Recent Developments in The Transmission of Human Life

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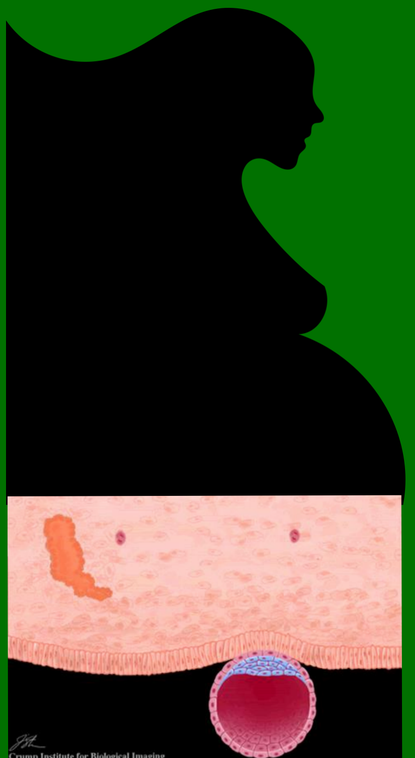
Live Streaming of The Conference
http://bit.ly/3UAcOgn



OVERVIEW ON THE POWER OF DIAGNOSTIC TOOLS USED TO ESTABLISH ENDOMETRIAL ADEQUACY FOR IMPLANTATION

Speaker

Carlo Bulletti

I HAVE NOT POTENTIAL
CONFLICT OF INTEREST WITH THIS LECTURE

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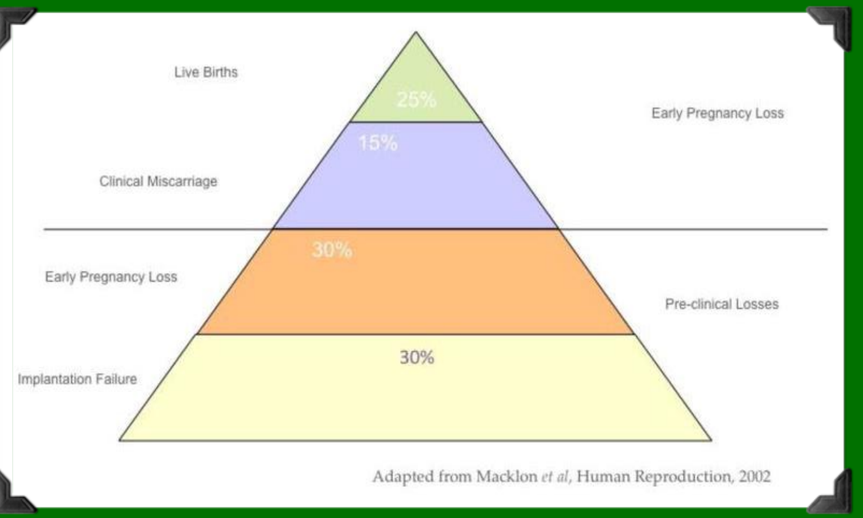
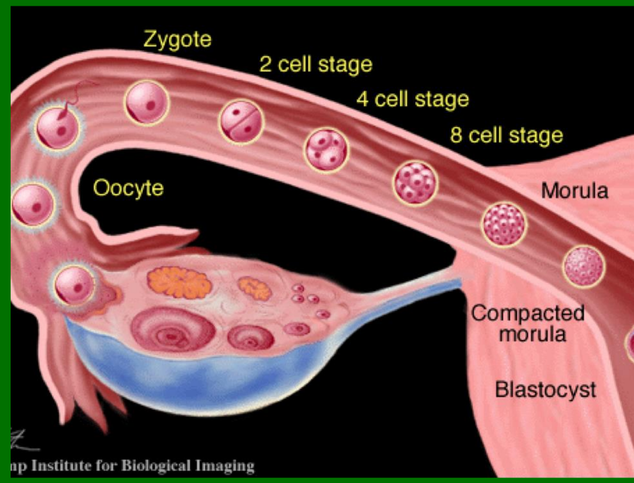
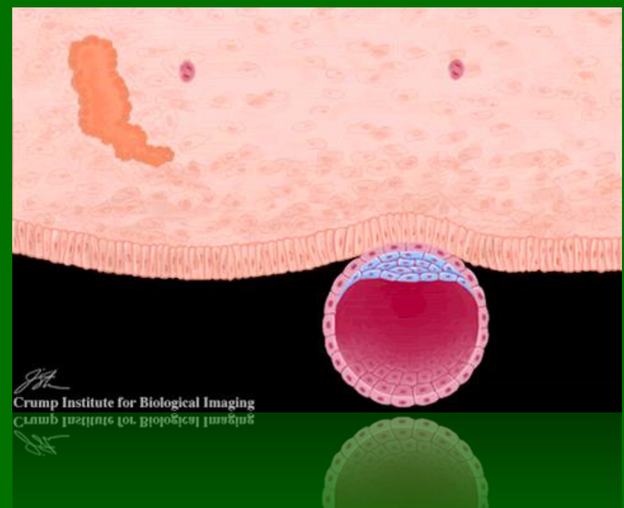
Embryos Implantation



Efficiency

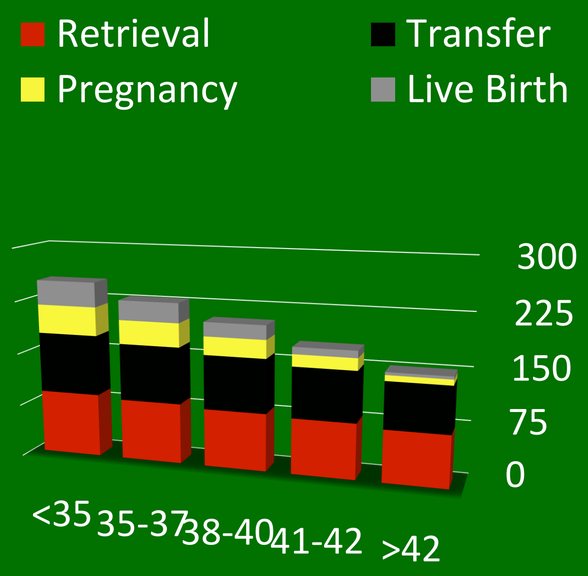
- The endometrium becomes receptive to blastocyst implantation 6 days after ovulation and remains receptive for 4 days (cycle days 20–24; Bergh and Navot, 1992)
- Successful implantation requires a receptive endometrium, a functional embryo at the blastocyst developmental stage and a synchronized dialog between maternal and embryonic tissues (Bulletti et 1987).
- Implantation has three stages: apposition, adhesion and penetration. Apposition is an unstable adhesion of the blastocyst to the endometrial surface. During this stage, the trophoblast becomes closely apposed to the luminal epithelium (Tabibzadeh and Babaknia, 1995).
- In response to this invasion and the presence of progesterone stimulation, the endometrial stromal cells and endometrial extracellular matrix undergo decidualization that is essential for the viability of the pregnancy.

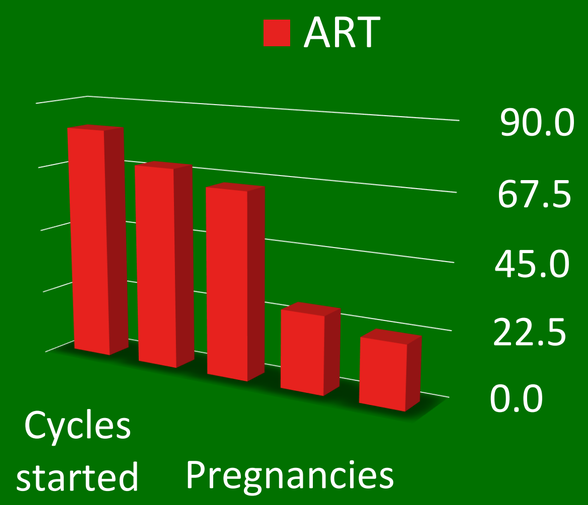


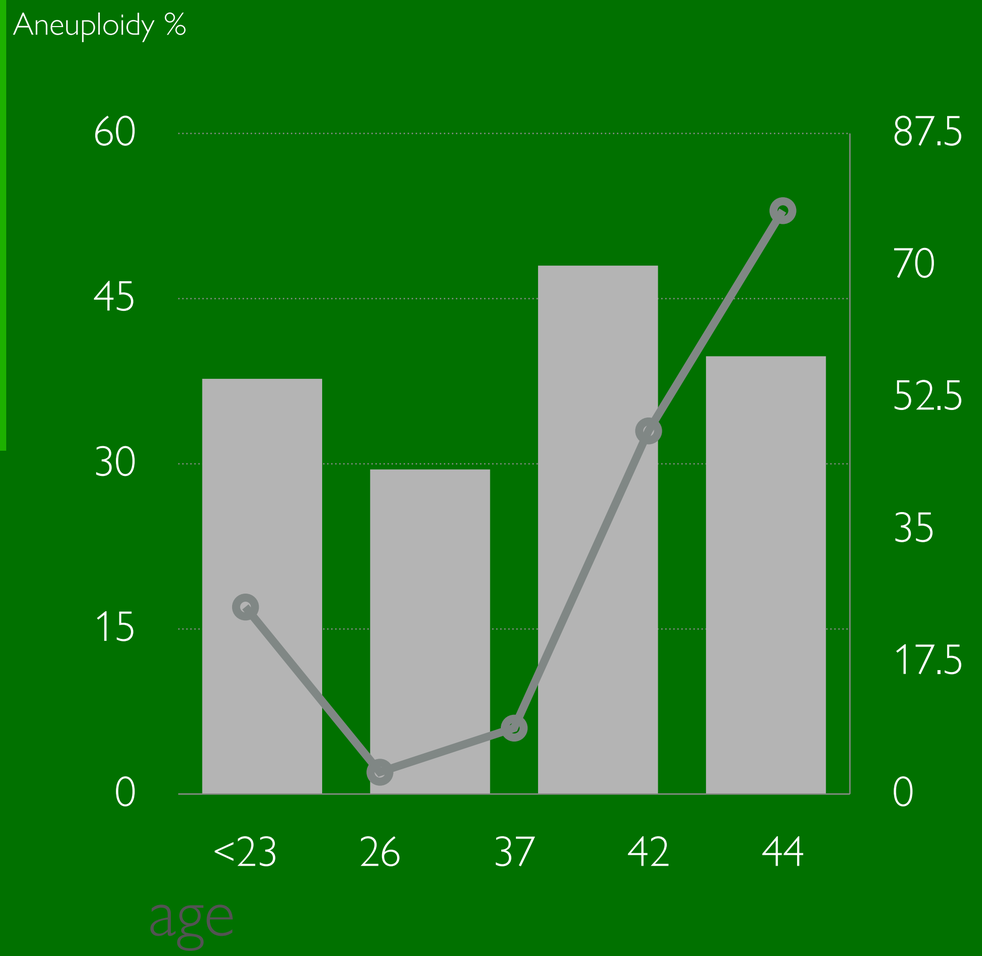


THE NATURE OF ANEUPLOIDY WITH INCREASING AGE OF FEMALE PARTNER

- The lowest risk for embryonic aneuploidy is between 26 and 30
- Both younger (<23) and older (>37) age groups had higher aneuploidy and an increased risk for more complex aneuploidy
- The overal risk did not measurably change after age 43.
- Trisomy and monosomy are equally prevalent







Franasiak JM et al Fertil Steril 2014;101:656-663

Implantation Failure Incidence

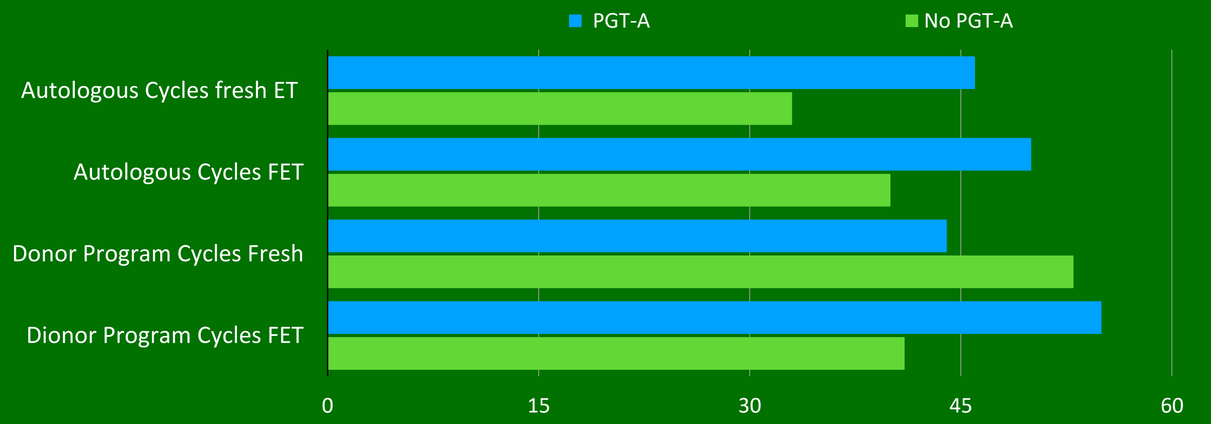
Embryo & Endometrium

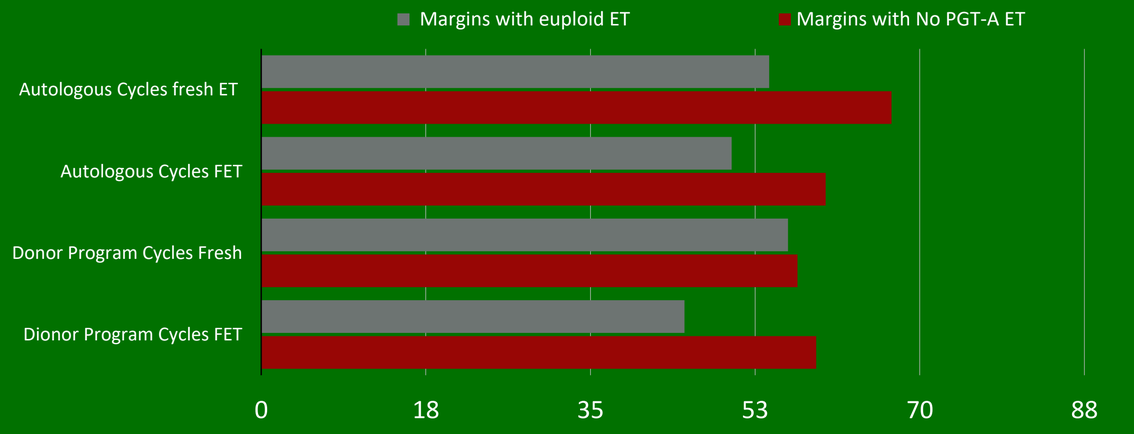
- *Repeated Implantation Failure (RIF) with only euploid embryos transferred is ranging from 5 % to 15% after three attempts (Pirtea et al , 2021; Busnelli A et al 2021; Ata B et al, 2022) . A marginal rate. However*
- *Implantation Rate of an euploid embryo transfer (after an implantation failure, Cozzolino et al 2022) emphasize the role still not fully understood of the endometrium in the successful embryo's implantation*
- *With margins of improvement rather than euploidies ranging from 45% to 55%*
- *Unless we want to accept with resignation the biological evidence that brings us 0.5 children for 1 euploid embryo transferred. Accepting it as "normality".*
- *But... the real question is: Is resignation perhaps the researcher's comet?*

Implantation Rate

	PGT-A	No PGT-A	Margins with euploid ET	Margins with No PGT-A ET
Autologous Cycles fresh ET	46	33	54	67
Autologous Cycles FET	50	40	50	60
Donor Program Cycles Fresh	44	53	56	57
Donor Program Cycles FET	55	41	45	59

From: Cozzolino et al, 2022 Modified





The Aneuploidy Contribution to the Implantation Failure

Blastocyst euploidy rates with comprehensive chromosome analysis according to female partner's age. (From Ata B et al , 2021 modified)

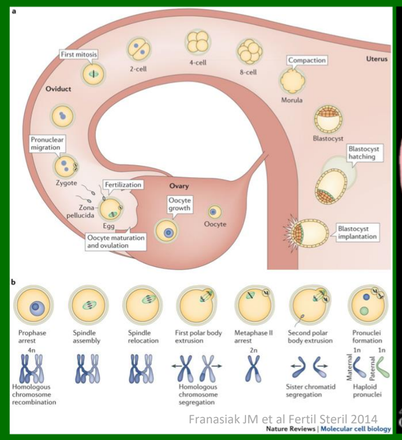
- Although PGT-A reduces implantation failure and miscarriages per transfer the euploid embryo does not guarantee success in IVF cycles. A receptive endometrium must interact with the euploid embryo to achieve an ongoing pregnancy

Study	<35 y	35–37 y	38–40 y	41–42 y	>42 y
Ata et al. 2012 (4)[a]	60%–63%	51%–55%	36%–39%	21%–25%	13%–17%
Demko et al. 2016 (6)[b,c]	60%	55%	45%	35%	20%
Barash et al. 2017 (3)[c]	62%	57%	44%	30%	
Hong et al. 2019 (7)[d]	74%	65%	47%	29%	15%
Irani et al. (5) 2020	55%	45%	32%	18%	8%
Mean Value	62.5%	55%	41.1%	27%	14.5%

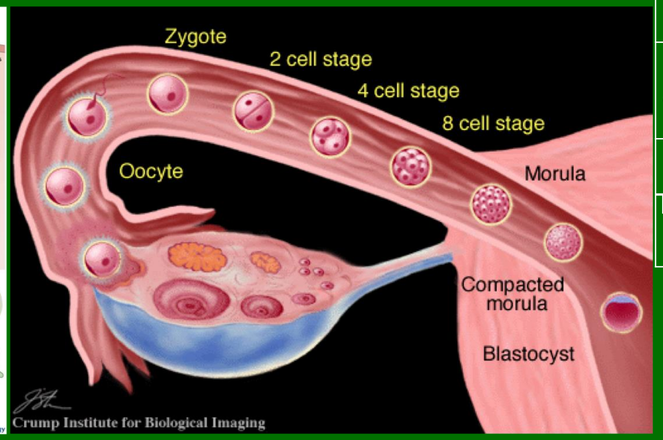
Total Blastocysts required to obtain al least one euploid blastocyst with 90% probability According to Esteves SC et al, 2018

Age groups	28	35	37	39	41	43	45
Total Blastocysts Required	3	4	5	6	9	16	29

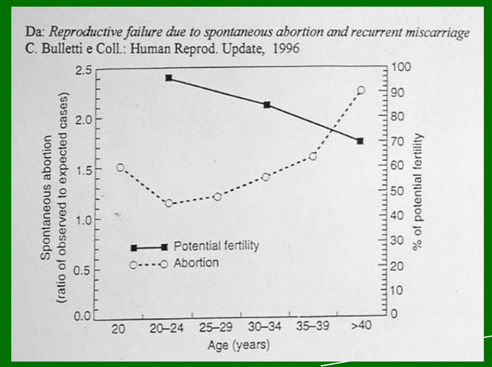
[a]Published and unpublished data.
b Figures for only day five embryos.
c Based on approximations from the published figures and graphs in the original publications.
d Only women who underwent ovarian stimulation are included in the table.

