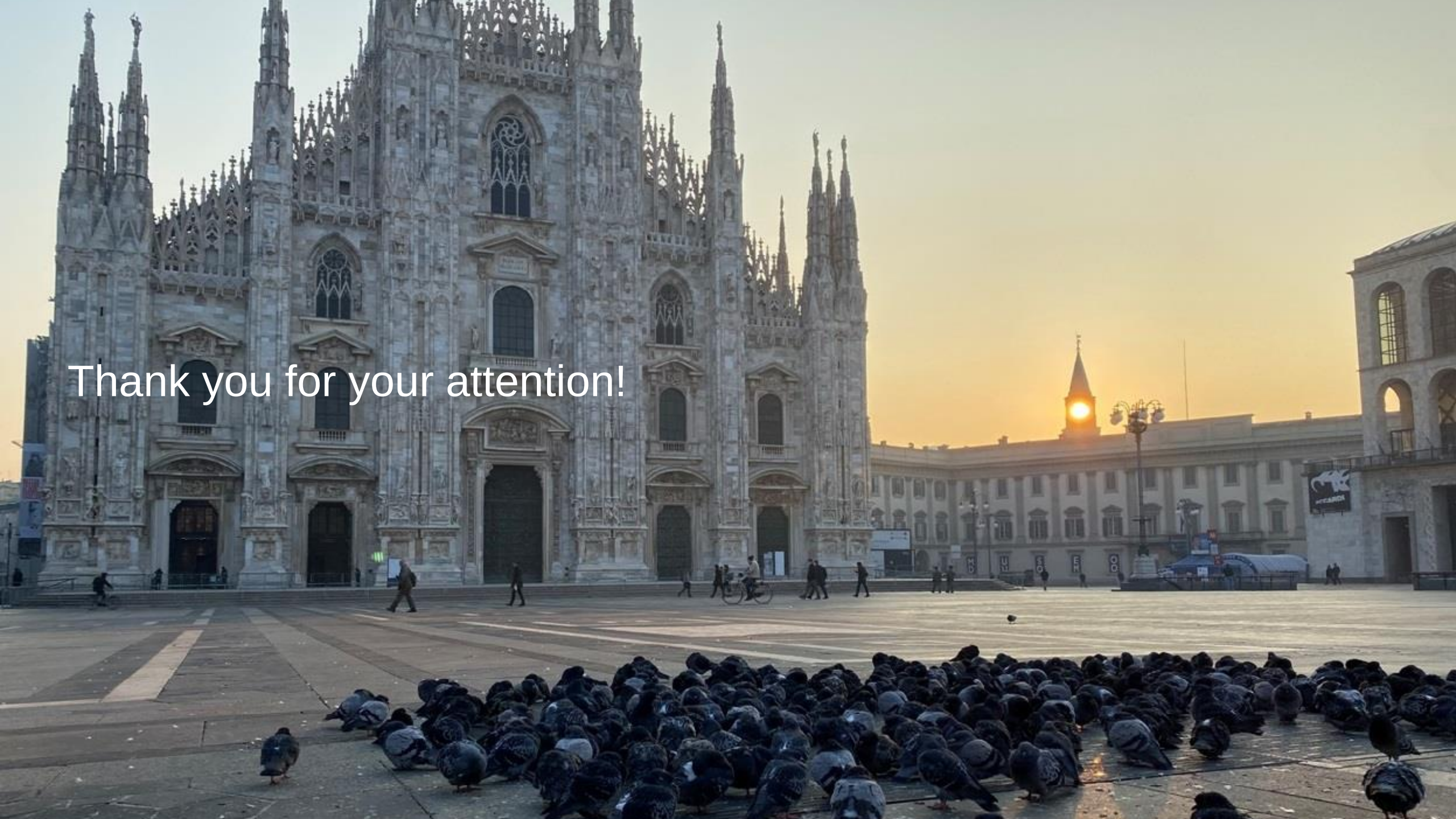
- Basic models of endometriosis
- Clinical studies on endometriosis

Limitations of this presentation:

- *Endometriosis considered as a unique entity* →
- *Quality is an intermediate outcome: risky!*

Peritoneal disease
Non-treated unilateral endometrioma
Treated unilateral endometrioma
Non-treated bilateral endometriomas
Treated bilateral endometriomas
Untreated DE
Treated DE
Peritoneal disease + DE
DE + non-treated unilateral endometrioma
DE + treated unilateral endometrioma
DE + non-treated bilateral endometriomas
DE + treated bilateral endometriomas
Peritoneal disease + adenomyosis
Ovarian disease + adenomyosis
DE + adenomyosis

DE, deep endometriosis; PR, pregnancy rate


Thank you for your attention!