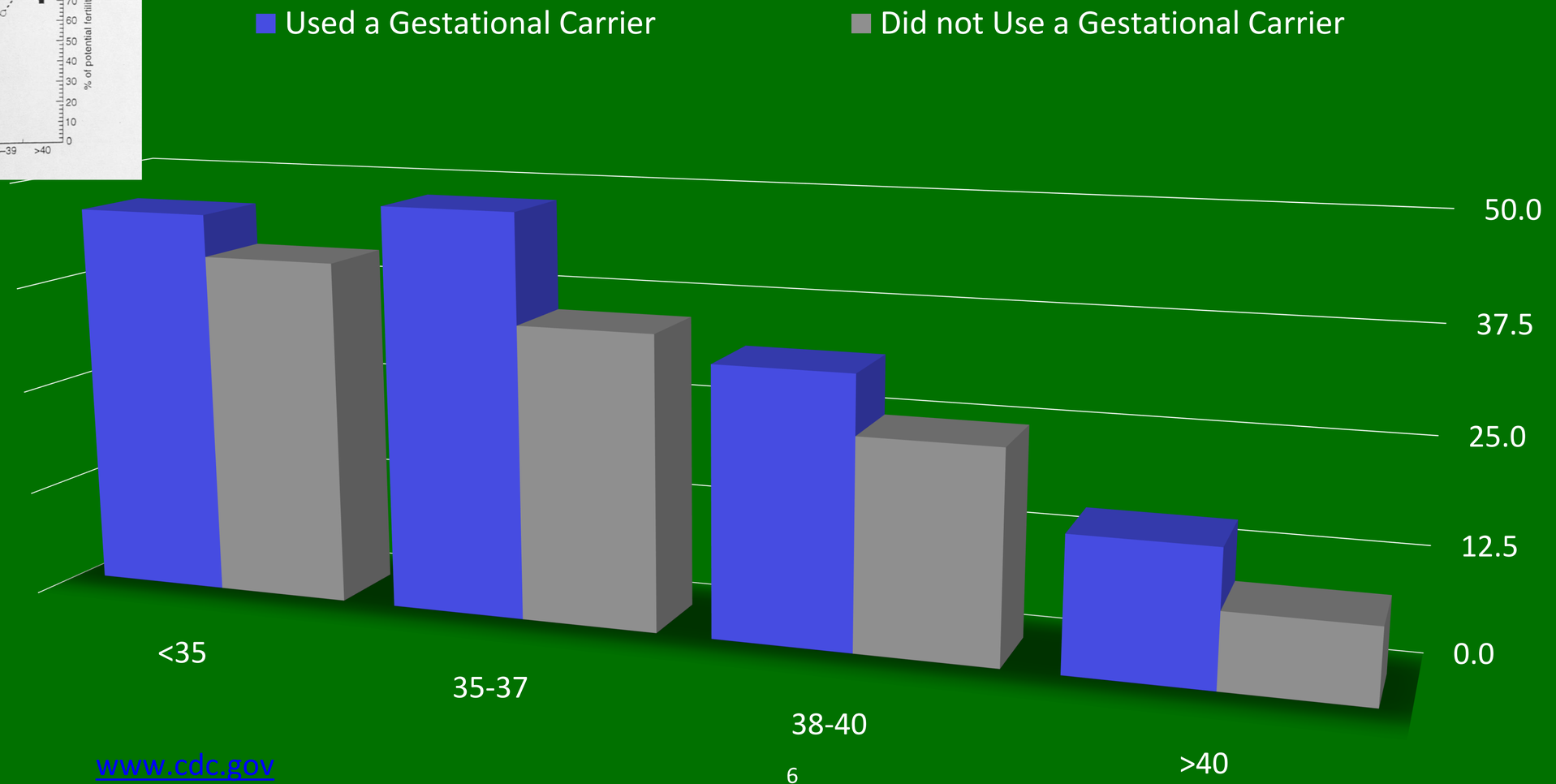


Franasiak JM et al Fertil Steril 2014
Nature Reviews | Molecular cell biology



Comparison of Live Birth per Transfer Between Cycles That Used Gestational Carriers and Those That Did Not (Both Using Fresh Non Donor Embryos), by ART Patient Age.





www.cdc.gov

Factors Influencing Good Embryo Nidation

Life Style Factors

- **<u>Smoking Habit</u> Tobacco consumption determines reduced uterine receptiveness. Endometrial decidualization may be compromised in smokers.** Occasionally/daily cigarette smoking women had similar chance of achieving a clinical pregnancy or a live birth as the nonsmokers when receiving medically assisted reproduction treatments. However, tobacco use before and during pregnancy remains a major cause of reduced fertility as well as maternal, fetal, and infant morbidity and mortality, and should strongly be discouraged. (Dechanet C, et al, 2011, C S Pietersma, et al 2022)
- Is increased **<u>alcohol intake</u>** in different phases of the menstrual cycle associated with fecundability in women? There is an inverse association between alcohol and fecundability. **Moderate to heavy drinking during the luteal phase, and heavy drinking in the ovulatory window, could disturb the delicate sequence of hormonal events, affecting chances of a successful conception**. Mohammad Yaser Anwar, et al 2021 https://doi.org/10.1093/humrep/deab121
- **<u>BMI</u>** and **<u>Female Dietary</u>** At the clinical level, **findings from this review do not support recommending any single dietary pattern for the purpose of improving pregnancy or live birth rates in women undergoing IVF treatment** (Sanderman, E.A., et al, 2022)

Cause	relationship	Diagnostic method	before RIF?	treatment	before first ART cycle?	management?	Refrences
	Lifestyle factors						
Smoking	X	questionnaire	yes	No Robust	yes	yes	• Dechanet C, et al, 2011, C S Pietersma, et 2022
Alcohol	X	questionnaire	yes	No Robust	yes	yes	• Mohammad Yaser Anwar, et 2021 https://doi.org/10.1093/humrep/dea 121
Obesity	?	questionnaire		No Robust	yes	yes	• Sanderman, E.A., et al, 2022
	Gamete quality						
Oocyte	yes	microscopical evaluation	yes	yes	yes	yes	
Spermatozoa	yes	microscopical evaluation	yes	yes	yes	yes	
	Embryo quality						
Morphology	Yes	Microscope classification	Yes	Yes	Yes	Yes	
Aneuploidy Screening	Yes	Genetic Assessment	Yes	Yes	Yes	Yes	
	Uterine factors						
Uterine septum	yes	US	yes	yes	yes	yes	
Submucosal fibroids/polyps	yes	US	yes	yes	yes	yes	
Intramural fibroids	yes	US	yes	yes	yes	yes	
Intrauterine adhesions	yes	Hysteroscopy	yes	yes	yes	yes	
Ademyosis	yes	US	yes	yes	yes	yes	
Impaired receptivity	yes	Histology	yes	yes	yes	yes	
	Adnexal pathology & Endometriosis						
Hydrosalpinx	yes	US	yes	yes	yes	yes	
Endometriosis	yes	US/ Questionnaire/Laparoscopcy	yes	yes	yes	yes	
	Endometrium						
Thickness at embryo transfer	yes	US	yes	yes	yes	yes	
Difficult embryo transfer	yes	Previous attempts	no	yes	yes	yes	
Histology In/out of phase	yes	In phase	yes	yes	yes	yes	
Genomic endometrial assessment	no	gene sequences estabishment	yes	no	yes	yes	
Metabolomics	may be	no	yes	no	yes	yes	
PINOPODES	may be	no	yes	no	yes	yes	
Endometritis							
	Systemic Factors						
Trombophilia	yes	blood samples analysis	yes	yes	yes	yes	
Immulogical factors	yes	blood samples analysis	yes	yes	yes	yes	
Progesterone serum levels at the trigger	yes	blood sample	yes	yes	yes	yes	
Progesterone serum levels at midsecretory or +5/6 d with P supplementation of natural, and HRT cycles	yes	blood sample	yes	yes	yes	yes	
TSH	yes	blood samples	yes	yes	yes	yes	

Factors Influencing Good Embryo Nidation

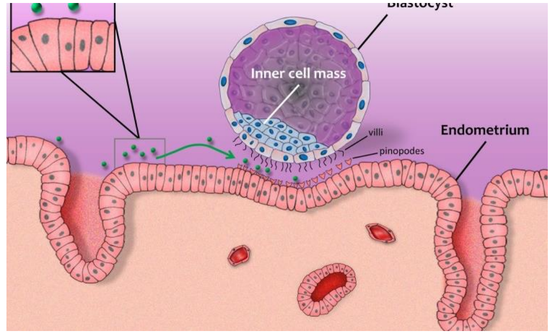
Gamete quality

- **Sperm The abnormal spermatozoa adversely affect** fertilization events, which results in reduced cleavage/blastocyst/ i**mplantation** and pregnancy rate during assisted reproductive techniques.(Chorya JB, Sutaria TV, Chaudhari RK, Chaudhari CF. Impact of gamete health on fertilization and embryo development: An overview. Asian Pac J Reprod 2022;11:201-7)
- **Oocytes Poor oocyte quality is also one of the reasons for infertility**, although the oocyte has an innate capacity to repair a certain amount of abnormality of both oocyte and spermatozoa. Therefore, oocyte health carries more responsibilities during fertilization events(Chorya JB, Sutaria TV, Chaudhari RK, Chaudhari CF. Impact of gamete health on fertilization and embryo development: An overview. Asian Pac J Reprod 2022;11:201-7)

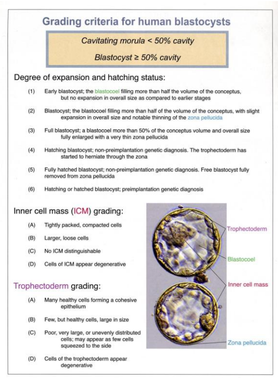
Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Gamete quality						
Oocyte	yes	microscopical evaluation	yes	yes	yes	yes	• Chorya JB et al, 2022
Spermatozoa	yes	microscopical evaluation	yes	yes	yes	yes	• Chorya JB et al, 2022

Factors Influencing Good Embryo Nidation

Embryo quality



Extracellular vesicles and the cross-talk between the blastocyst and endometrium during implantation.
Ronit Machtinger, Louise C. Laurent, Andrea A. Baccarelli
*Hum Reprod Update. 22(2): 182-193, 2017



- **<u>Morphology</u>** Embryo's Morphological Parameters were explored by Wang X et al , 2022 through 3 blastocysts parameters : **degree of expansion (** expansion), **appearance of tophectoderm** (TE) and i**nner cell mass** (ICM).**Blastocyst morphology was graded according to the Gardner-Schoolcraft system**, an alpha numeric composite score of blastocyst expansion (1–6), inner cell mass (ICM) (A–C), and TE (A–C) appearance.T**he study report that the association of blastocyst morphological parameters with live birth may be affected by blastocyst biopsy and/or genetic testing, and its association with birth weight may be affected by blastocyst freezing and biopsy and/or genetic testing.**However the overall study population consists of good prognosis patients with average age <35 years, nonobese body mass indices, antral follicle count >19, and an average anti-müllerian hormone level >3.8 ng/mL. **Findings from this study may not be generalizable to the patients with a poor prognosis whose autologous blastocysts may confer different reproductive competence after undergoing TE biopsy and/or the freeze-thawing process** (Liu AH, et al, .2023)

- **<u>Blastocyst: euploid versus unascertained.</u> Implantation rate according to Cozzolino M et al, 2022 modified**

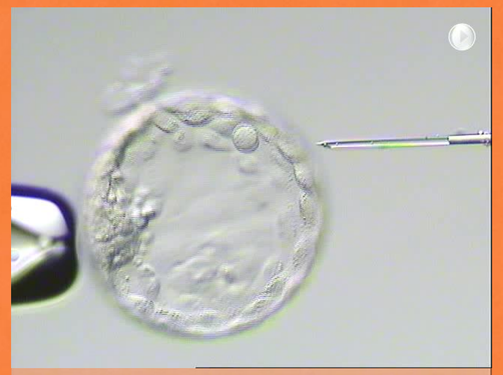
	PGT-A	No PGT-A	Margins of improvement with euploid ET	Margins of improvment with No PGT-A ET
AUTOLOGOUS CYCLE	46±50 FRESH	33±41 FRESH	54	67
	50±51 FET	40±45 FET	50	60
DONATED CYCLES	44±50 FRESH	53±46 FRESH	56	57
	55±50 FET	41±50 FET	45	59

Total Blastocysts required to obtain al least one euploid blastocyst with 90% probability
According to Esteves SC et al, 2018

Age groups	28	35	37	39	41	43	45
Total Blastocysts Required	3	4	5	6	9	16	29

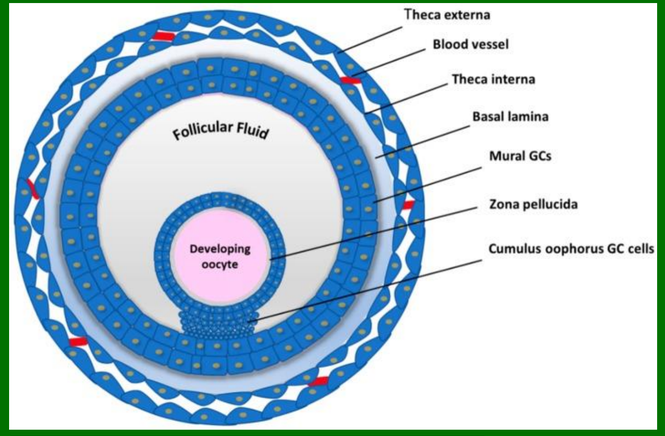
Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Embryo quality						• Chorya JB et al, 2022
Morphology	Yes	Microscope classification	Yes	No Robust	Yes	No	Wang X et al , 2022
Aneuploidy Screening	Yes	PGT-A	Yes	Yes	Yes	Yes	Cozzolino M et al 2022 ATA B et al 2021 Pirtea P et al , 2021 Busnelli A et al 2021

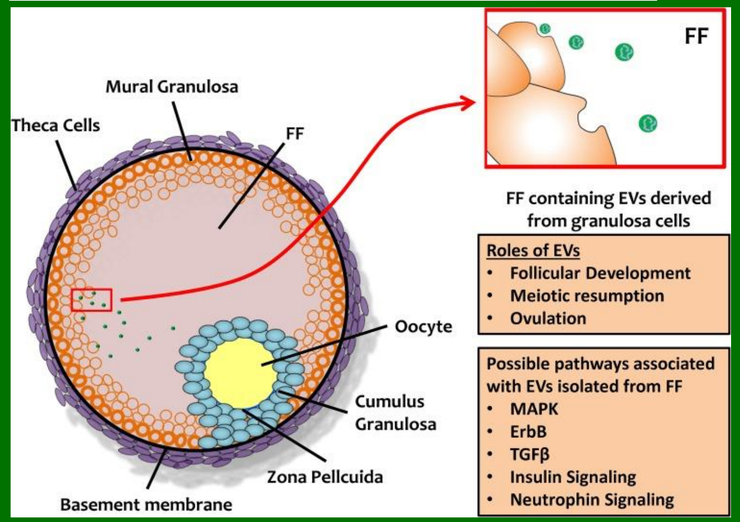
Blastocele Compartment 1



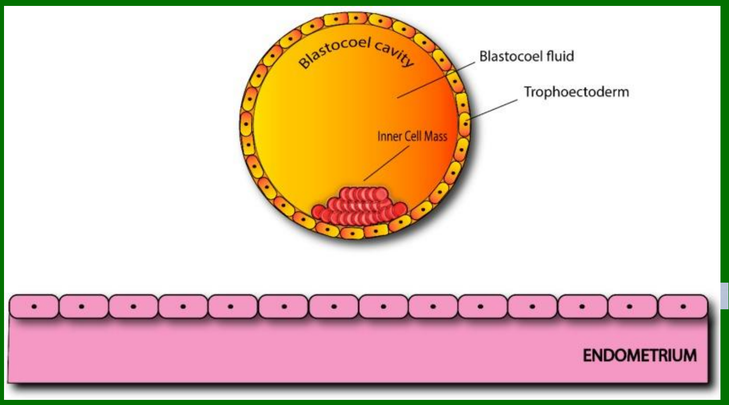
MCR--->Volume of biological fluid completely cleared of metabolites as measured in unit time. Elimination occurs as a result of metabolic processes in the compartment analyzed

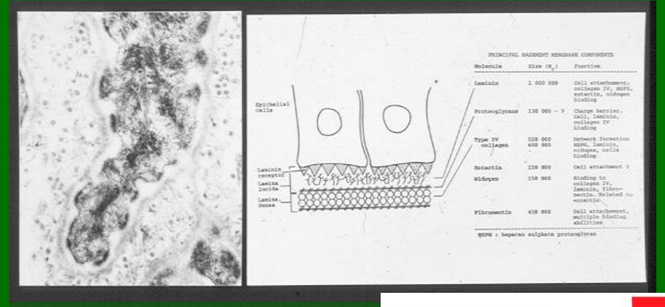
Medium Compartment 2

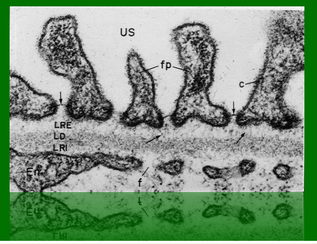


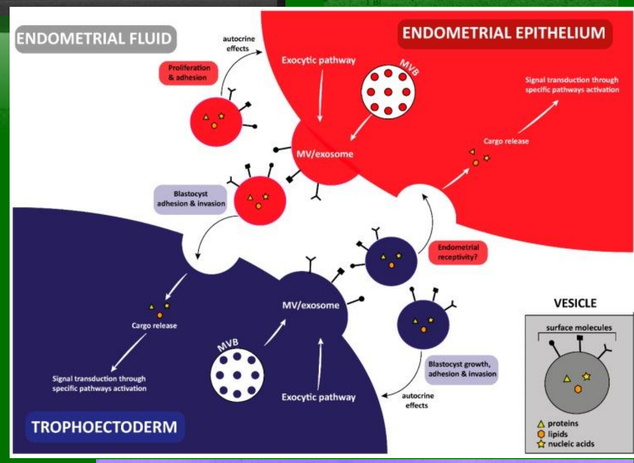


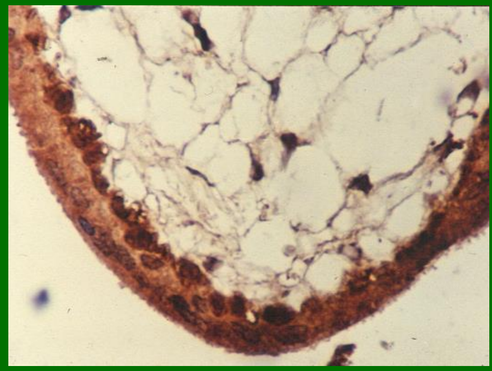
Extracellular vesicles and the ovarian follicle. EV: extracellular vesicles; FF: follicular fluid."
"Ronit Machtinger, Louise C. Laurent, Andrea A. Baccarelli
"Hum Reprod Update. 22(2): 182-193"2017









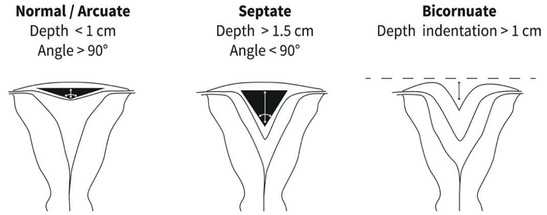


Factors Influencing Good Embryo Nidation

Uterine Factors

Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Uterine factors						
Uterine septum	yes	US	yes	yes	yes	yes	• Mollo A et al 2009 • Wang X et al, 2019
Submucosal fibroids/polyps	yes	US	yes	yes	yes	yes	BullettiC et al 1999 and 2004; Seracchioli R et al 2000 and 2006 Metwally M et al , 2020
Intramural fibroids	yes	US	yes	yes	yes	yes	Vitagliano A et al , 2021
Intrauterine adhesions	yes	Hysteroscopy	yes	yes	yes	yes	Hooker, A et al, 2016; Hooker, AB.et al, 2014 Grimbizis GF et al, 2011
Ademyosis	yes	US	yes	yes	yes	yes	Franasiak JM et al, 2021 Cakmak H et al,2010; Bourdon M et al, 2021
Impaired receptivity	yes	Histology	yes	yes	yes	yes	Treloar AE, et al 1967; Noyes RW, et al 1950; Gurpide E, et al 1991 Csapo AI et al , 1972 and 1973 Ubaldi F et al 1997; Kolibianakis EM et al 2005; Shapiro BS et al 2011 Doyle N et al, 2022

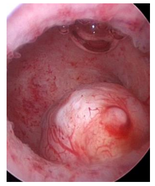
Septate uterus has been associated with an increase in the risk of miscarriage, premature delivery, and malpresentation; **however, there is insufficient evidence that a uterine septum is associated with infertility.** Uterine septum: a guideline Pfeifer, S etb al, 2016

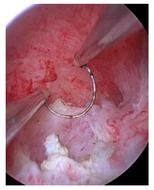


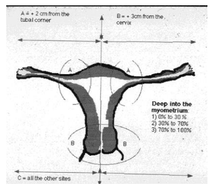
Fertility and Sterility Volume 106 Issue 3 Pages 530-54 (September 2016) DOI: 10.1016/j.fertnstert.2016.05.014

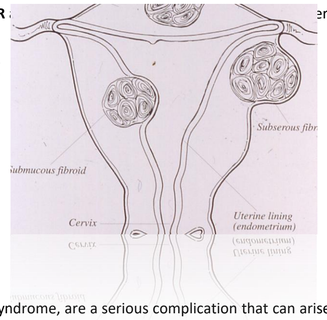
Fibroids Fibroids have been proposed to adversely impact implantation via several mechanisms, including venous congestion, diminished vascular supply, increased myometrial contractions, increased glycodelin level, and reduced HOXA10 and outcomes is correlated with location. (BullettiC et al 1999 and 2004; Seracchioli R et al 2000 and 2006)) The presence of submucosal fibroid decreases pregnancy rates after IVF (BullettiC et al 1999 and 2004; Seracchioli R et al 2000 and 2006; Metwally M et al , 2020), and myomectomy increase the cumulative pregnancy rate, CPRs (BullettiC et al 1999 and 2004; Seracchioli R et al 2000 and 2006; Metwally M et al , 2020).**A cochrane study examined the effects of myomectomy compared to no treatment. Results found insufficient evidence to determine a difference between treatment options for clinical pregnancy rate or miscarriage rate.**

Polyps may be releted to the endometritis as **detrimental effects on good embryo IR** ersal of that effect (Vitagliano A et al , 2021)









TYPE INTRAMURAL EXTENSION
0 NONE

Intrauterine Adhesions (IUAs) Intrauterine adhesions (IUAs), Asherman syndrome, are a serious complication that can arise after a miscarriage and intrauterine surgery, and **are associated with secondary infertility** and severe obstetric complications (Hooker, A et al, 2016; Hooker, AB.et al, 2014). IUAs develop following the destruction of the basal layer of the endometrium, arising in women undergoing postpartum dilation and curettage (D&C) . A systematic review of 36 studies reported a pregnancy rate of 63% after adhesiolysis, with an LBR of 75% among those who conceived (Grimbizis GF et al, 2011). The category of possible fertility symptoms in patients with IUAs includes secondary infertility and recurrent miscarriages, ectopic pregnancy, abnormal placentation, fetal growth restriction, fetal anomalies, premature delivery, and postpartum hemorrhage

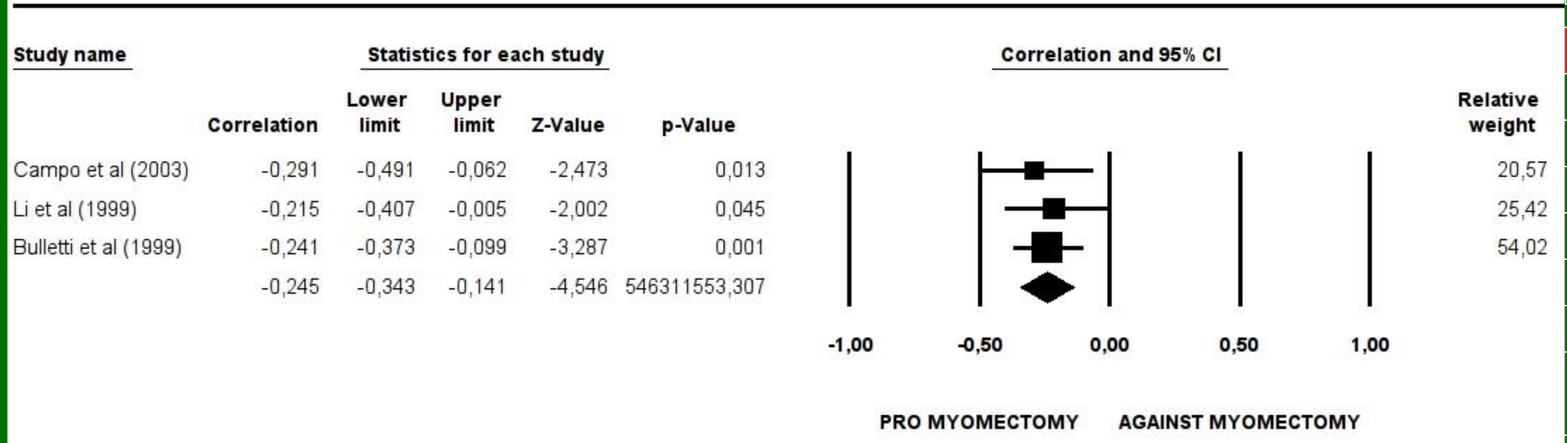
Adenomyosis. is a common gynecological disorder with unclear etiology that is characterized by the presence of ectopic endometrial glands and stroma within the myometrium with adjacent smooth muscle hyperplasia and fibrosis. The presenting clinical signs and symptoms include a soft and diffusely enlarged uterus with menorrhagia, dysmenorrhea and metrorrhagia. **When adenomyosis is encountered in younger reproductive age women, it is likely to reduce endometrial receptivity in a manner similar to endometriosis (Franasiak JM et al, 2021) T**he estrogen receptor expression, lack of progesterone receptor expression, and secondary progesterone resistance are related to embryo losses suffered by an affected patient. In adenomyosis, **the sex steroid hormone aberration's impaired immune response is related to implantation failure** (Cakmak H et al,2010; Bourdon M et al, 2021)

Impaired Receptivity . Cyclic Changes The unique aspect of this enigmatic tissue is that it undergoes proliferation and differentiation under the influence of steroid hormones, followed by tissues breakdown and disposal in the absence of a pregnancy. Progesterone is a pre-requisite for embryo implantation and its absence for pregnancy loss (Csapo AI et al , 1972 and 1973) The potential role of this mechanism in implantation failure is supported by data that demonstrate a restoration of normal pregnancy rates when embryos created in cycles with prematurely elevated progesterone levels are vitrified and transferred in a subsequent cycle (Ubaldi F et al 1997; Kolibianakis EM et al 2005; Shapiro BS et al 2011). Wahtever is the importance of each factor influencing a successful embryo implantation, timing and length of progesterone administration in frozen embryo transfer cycles become crucial in implantation failure but the inadequacy of the tests designed to identify the gold standard leave this subject in the fog. In a recent RCT including 878 partecipants, 58.5% of transfers in the endometrial receptivity timed group and 61.9% of transfers in the standard timing group resulted in live birth, a difference that was not statistically significant **Thus demonstrating that the use of endometrial receptivity testing to time transfer of a frozen euploid blastocyst created by in vitro fertilization did not improve the chances of achieving live birth. These findings do not support routine use of endometrial receptivity testing to guide the timing of frozen embryo transfer (Doyle N et al, 2022) .**

Effect of Myomectomy on IVF procedures
PREGNANCY RATE(%)

Study	Myiomectomy	Without Myomectomy	D
STOVALL 1998	37%(34/91)	53%(48/91)	-
ELDAR-GEVA 1998	27,2%(24/88)	39.3%(98/249)	-
RAMZY 1998	38.5%(15/39)	33.5%(123/367)	+
DIETTERICH 2000	55%(5/9)	63.6%(7/11)	-
JUN 2001	30.5%(43/141)	41.6%(169/406)	-
SURREY 2001	50.7%(37/73)	58.4%(191/327)	-
HART 2001	23.3% (26/112)	34.1%(110/322)	-
CHECK 2002	34.4%(21/61)	47.5%(29/61)	-
YARALI 2002	21.9%(16/73)	27.7%(90/324)	-
OLIVEIRA 2004	48%(117/245)	45%(110/245)	+
WANG 2004	59.2%(29/49)	46.6%(34/73)	-
NG 2005	22.9%(11/48)	14.9%(7/47)	+
GIANAROLI 2005	60%(45/75)	41.7%(53/127)	+
KHALAF 2006	23.3% (26/112)	32.9%(106/322)	-
KLATSKY 2007	47%(44/94)	54%(149/275)	-
VIMERCATI 2007	33.3%(17/51)	58%(98/169)	-
HORCAJADAS 2008	53%(429/807)	57%(130/228)	-
BOZDAG 2009	36%(22/61)	38%(168/444	-
SOMIGLIANA 2011	24%(28/119)	19%(22/119)	+

PREGNANCY OUTCOME BEFORE AND AFTER MYOMECTOMY



Pregnancy Rate

Study	Study | RR [95% Conf. Interval] % Weight
1	CAMPO 2003 | 1.78571 1.09335 2.91652 5.64107
2	LI 1999 | 1.52632 .993922 2.34389 7.65574
3	BULLETTI 1999 | 2.13636 1.3914 3.28018 8.86454
4	ROY 2010 | 1.13235 1.0154 1.26278 54.799
5	SHOKEIR 2010 | 2.2506 1.59826 3.1692 11.5705
6	CASINI 2006 | 1.38199 .940266 2.03122 11.4691
	M-H pooled RR | 1.44639 1.30043 1.60873
	Heterogeneity chi-squared = 29.78 (d.f. = 5) p = 0.000
	Test of RR=1 : z= 6.80 p = 0.000

Delivery rate

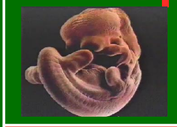
Study	Study | RR [95% Conf. Interval] % Weight
2	CAMPO 2003 | 2.5 1.38311 4.51882 12.7717
3	LI 1999 | 1.5625 .954308 2.5583 20.4347
4	BULLETTI 1999 | 3.5 1.95509 6.2657 15.326
5	ROY 2010 | 5.18182 3.44353 7.7976 28.0977
6	CASINI 2006 | 1.3436 .789914 2.28539 23.37
	M-H pooled RR | 2.94496 2.35523 3.68236

Abortion Rate

	Study | RR [95% Conf. Interval] % Weight
1	CAMPO 2003 | .241379 .091982 .633429 13.5471
2	LI 1999 | .40404 .210072 .777107 18.0553
3	BULLETTI 1999 | .312057 .167937 .579856 17.0036
4	ROY 2010 | .463636 .308205 .697453 41.6047
6	CASINI 2006 | 1.05 .55478 1.98728 9.78933
M-H pooled RR	| .454394 .350389 .58927
	Heterogeneity chi-squared = 9.82 (d.f. = 4) p = 0.044
	Test of RR=1 : z= 5.95 p = 0.000

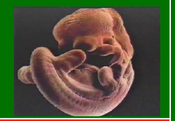
- The meta-analysis did not present intertrial qualitative heterogeneity, except for study size.
- All studies indicate a prevalence of incidence (pregnancies) in treated, ie post-intervention patients
- The relative risks of achieving pregnancy after surgery are almost all significant, except for the 2nd study (nearly 95% significant) and the 6th
- The Overall is significant and indicates a better post-intervention result, where the "RISK OF PREGNANCY" increases by 45% and is significant at 95%
- Therefore the studies are sufficiently similar to each other and allow for a global measure of the efficacy of the treatment (intervention), more precise and reproducible than that of each of the trials analysed.

Proposed mechanisms of implantation failure in gynecological diseases



Gynecological Disease	*Proposed Mechanisms of implantation failure*
Endometriosis	1.Reduced avb3 integrin and LIF expressions in the window of implantation 2.Lack of IL-11 and IL-11Ra expressions in secretory phase 3.Absence of HOXA10 and HOXA11 peak in secretory phase 4.Elevated EMX2 expression 5.Progesterone resistance 6.Alteration in PR-A to PR-B ratio 7.Decreased HOXA10 expression due to hypermethylation of its promoter region
Hydrosalpinx	1.Mechanical interference to apposition by bathing of endometrial lining with hydrosalpinx fluid intermittently 2.Reduced avb3 integrin and LIF expressions 3.Decreased HOXA10 expression
Leiomyoma	1.Distorting endometrial lining 2.Obstructing the tubal ostia or cervical canal 3.Decreased HOXA10 and BTEB1 expressions
Endometrial Polyps	1.Mechanical interference with sperm transport and embryo implantation 2.Low IGFBP-1 and osteopontin levels in secretory phase 3.Low progesterone receptor levels in secretory phase
PCOS	1.Decreased avb3 integrin, HOXA-10 and IGFBP-1 during secretory phase 2.Overexpression of androgen receptors 3.Failure to downregulate estrogen receptor-a in the window of implantation 4.Overexpression of the steroid receptor coactivators AIB1 and TIF2

Available methods to improve implantation in gynecological diseases.



Gynecological Disease	*Proposed Mechanisms of implantation failure*
Endometriosis	1.Excision or laser/diathermy ablation of endometriosis implants
Leiomyoma	1.Myomectomy
Hydrosalpinges	1.Salpingectomy 2.Proximal tubal occlusion(if salpingectomy is technically 3.difficult or not feasible)
Endometrial Polyps	1.Hysteroscopical polipectomy
Adenomyosis	1.GnRH agonist treatment 2.Surgical excision
Endometritis	1.Antibiotic Therapy
Endometrial dysfunction due to ovarian stimulation	1.Cryopreservation of embryos 2.Reduced ovarian stimulation

Main results and the role of chance:

- The congenital anomaly involved a single organ in 12 (30.8%) out of the 39 types of anomalies, while multiple organs and/or segments of Mu¨llerian ducts (complex anomaly) were involved in 27 (69.2%) types.
- Uterus was the organ most frequently involved (30/39: 76.9%), followed by cervix (26/39: 66.7%) and vagina (23/39: 59%).
- In all 39 types, the ESHRE/ESGE classification system provided a comprehensive description of each single or complex anomaly.
- A precise categorization was reached in 38 out of 39 types studied.
- Only one case of a bizarre uterine anomaly, with no clear embryological defect, could not be categorized and thus was placed in Class 6 (un-classified) of the ESHRE/ESGE system.

Congenital abnormalities of the genital tract can lead to a significant increase in spontaneous abortions and the repeated failure of embryo implantation

Uterine anomalies			Cervical/Vaginal Anomalies	
Main Class		Sub Class	Main Class	Sub Class
U0	Normal Uterus		C0	Normal Cervix
U1	Dysmorphic Uterus	A. T-Shaped B. Infantilis C. Others	C1	Septate Cervix
U2	Spetate Uterus	A. Partial B. Complete	C2	Double Normal Cervix
U3	Bicorporeal Uterus	A. Partial B. Complete C. Bicorporeal Septate	C3	Unilateral Cervical Aplasia
U4	Hemi-uterus	A. With rudimentary cavity(communicating or not horn) B. Without rudimentary cavity(horn without cavity/no horn)	C4	Cervical Aplasia
U5	Aplastic	A. With rudimentary cavity(bi or unilateral Horns) B. Without rudimentary ca…al re…		
U6	Unclassified Malformations		V0	Normal Vagina
			V1	Longitudinal non-obstructing vagina septum
			V2	Longitudinal obstructing vaginal septum
			V3	Transverse Vaginal Septum and/or Imperforate hymen

CLASSIFICATION OF CONGENITAL ANOMALIES OF THE GENITAL TRACT

UO normal uterus

U1 uterus dysmorphic

U2 Uterus Septum

U3 Double Body Uterus

U4 half Uterus

U5 uterus aplastic

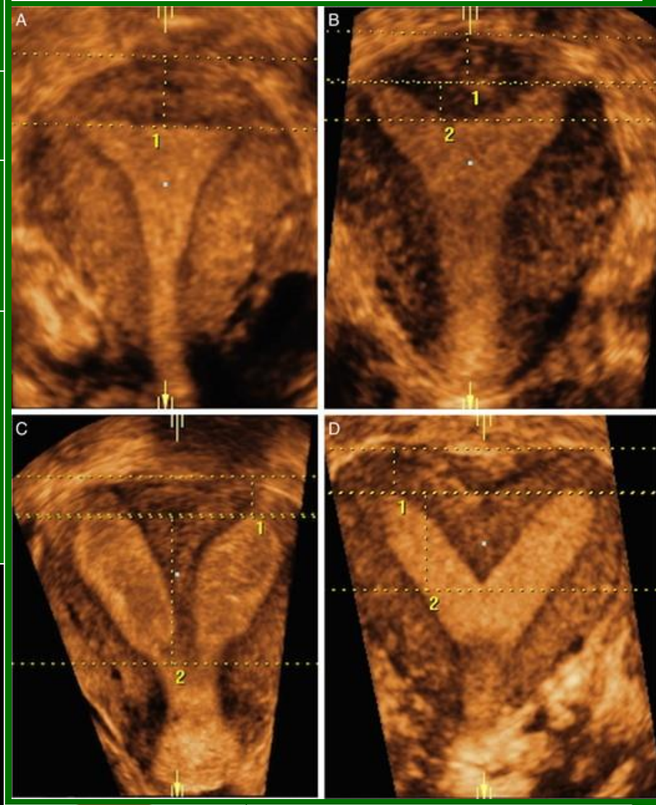
14

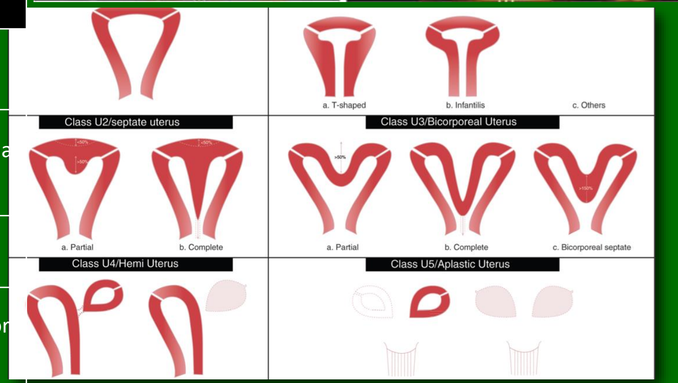
Grigoris F. Grimbizis, et al, The Thessaloniki ESHRE/ESGE consensus on diagnosis of female genital anomalies, *Human Reproduction*, Volume 31, Issue 1, January 2016, Pages 2–7, https://doi.org/10.1093/humrep/dev264

Diagnostic accuracy of magnetic resonance imaging compared with hysteroscopy ± laparoscopy in diagnosing female genital tract congenital anomalies.

Sensitivity, specificity, PPV and NPV cannot be assessed for magnetic resonance imaging as this was not used as a screening tool in the studies identified.

A Di Spiezio Sardo et al, The comprehensiveness of the ESHRE/ESGE classification of female genital tract congenital anomalies: a systematic review of cases not classified by the AFS system Human Reproduction, Vol.30, No.5 pp. 1046–1058, 2015 doi:10.1093/humrep/dev061





Factors Influencing Good Embryo Nidation

Hydrosalpinx and Endometriosis

- The presence of a hydrosalpinx decreases the implantation rate, CPR, and LBR by 50% in women undergoing IVF (Franasiak JM et al 2021; Cakmak H et al, 2010;Melo P et al, 2020). This adverse impact is mediated by hydrosalpinx fluid, which may cause direct embryotoxicity, impaired endometrial receptivity, and mechanical flushing (Franasiak JM et al 2021; Cakmak H et al, 2010;Melo P et al, 2020). **The recent Cochrane review (Melo P et al, 2020) exhibit a moderate-quality evidence that salpingectomy prior to ART probably increases the CPR compared to no surgery in women with hydrosalpinges.** When comparing tubal occlusion to no intervention, the same study report that tubal occlusion may increase CPR, although the evidence was of low quality

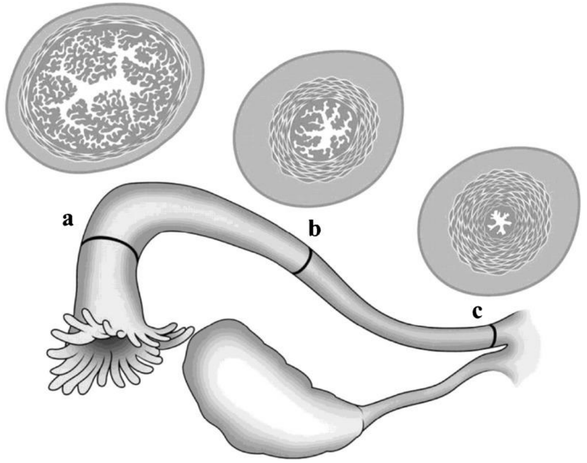
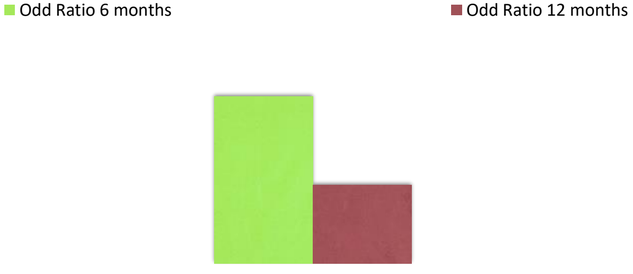


Illustration of the human fallopian tube, showing the longitudinal folds in cross-section at the (a) infundibulum, (b) ampulla and (c) isthmus. (with kind permission from Oxford University Press [3]) J Assist Reprod Genet. 2014 Oct; 31(10): 1337–1347. Published online 2014 Aug 13. doi: 10.1007/s10815-014-0309-x

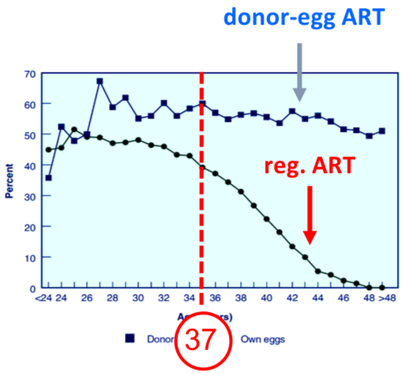


OR Recurrences of Women Who Delivered Vs Those Who Did Not

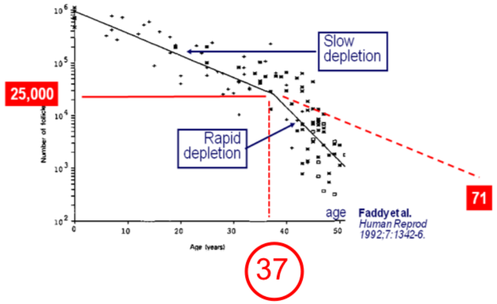
Bulletti C et al, Fertil Steril, 2005

- **Endometriosis** Ovarian steroid hormones induce proliferative (estrogens) and antiproliferative (progesterone) effects on endometrial epithelial cells of eutopic endometrium with an unique genome-wide effects in normal human endometrial stromal fibroblasts that become abnormal in endometriosis (Koninckx PR et al, 2022). The endometriotic effect of progesterone are dissociated from that of eutopic endometrium (Koninckx PR et al 2022 Vallve-Juanico J, et al 2019) Proinflammatory changes in the uterine immune profile have been observed in patients affected by endometriosis (Koninckx PR et al 2022; Vallve-Juanico J, et al, 2019; Cakmak H et al,2010) with some implication with implantation process.HOX genes are essential for endometrial growth, differentiation and receptivity by mediating some functions of the sex steroids infertility in endometriosis as the result of defective implantation. **Decreased expression of implantation markers during the mid secretory phase of menstrual cycle , silencing of the HOXA10 gene, due to hypermethylation and progesterone resistance may lead to impaired implantation in endometriosis (Cakmak H et al,2010)**

oocyte quality

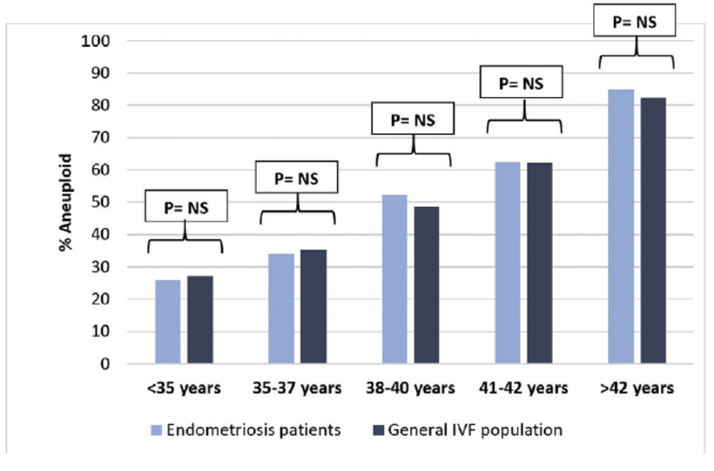


oocyte quantity

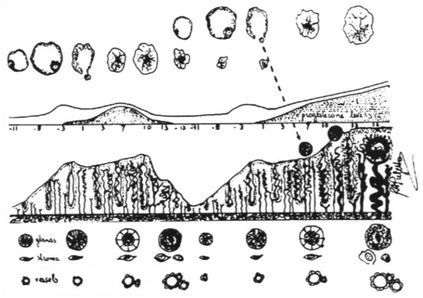


Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Adnexal pathology & Endometriosis						
Hydrosalpinx	yes	US	yes	yes	yes	yes	Franasiak JM et al 2021; Cakmak H et al, 2010;Melo P et al, 2020
Endometriosis	yes	US/ Questionnaire/Laparoscopcy	yes	yes	yes	yes	Koninckx PR et al 2022 Vallve-Juanico J, et al 2019 Cakmak H et al,2010

Embryo quality (aneuploidy rate) in endometriosis



Factors Influencing Good Embryo Nidation



Endometrium

IF

Authors(year)	Recognized Pregnancies	Unrecognized Pregnancies
Roberts CJ et al , 1975	15%-20%	78%
Miller JF et al,1980	15%-20%	43%
Edmonds et al, 1982	15%-20%	60%
Wilcox AJ et al, 1988	15%-20%	60%

Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Endometrium						
Thickness at embryo transfer	yes	US	yes	yes	yes	yes	Mahutte N, et al. 2022
Difficult embryo transfer	yes	Previous attempts	no	yes	yes	yes	Arora P et al 2018 Levi Setti PE et al , 2021
Histology In/out of phase	yes	In phase OM Features	yes	yes	yes	yes	Wang W et al, 2020 Lucas ES et al 2020 Gellersen B et al 2014 Li Y , et al 2020
Genomic endometrial assessment	no	gene sequences estabishment	yes	no	yes	yes	Díaz-Gimeno P, et al,2011 and 2013; Horcajadas JA, et al, 2005; Van Vaerenbergh I, et al 2009; Lipecki J et al 2022; Simon C, et al 2020; Cozzolino M, et al 2020; Riestenberg C, 2021; Doyle N et al, 2022
Metabolomics	may be	no	yes	no	yes	yes	Poh QH et al , 2023
PINOPODES	may be	no	yes	no	yes	yes	Quin KE et al, 2020
Endometritis							Carson SA et al, 2021; Buzzaccarini, G. et al, 2020; Franasiak JM et al, 2021; Darici E et al, 2023; Puente , et al 2020
Microbiome	May be	Still uncertain	Yes	No	Yes	Yes	Benner M et al , 2018 Franasiak JM et al, 2021

Endometrial Thickness at embryo transfer In cycles with a fresh embryo transfer, live birth rates increase significantly until an endometrial thickness of 10–12 mm, while in FET cycles live birth rates plateau after 7–10 mm. However, an endometrial thickness <6 mm was associated clearly with a dramatic reduction in live birth rates in fresh and frozen embryo transfer cycles.(Mahutte N, et al, 2022)

Difficult Embryo Transfer We concluded that cervical dilation was effective at managing difficult ET. Hegar dilators used a minimum of 3 weeks before ET showed to have higher PR Arora P, Mishra V. Difficult Embryo Transfer: A Systematic Review. J Hum Reprod Sci. 2018 Jul-Sep;11(3):229-235. doi: 10.4103/jhrs.JHRS_59_18. PMID: 30568351; PMCID: PMC6262663. The afterload technique seems to reduce the rate of difficult ETs.Levi Setti PE, Cirillo F, Morenghi E, Immediata V, Caccavari V, Baggiani A, Albani E, Patrizio P. One step further: randomised single-centre trial comparing the direct and after load techniques of embryo transfer. Hum Reprod. 2021 Aug 18;36(9):2484-2492. doi: 10.1093/humrep/deab178. PMID: 34323282.

Histology In/Out of Phase Endometrial cycle include a sequence of gene expression, omics and structural changes of epithelial, stromal and vascular cells in close relationship to the cyclical ovarian steroid productions ending and restarting with the menstruation (Wang W et al, 2020) The shedding of functional endometrium and the progressive cyclical proliferation of epithelial glandular and luminal cells offer to undifferentiated stromal cells the ground to become pre-decidual (round shape and larger)(Lucas ES et al 2020) in mid secretive phase when, usually, embryo implant. The microvascular cast supporting both basal and functional endometrium undergo intense proliferation first and size modification later. After few days priming of progesterone, within mid secretive endometrium, the presence of an interacting blastocyst switch pre-decidual into decidual cells (Gellersen B et al 2014). **Histologic endometrial dating was used to optimize timing in fertility treatments by embryo transfer in the "in phase" endometrium postulating that an out of phase endometrium fail to accept embryo nidation. Despite several inter/intra observer discrepancies still histologic endometrial dating of RIF patients in natural cycles may be used as a biomarker for a receptive endometrium in diagnosing the timing for ET (Li Y , et al 2020).**

Genomic endometrial assessment Gene expression signatures for endometrial dating have been applied to clinical practice for use as diagnostic tools; these include the ERA test (Díaz-Gimeno P, et al,2011 and 2013; Horcajadas JA, et al, 2005; Van Vaerenbergh I, et al 2009.) and Endometrial Receptivity (ER) Map (Horcajadas JA, et al, 2005; Van Vaerenbergh I, et al 2009) or other gene sequence assessment (Lipecki J et al 2022). The ER Map classifies endometrial samples as prereceptive, receptive, or postreceptive (Horcajadas JA, et al, 2005; Van Vaerenbergh I, et al 2009). Unfortunately, a number of studies showed that the test does not clearly and strongly help to improve implantation in women with infertility (Simon C, et al 2020; Cozzolino M, et al 2020; Riestenberg C, 2021) **In a recent RCT including 878 partecipants, results do not support routine use of endometrial receptivity testing to guide the timing of frozen embryo transfer** (Doyle N et al, 2022)

Metabolomic. Functionally,the signalling reciprocity of endometrial and embryo EVs regulates the site of implantation, preimplantation embryo development and hatching, antioxidative activity, embryo attachment, trophoblast invasion, arterial remodelling, and immune tolerance. This has also led to discovery of potential cargo in EVs in human uterine fluid (UF) and embryo spent media (ESM) of diagnostic and therapeutic value in implantation success, fertility, and pregnancy outcome. Poh QH, et al, Omics insights into extracellular vesicles in embryo implantation and their therapeutic utility. Proteomics. 2023 Jan 2:e2200107. doi: 10.1002/pmic.202200107. Epub ahead of print. PMID: 36591946.

Pinopodes Multiple endocrine-driven factors are important for controlling the timely receptivity of the uterus, and this complexity underscores implantation failure as a major cause of recurrent infertility associated with assisted reproductive technologies. **One particular cellular structure often hypothesized to promote receptivity is the pinopode or uterodome - a hormonally regulated, large cellular protrusion on the uterine epithelial surface. Recent clinical studies associate pinopodes with favorable fertility outcomes in women**, and because they are directly linked to an increase in progesterone levels, the potential utility of these hormone-regulated cell biological structures in predicting or improving implantation in a clinical setting holds promise.Quinn KE, et al Pinopodes: Recent advancements, current perspectives, and future directions. Mol Cell Endocrinol. 2020 Feb 5;501:110644. doi: 10.1016/j.mce.2019.110644. Epub 2019 Nov 15. PMID: 31738970; PMCID: PMC6962535.

Endometritits Chronic endometritis (CE) is a persistent inflammatory disorder of the functional and basal endometrium, characterized by infiltration of endometrial stromal plasmacytes (ESPCs) edema, high stromal cell density, dissociated epithelial/stroma differentiation (Carson SA et al, 2021; Buzzaccarini, G. et al, 2020; Franasiak JM et al, 2021). **Its prevalence is reported between 2% and 60%** (Darici E et al, 2023) with higher representation in recurrent early pregnancy (REP) and recurrent implantation failure (Puente , et al 2020) Chronic Endometritis is primarily caused by infections, which fight to the noxa by producing specific cytokine and leukocyte pattern. This immunoreaction, is counteracting a correct embryo implantation (Carson SA, et al 2021; Buzzaccarini, G., et al 2020; Franasiak JM, et al 2021). **Untill we do not standardize the chronic endometritis features, RCT for adequate medicalization with reproductive success would be not established by using selected patient populations who failed implantation of euploid embryos but only by evaluating histological disappearance of plasma cells as the primary outcome parameter, the evidence for diagnostic and therapeutical tools proposed for ART couples is very questionable (Darici E et al, 2023) A significant delay in the initiation of reproductive treatments with the relative time to pregnancy prolongation, dramatic changes of healthy uterine bacterial population with wide spectrum antibiotic use, antibiotic resistance, additional expenses required, and the invasive endometrial biopsy and hysteroscopy should be not ignored (Darici E et al, 2023).**

Microbiome . An endometrial microbial composition with high numbers of Lactobacillus is associated with successful implantation, whereas a composition with a lower number of Lactobacillus and high percentage of Gardnerella vaginalis and Streptococci resulted in adverse reproductive outcome (Benner M et al , 2018). For studies investigating RIF, the number of vaginal Lactobacillus, compared with endometrial lactobacilli, seems to be a predictive biomarker (Franasiak JM et al, 2021). **However, based on current data, future research needs to include the uterine microbiome as a relevant factor in order to understand the players needed for healthy pregnancy.**

Difficult Transfer

- **Choosing different catheters**
- **Pre-medicalization with uterorelaxant**
- **The use of tentacular traction and rigid catheters**
- **Dilatation without anestesia**
- **Delayed ET and dilatation of the cervix under anestesia**
- **Delayed ET and Hysteroscopy**
- **Trans-myometrial ET**
- **Tubal ET**

Seven RCTs have been identified comparing a number of different catheters. The results of these trials suggest that the choice of embryo transfer catheter can affect pregnancy rates.

In particular, **data from large trials suggest that certain types of soft catheter are more effective that other types of catheter. [Evidence level 1b**]

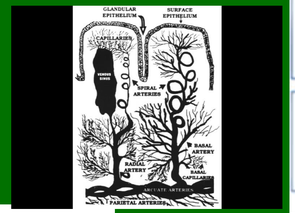
Data from the various studies could not be aggregated due to significant clinical heterogeneity and differences between individual catheters.

Endometrial Thickness

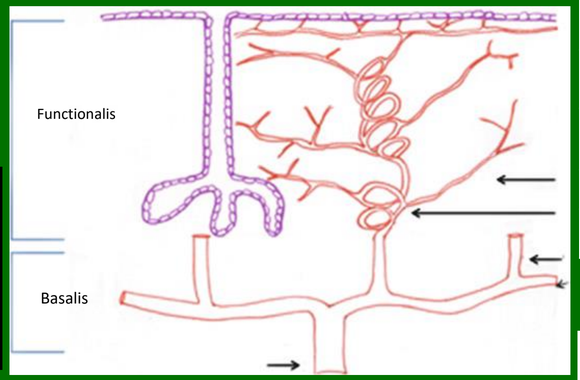
Clinical outcomes in fresh IVF-ET cycles by endometrial thickness

Endometrial thickness (mm)	Clinical pregnancy rate	Live birth rate	Pregnancy loss rate	Mean term singleton birth weight in grams (SD)
≥18	44.9% (105/234)	33.8% (79/234)	34.7% (42/121)	3,400 (427)
16–17.9	47.6% (288/605)	37.7% (228/605)	31.7% (106/334)	3,310 (395)
14–15.9	43.8% (966/2,207)	33.7% (743/2,207)	34.3% (388/1,131)	3,399 (420)
12–13.9	43.0% (2,899/6,739)	33.4% (2,250/6,739)	34.0% (1,159/3,409)	3,351 (434)
10–11.9	41.1% (5,620/13,672)	31.8% (4,345/13,672)	34.0% (2,239/6,584)	3,337 (430)
8–9.9	37.5% (5,415/14,444)	28.1% (4,059/14,444)	37.5% (2,432/6,491)	3,317 (427)
6–7.9	31.0% (1,574/5,084)	22.1% (1,126/5,084)	42.2% (822/1,948)	3,262 (438)
4–5.9	24.4% (97/398)	15.8% (63/398)	54.3% (75/138)	3,215 (547)
*P**	<.001	<.001	<.001	<.001

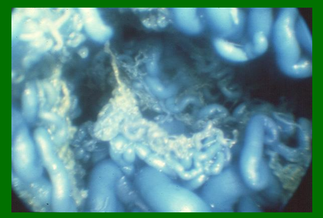
Neal Mahutte, et al Optimal endometrial thickness in fresh and frozen-thaw in vitro fertilization cycles: an analysis of live birth rates from 96,000 autologous embryo transfers, Fertility and Sterility, Volume 117, Issue 4, 2022, Pages 792-800, ISSN 0015-0282, https://doi.org/10.1016/j. fertnstert.2021.12.025. (https://www.sciencedirect.com/science/article/pii/S0015028221023189)

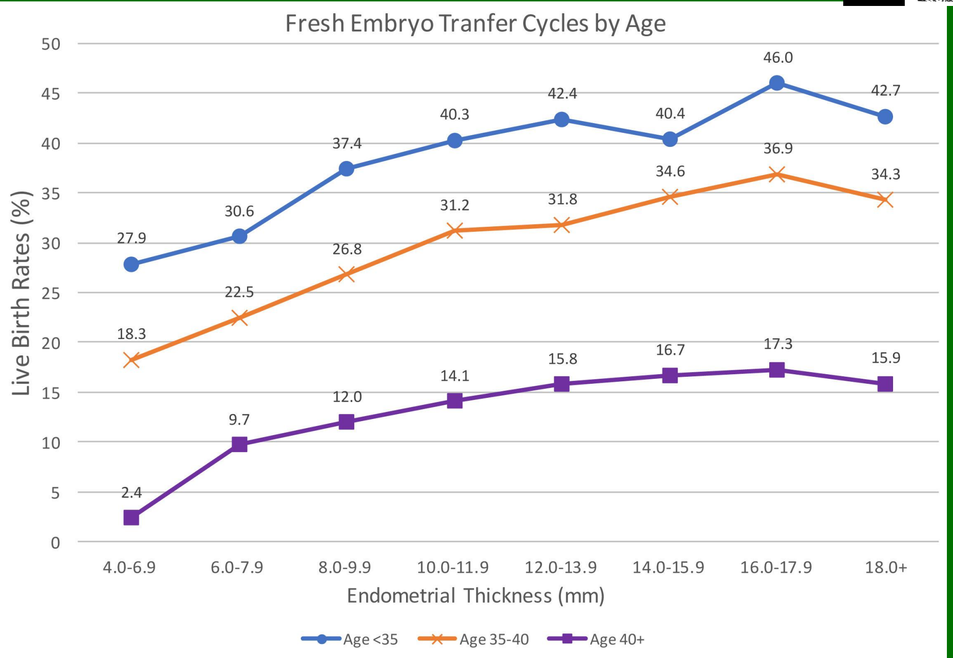


Endometrium



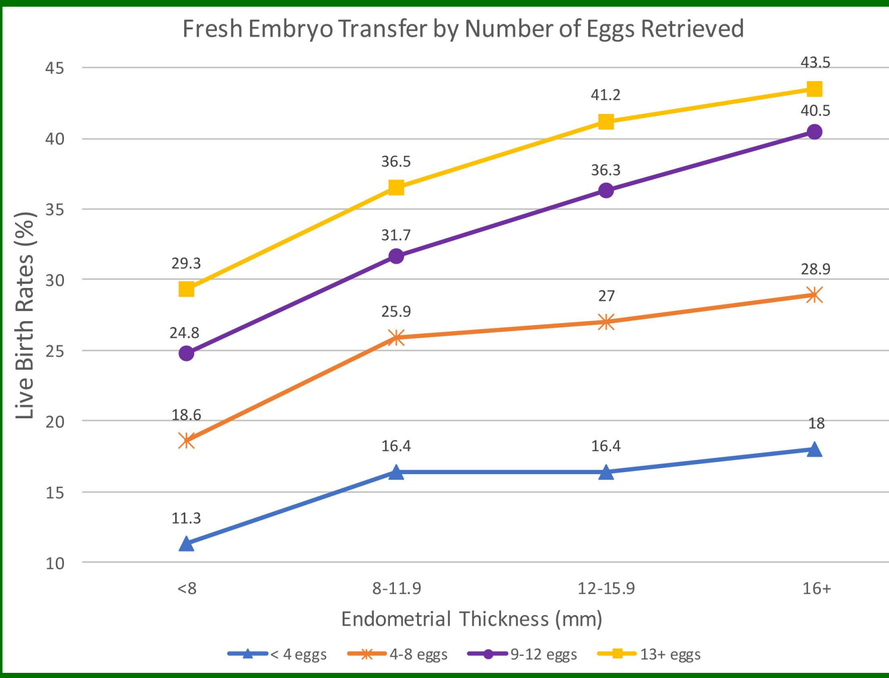
Myometrium



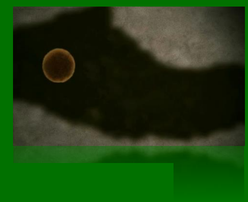


Clinical outcomes in FET cycles by endometrial thickness

Endometrial thickness (mm)	Clinical pregnancy rate	Live birth rate	Pregnancy loss rate	Mean term singleton birth weight in grams (SD)
≥18	44.1% (60/136)	30.9 (42/136)	41.7% (30/72)	3,496 (432)
16–17.9	45.0% (159/353)	32% (113/353)	38.9% (72/185)	3,529 (563)
14–15.9	42.1% (604/1,434)	29.2% (419/1,434)	41.6% (299/718)	3,474 (450)
12–13.9	41.9% (2,134/5,094)	30.7% (1,566/5,094)	38.9% (998/2,564)	3,486 (441)
10–11.9	42.3% (5,728/13,539)	30.8% (4,169/13,539)	40.8% (2,875/7,044)	3,452 (442)
8–9.9	40.7% (10,218/25,089)	29.4% (7,375/25,089)	41.3% (5,197/12,572)	3,451 (445)
7–7.9	39.3% (2,476/6,302)	28.4% (1,791/6,302)	41.9% (1,293/3,084)	3,407 (424)
6–6.9	31.5% (334/1,059)	22.6% (239/1,059)	46.0% (204/443)	3,378 (440)
<6	29.1% (108/371)	15.1% (56/371)	62.2% (92/148)	3,412 (394)
*P**	<.001	<.001	<.001	<.001



Embryo Transfer - Nice Clinical Guidelines



- Ultrasound-guided embryo transfer is a complex intervention. Four RCTs and four quasiRCTs comparing ultrasound-guided embryo transfer versus clinical touch embryo transfer were identified. [Evidence level 1b–2a]
- We performed a meta-analysis using data from all eight studies. This showed a significant increase in pregnancy rates with routine ultrasound-guided embryo transfer (pooled OR 1.46, 95% CI 1.25 to 1.70, n = 3358 embryo transfers).
- When the quasi-RCTs were excluded, there was still a significant increase in pregnancy rates with routine ultrasound-guided embryo transfer (pooled OR 1.42, 95% CI 1.17 to 1.73, n = 2051 embryo transfers).
- Overall, the meta-analyses suggest that use of ultrasound at the time of embryo transfer increases pregnancy rates.
- **However, there was clinical heterogeneity among different groups of women and in the specific role of ultrasound in each trial. [Evidence level 1a]**

Embryo transfer techniques Use of ultrasound

Arch Gynecol Obstet. 2015 Aug;292(2):255-62. doi: 10.1007/s00404-015-3657-6. Epub 2015 Feb 17.
Methods employed to overcome difficult embryo transfer during assisted reproduction treatment.
Akhtar MA et al

Multiple steps, at both scientific and clinical level, are involved in assisted reproduction technology (ART). Optimization of each of these steps contributes to maximize the success of ART. Embryo transfer is one of the vital steps in ART. An easy embryo transfer increases the success of ART. Adequate training is required to undertake embryo transfer, but **anatomical difficulties in completing this procedure alter the success of treatment**. Difficult embryo transfer is challenging for clinicians but has an overwhelming negative impact on patients. **Difficult embryo transfer may cause cervical or endometrial trauma with uterine contractions which can lead to unsuccessful implantation and poor outcome after ART.**

METHODS:

Literature review of published material looking at all interventions that were employed to overcome difficult embryo transfers during ART.

RESULTS:

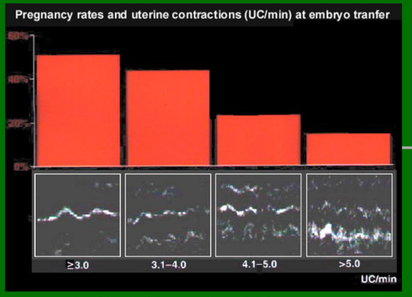
We identified 5 methods (17 studies) were employed to overcome difficult embryo transfer which are: Hysteroscopic methods in 4 studies (2 case reports, 2 case series). Malecot catheters after hysteroscopic evaluation were used in 2 studies (1 case report, 1 case series). Dilators including hygroscopic dilators were used in 2 studies (1 case report, 1 case series) and mechanical dilators were used in 3 studies (2 case series, 1 RCT-patients 367). Intrafollopian transfer was reported in 1 case report. Transmyometrial embryo transfers were reported in 5 studies (2 case reports, 2 case series, 1 RCT).

CONCLUSION:

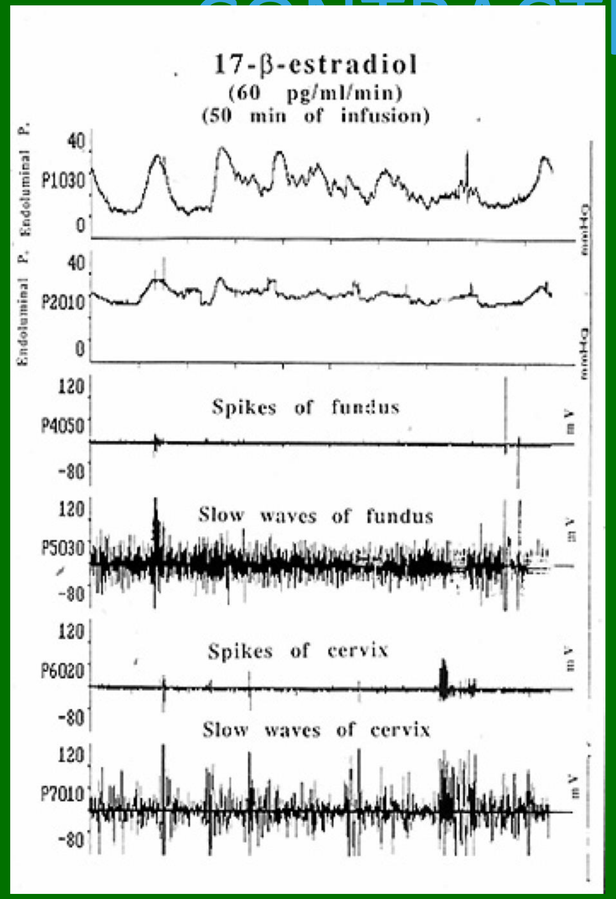
There were only two randomized controlled studies (RCTs) identified in the review. All other were either case series or case reports. We found that there was no uniform classification or grading of difficulty of embryo transfer in the literature. A grading system has been suggested in this review. We recommend that there should be a consensus guideline formulated for interventions to overcome difficult embryo transfer. A large multicenter randomized controlled study is required to compare different methods for women with difficult embryo transfer.

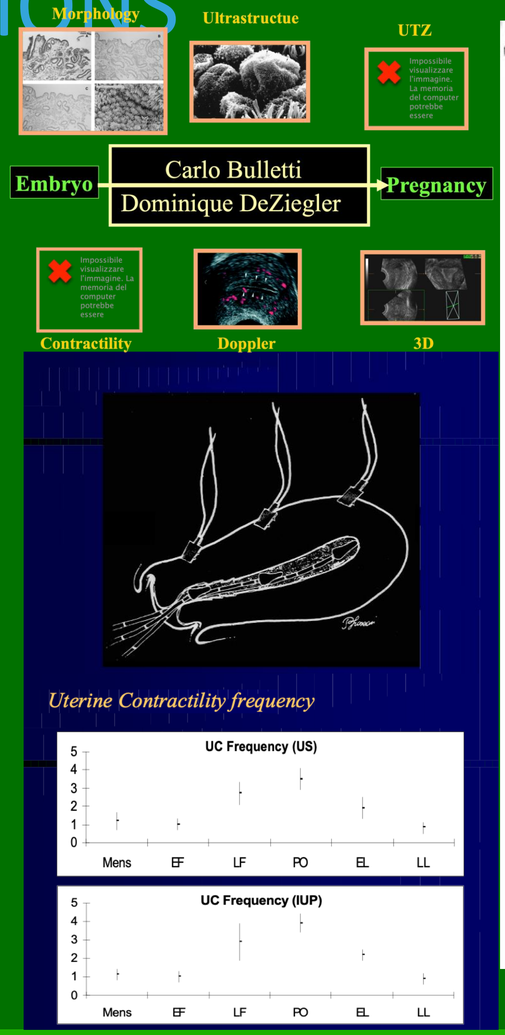
In a **prospective cohort study Atosiban reduced the number of uterine contractions in these patients and also increased the implantation and pregnancy rates. The pregnancy rate went from zero to 43.7%. The beneficial effects of atosiban were observed not only in patients who had a high frequency of uterine contractions at baseline but also in those who had a low frequency. These findings suggest that atosiban may have other benefits in addition to its effect on contractions of the uterus. More studies are required to find out exactly how atosiban works and to increase the knowledge of its use in patients with RIF undergoing IVF/embryo transfer.** Lan VT **et al, 2012 RBMonline**

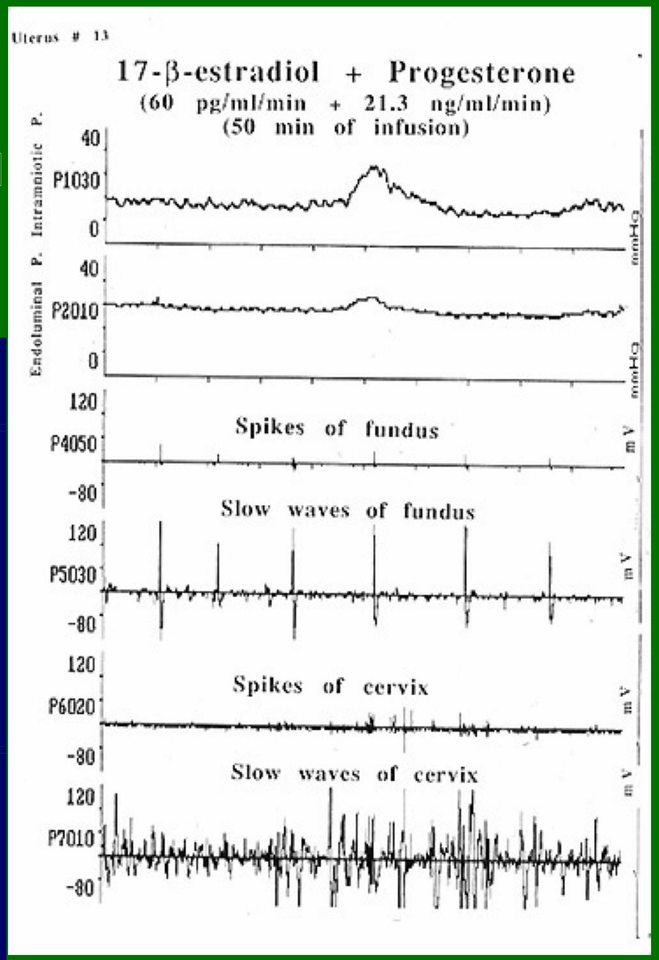
STEROID HORMONES & UTERINE CONTRACTIONS



Fanchin, et al, Human Reproduction







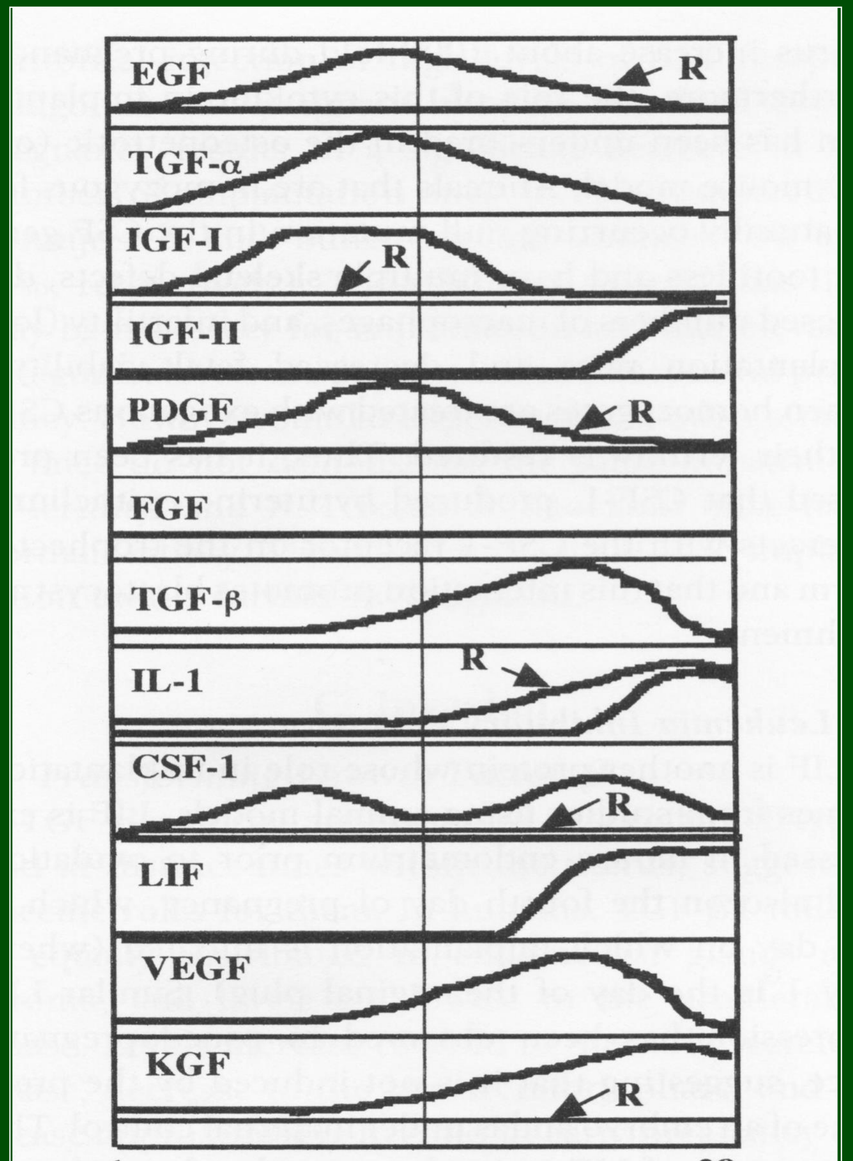
Hum Reprod. 1993 Oct;8(10):1558-63.
Electromechanical activities of human uteri during extra-corporeal perfusion with ovarian steroids.
Bulletti C, et al

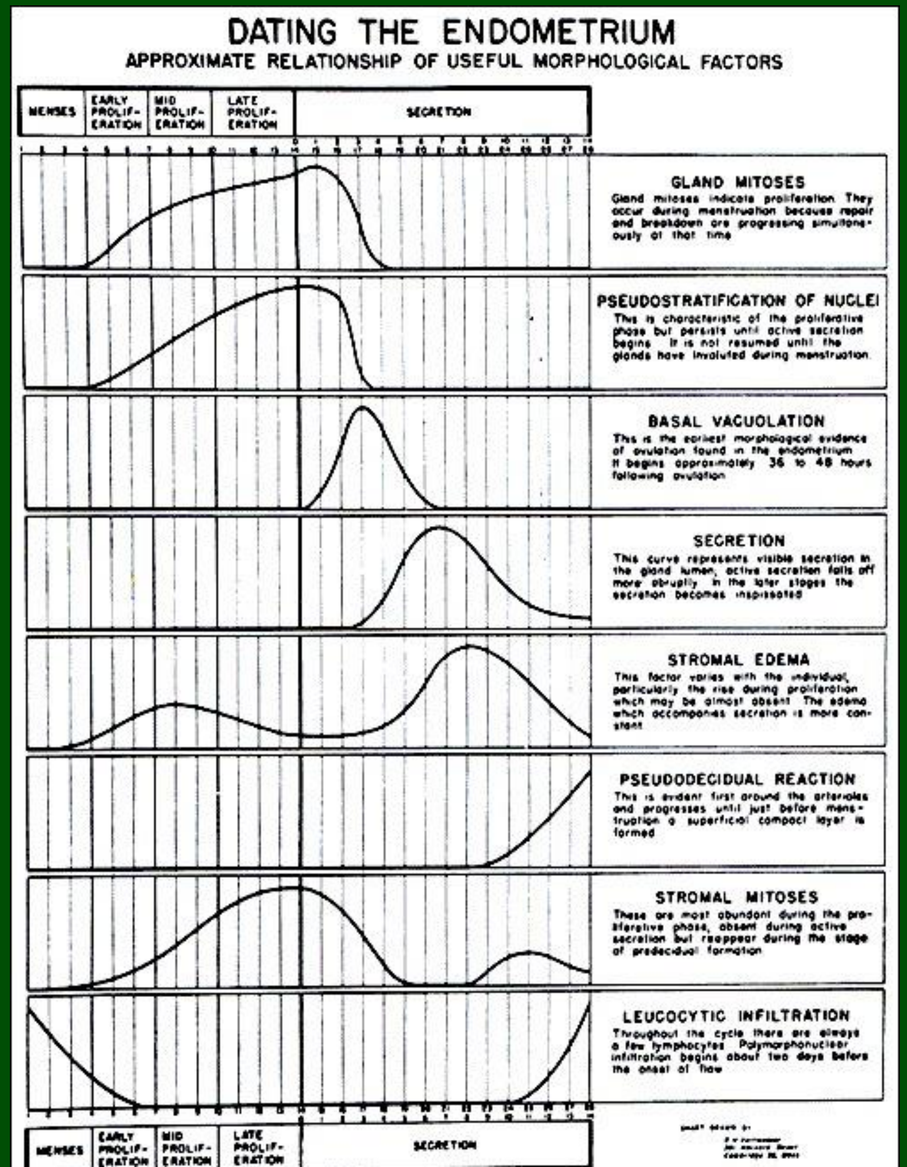
Endometritis

It is a treatable disease?

- Chronic endometritis (CE) is a persistent inflammatory disorder of the functional and basal endometrium, characterized by infiltration of endometrial stromal plasmacytes (ESPCs) edema, high stromal cell density, dissociated epithelial/stroma differentiation (Carson SA et al, 2021; Buzzaccarini, G. et al, 2020; Franasiak JM et al, 2021).
- Its prevalence is reported between 2% and 60% (Darici E et al, 2023) with higher representation in recurrent early pregnancy (REP) and recurrent implantation failure (Puente , et al 2020)
- Chronic Endometritis is primarily caused by infections, which fight to the noxa by producing specific cytokine and leukocyte pattern.
- This immunoreaction, is counteracting a correct embryo implantation (Carson SA, et al 2021; Buzzaccarini, G., et al 2020; Franasiak JM, et al 2021). Endometrial histological analysis, with detection of plasma cells within endometrial stroma is the feature's diagnostic marker.
- Untill we do not standardize the chronic endometritis features, RCT for adequate medicalization with reproductive success would be not established by using selected patient populations who failed implantation of euploid embryos but only by evaluating histological disappearance of plasma cells as the primary outcome parameter, the evidence for diagnostic and therapeutical tools proposed for ART couples is very questionable (Darici E et al, 2023)
- The significant delay in the initiation of reproductive treatments with the relative time to pregnancy prolongation, dramatic changes of healthy uterine bacterial population with wide spectrum antibiotic use, antibiotic resistance, additional expenses required, and the invasive endometrial biopsy and hysteroscopy should be not ignored (Darici E et al, 2023).

Ednometrium in
Repeated Implantation Failure (RIF) & Recurrent Pregnancy Loss(RPL





Factors Influencing Good Embryo Nidation

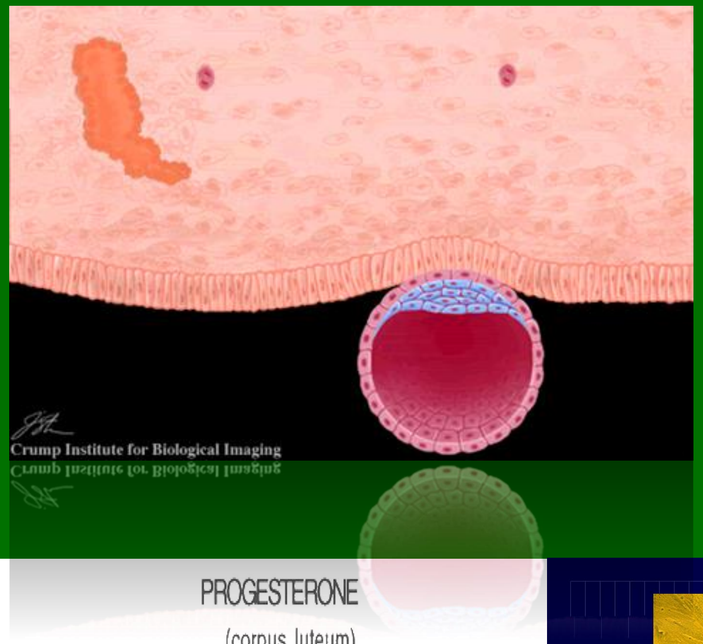
Systemic Factors

- **Trombophilia**The association of their impact on RIF has been proposed with possible mechanism of the impairment of the initial vascularization process of the embryo because of disturbed blood flow to decidual or chorionic vessels (Simon A et al 2012; Norwitz ER et al 2001; Shaulov T et al, 2020; Qublan H et al, 2008) **Testing for inherited or acquired thrombophilia in patients with RIF is not recommended because there is insufficient evidence that patients with RIF are more likely to be affected by either inherited or acquired thrombophilias** In an RCT of 83 women with a history of 3 or more previous IVF failures who carried a diagnosis of an acquired or inherited thrombophilia, prophylactic enoxaparin from the day of embryo transfer until delivery or fetal demise significantly improved the implantation rates, CPRs, and LBRs (Simon A et al 2012; Norwitz ER et al 2001; Shaulov T et al, 2020; Qublan H et al, 2008). **However no definitive clinical evidence currently exists to suggest empiric treatment for patients with RIF without diagnosed trombophilia**
- **Immunological Factors** Trophoblast invasion is a complex process involving interactions with stromal cells, glands, arteries, macrophages, and uterine natural killer (uNK) cells. **Abnormal immune responses are described in women with uterine tissue pathologies such as adenomyosis, endometriosis, and chronic endometritis (CE) (Bourdon M et al, 2021, Li Y et al, 2020; Vallve-Juanico J et al, 2019) or systemic disorders such as obesity (Comstock IA et al , 2017), polycystic ovary syndrome (PCOS) (Liu S et al, 2021), and autoimmunity (Khizroeva J et al 2019). Unfortunately there are not validated therapy reverting implantation failures with successful embryo nidation.**
- **Progesterone Serum Levels at The trigger** Progesterone is a pre-requisite for embryo implantation and its absence for pregnancy loss (Csapo AI et al , 1972 and 1973) . **However supraphysiologic estrogenic plasma levels during controlled ovarian hyperstimulation may result in a premature rise of progesterone levels and secretory transformation with lower efficiency of good embryo transfer (Bosch E et al, 2010).**
- **Progesterone serum levels at the mid secretory phase** In frozen embryo transfer (FET) cycles obtained with hormonal replacement therapy (HRT), low serum progesterone levels on the day of embryo transfer (ET) is associated with poorer pregnancy outcome thus serum progesterone levels across luteal phase days are associated with pregnancy outcome: **serum progesterone might be playing an important role not only during implantation, but also in pregnancy maintenance (Labarta et al, 2021) and this should be related to unknown extra uterine factors because of higher endometrial concentration of progesterone administered by vaginal route (Bulletti C et al, 1997) versus both oral or IM (Miles RA et al, 1994) and the similar IR between ET after endometrial preparation with vaginal versus vaginal plus IM progesterone administration every three days with a significant increase of life birth rate (LBR) in this last group.**
- **TSH. Sub clinical hypothyroidism is related to lower implantation rate and the tyrosine replacement therapy is able to restore normal values of implantation rate.** That when the hypothyroidism come out with or without the autoimmune stigma with different degree of evidence(Unuane D, et al 2020). Hypothyroidism and hyperthyroidism can result in menstrual irregularities and anovulatory cycles, thus affecting the fertility. There is a significant high prolactin (PRL) level in infertile women with hypothyroidism when compared to euthyroid patients, indicating the relation between hypothyroidism and hyperprolactinemia. The etiology of infertility is multifactorial with thyroid disorders as the most common presenting factor, hypothyroidism in particular, with or without abnormal presence of antibodies. Women planning for pregnancy and infertile women should be assessed for thyroid hormones and serum PRL and those who are looking for babies should restore TSH <2,5 .(Koyyada A, Orsu P. Role of hypothyroidism and associated pathways in pregnancy and infertility: Clinical insights. *Tzu Chi Med J*. 2020;32(4):312-317. Published 2020 Apr 10. doi:10.4103/tcmj.tcmj_255_19).

Cause	Causal relationship	Diagnostic method	Can be diagnosed before RIF?	Evidence-based treatment	Can be addressed before first ART cycle?	RIF alters management?	Refrences
	Systemic Factors						
Trombophilia	yes	blood samples analysis	yes	Insufficient	yes	yes	Simon A et al 2012; Norwitz ER et al 2001; Shaulov T et al, 2020; Qublan H et al, 2008
Immulogical factors	yes	blood samples analysis	yes	yes	yes	yes	Bourdon M et al, 2021, Li Y et al, 2020; Vallve-Juanico J et al, 2019; Comstock IA et al , 2017; Liu S et al, 2021; Khizroeva J et al 2019
Progesterone serum levels at the trigger	yes	blood sample	yes	yes	yes	yes	Csapo AI et al , 1972 and 1973; Bosch E et al, 2010
Progesterone serum levels at midsecretory or +5/6 d with P supplementation of natural, and HRT cycles	yes	blood sample	yes	yes	yes	yes	Labarta et al, 2021; Bulletti C et al, 1997; Miles RA et al, 1994
TSH	yes	blood samples	yes	yes	yes	yes	Unuane D, et al 2020; Koyyada A

Decidualization

- Stromal to decidual cells differentiation
- Angiogenesis to build up endometrial network usefull to support embryos implantation
- Epithelial Cell Differentiation



Human chorionic gonadotrophin or progesterone versus no treatment in down regulated cycles

- A meta-analysis of 18 RCTs showed significantly higher pregnancy rate per cycle in women treated with hCG compared with no treatment (OR 1.9, 95% CI 1.3 to 3.1, based on five RCTs) when used with GnRH agonist. [Evidence level 1a]
- A significantly higher pregnancy rate per cycle was also found in groups treated with intramuscular or oral progesterone compared with no treatment (OR 1.2, 95% CI 1.0 to 1.7, based on eight RCTs). n down regulated cycles

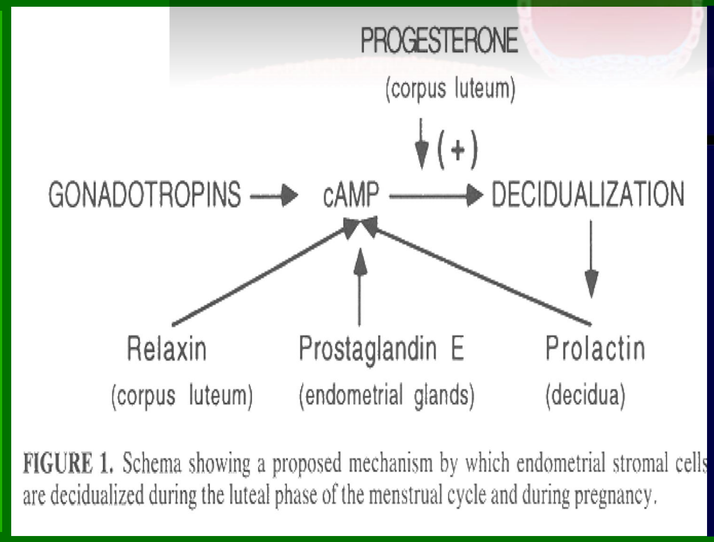
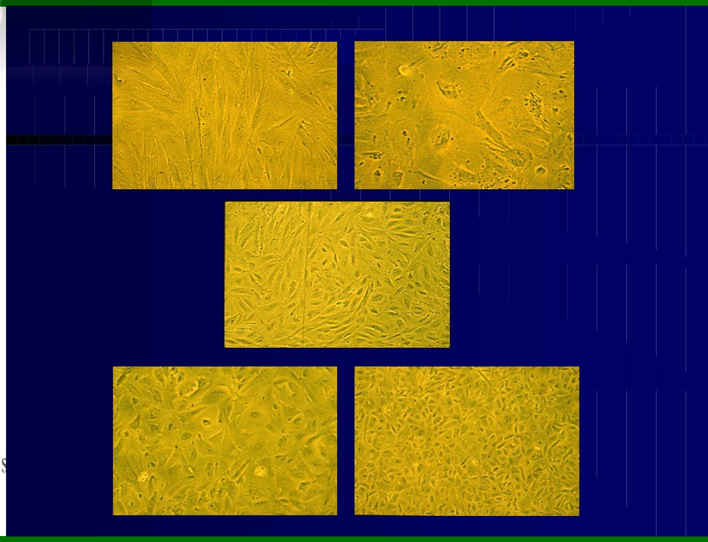


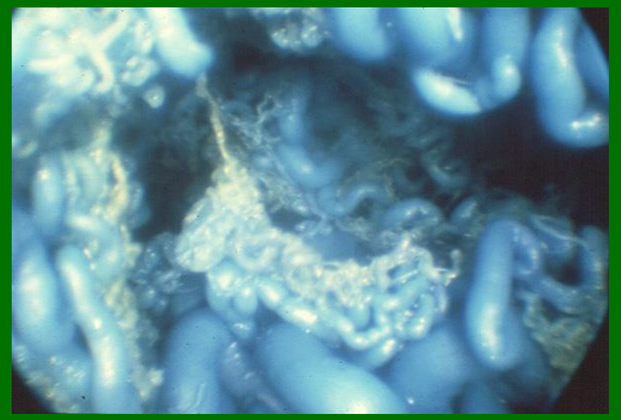
FIGURE 1. Schema showing a proposed mechanism by which endometrial stromal cells are decidualized during the luteal phase of the menstrual cycle and during pregnancy.

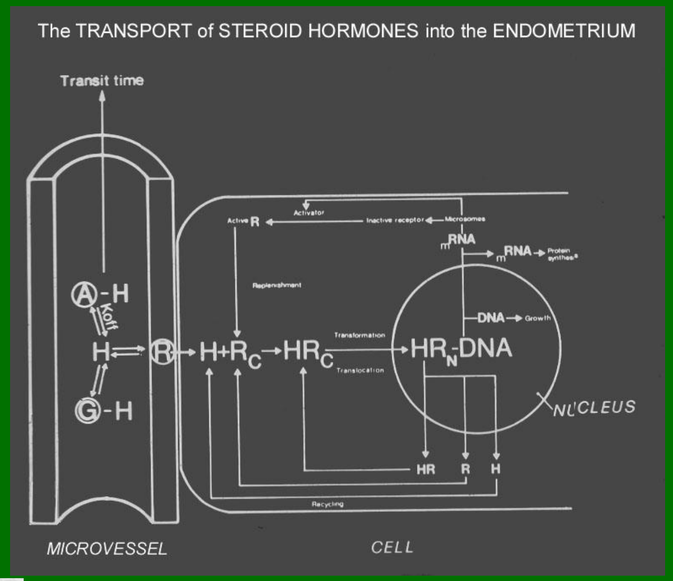


Endocrine, paracrine and autocrine functional controls

ormones:

- Production Rate
- Metabolic Clearance Rate
- Circulating Serum levels
- Transport from circulation to the target tissues





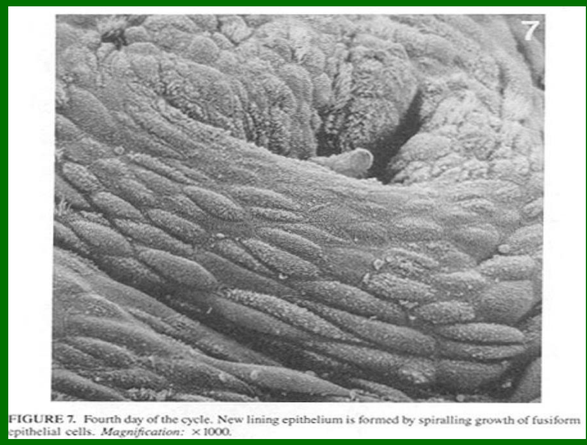
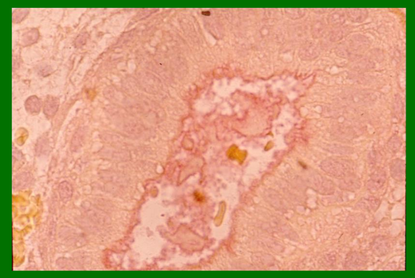
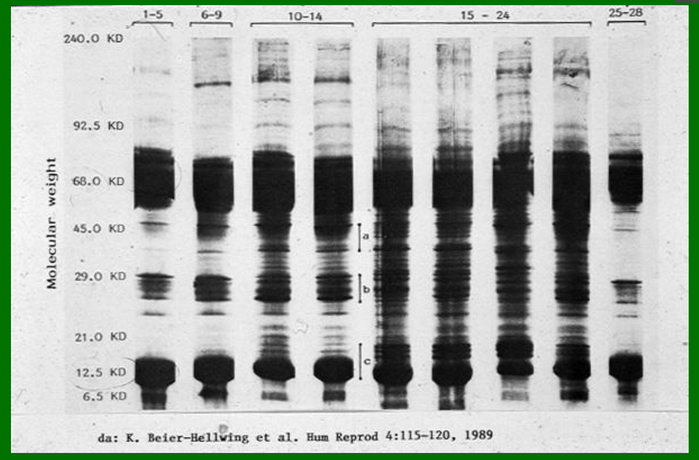
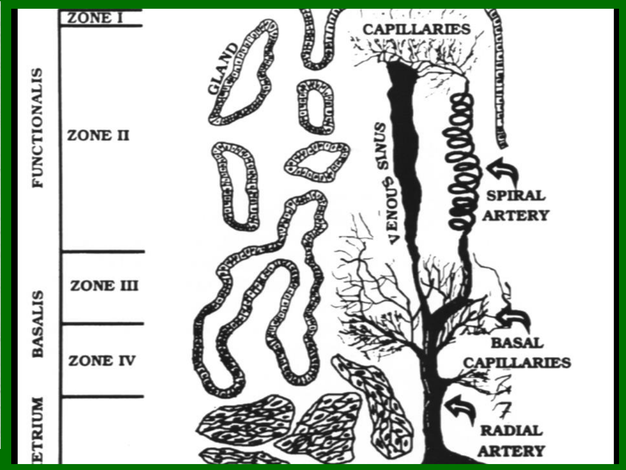


FIGURE 7. Fourth day of the cycle. New lining epithelium is formed by spiralling growth of fusiform epithelial cells. *Magnification:* ×1000.





da: K. Beier-Hellwing et al. Hum Reprod 4:115-120, 1989



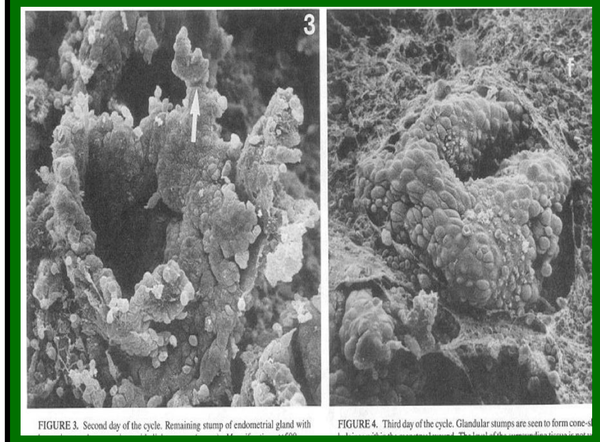


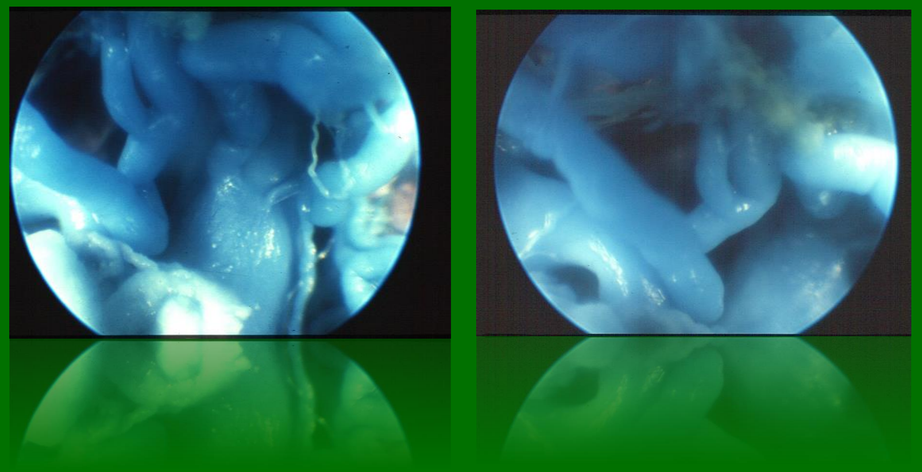
FIGURE 3. Second day of the cycle. Remaining stump of endometrial gland with

FIGURE 4. Third day of the cycle. Glandular stumps are seen to form cone-s

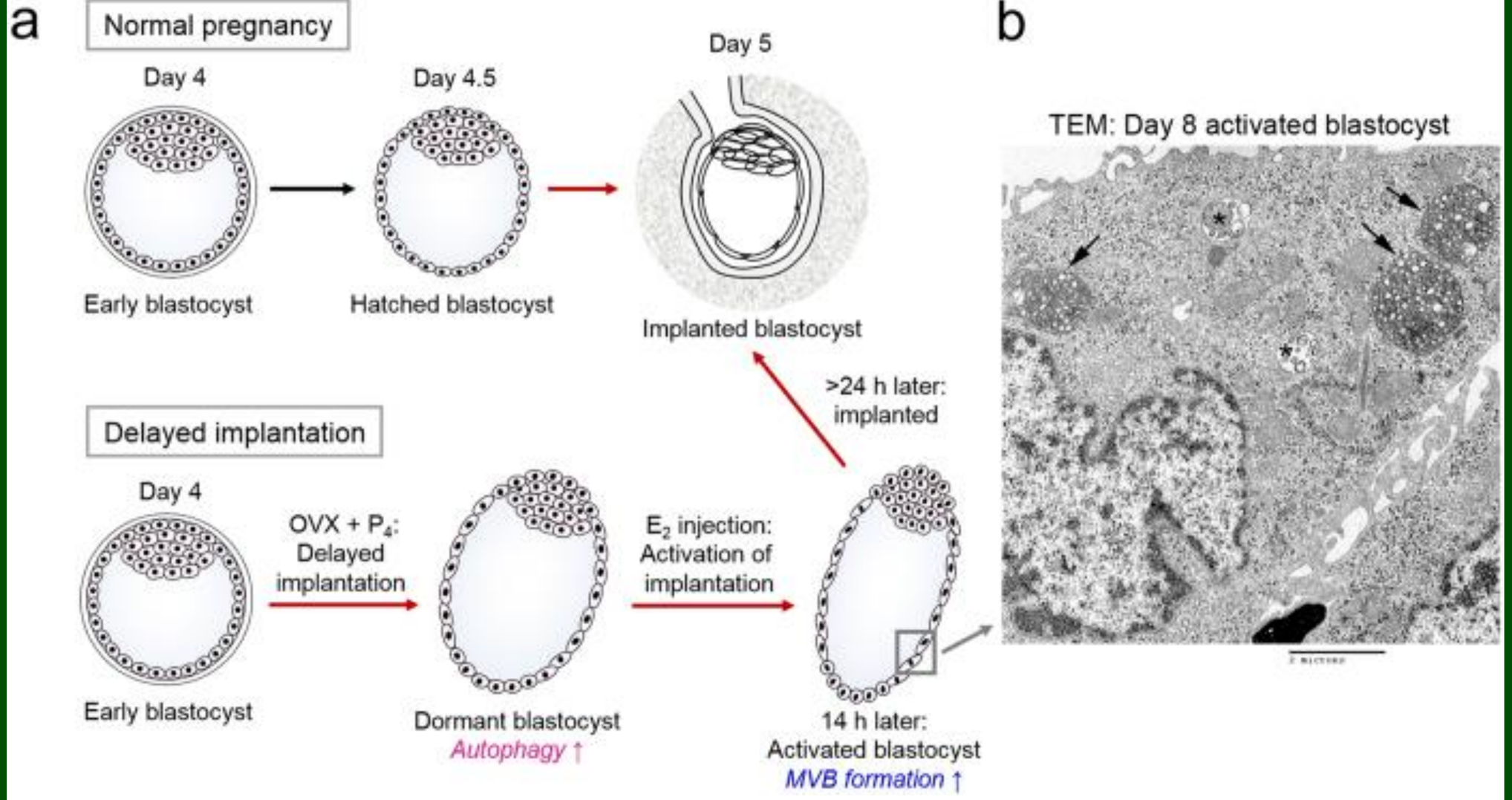
Consideration of clinical benefits and harms

How Progesterone effect on Human Uterus

- **Antiproliferative antagonist to estrogens(Lobo RA , 1992)**
- **Uterine Vascular Changes**
- **Endometrial Preparation to nidation (Navot D, 1986; Bergeron E, 2000)**
- **Reduction of uterine contractions (Bulletti C et al 1993, 2000)**
- **Inhibition of mother embryo's toxicity on embryonic trophoblast (PK siiteri, 1983)**



- **Triggering the Ovulation.** Despite LH should be the best determinant and GnRHa remain the most "physiological" signal, hCG remain the most used drug. Further studies are going on to elucidate the best practice.
- **Luteal phase support compared with no support** There is evidence that luteal phase support with progesterone is associated with significantly more live full-term singleton births and clinical pregnancies than placebo or no support.
- **Choice of drugs** There was no significant difference in the number of clinical pregnancies and live full-term singleton births when comparing the different types of drugs that are used for luteal phase support. However, the evidence showed that using hCG for luteal phase support was associated with an increased risk of OHSS compared with the use of progesterone.
- **Duration of support** Offering luteal phase support for an extended period of time did not appear to result in more clinical benefits, or to cause more harm, than a short period of luteal phase support. However, the evidence reported in this area is limited. It is biologically plausibile for luteal phase support to be effective for up to 8 weeks after embryo transfer, after which time the pregnancy is self supporting. Women should be informed that there is no evidence for continuing luteal phase support beyond 8 weeks.



Mouse model of implantation and delayed implantation.

Mouse embryos escape the zona pellucida on day 4.5 of pregnancy and implant. **In mice, implantation requires ovarian estrogen secreted on the morning of day 4. Ovariectomy (OVX) on the morning of day 4 of pregnancy removes the source of estrogen and thus delays implantation.** Pregnancy was maintained by daily injections of progesterone (P4) **but blastocysts enter a dormant state ("dormant blastocyst").** These embryos live longer than normal blastocysts and exhibit heightened autophagic activation . **When an injection of 17β-estradiol (E2) is given, dormant blastocysts are activated and implantation is initiated ("activated blastocyst").** We previously showed that dormant blastocysts exhibit heightened autophagy, and activated blastocysts accumulate MVB in the trophectoderm. (**b**) Transmission electron microscopy analysis of a day 8 activated blastocyst. A trophectoderm cell is shown at 3000X. Arrows, multivesicular bodies (MVB); asterisks, late endosomes.

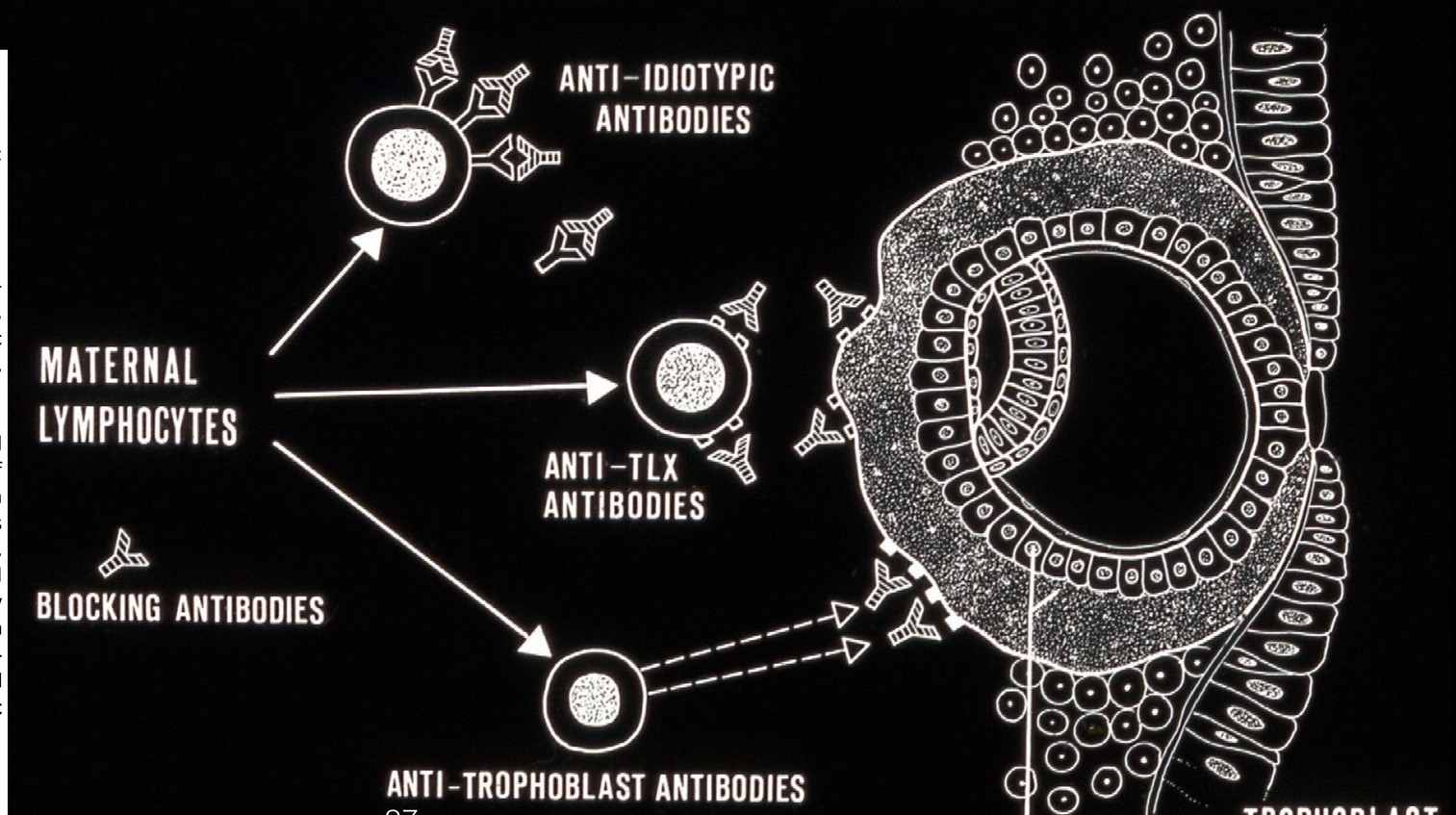
Shin H et al Sci Rep. 2017 Feb 3;7:41986. doi: 10.1038/srep41986. The formation of multivesicular bodies in activated blastocysts is influenced by autophagy and FGF signaling in mice.

Leukemia Inhibitory Factor

Embryo Implantation is Blocked by Intraperitoneal injection of Anti-LIF Antibody in Mice
Terakawa J et al, J of Reproduction and Development 57(6), 2011

- 7.5 mg/g body weight 3 times between D3 and D4 effectively blocked embryo implantation
- Growth-arrested blastocysts were recovered from the uterus whitout any implantation sites in both strains.
- Uterine stromal cells did not undergo decidual transformation

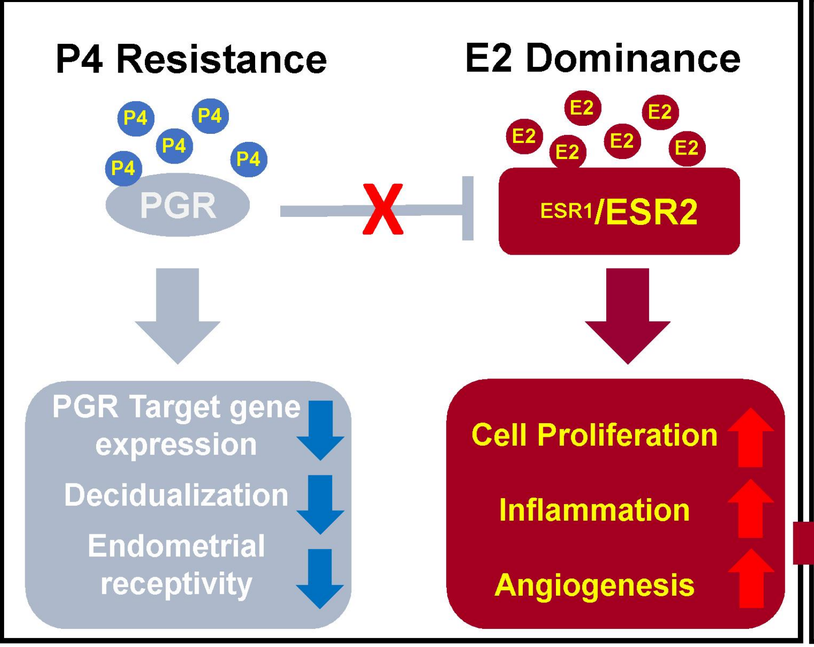
Preventing of Embryo Loss By Blocking antibodies

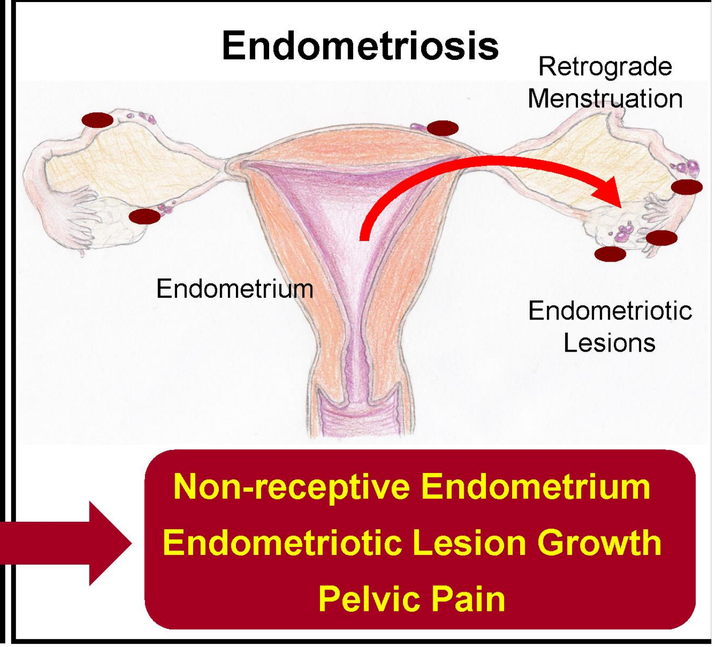


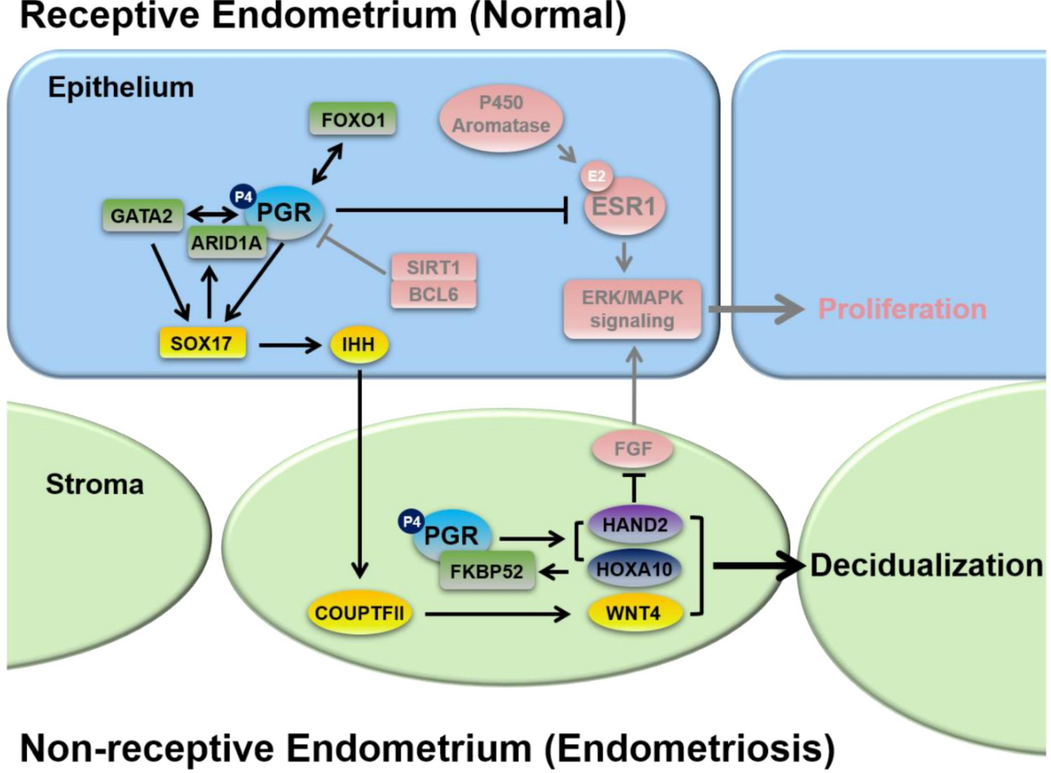
Best Pract Res Clin Obstet Gynaecol. 2019 Jul 5. pii: S1521-6934(19)30089-6. doi: 10.1016/j.bpobgyn.2019.07.001. [Epub ahead of print]
Progestogens and immunology.
Szekeres-Bartho J1, Schindler AE2.

Department of Medical Biology, Medical School, Pecs, Hungary; MTA - PTE Human Reproduction Research Group, Hungary; János Szentágothai Research Centre, University of Pecs, Hungary; Endocrine Studies, Centre of Excellence, Hungary. Electronic address: Szekeres.julia@pte.hu. Institute for Medical Research and Education, Essen, Germany.

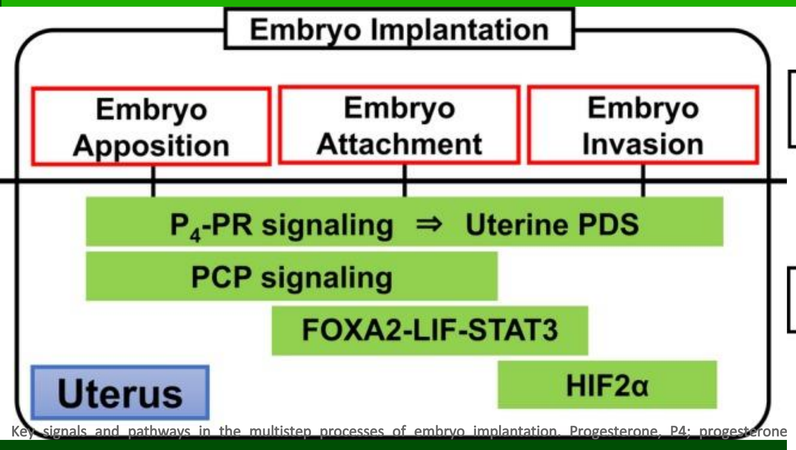
Abstract
Fifty percent of fetal antigens are of paternal origin. These are recognized by the maternal immune system, thereby resulting in lymphocyte activation and the induction of progesterone receptors (PRs) in immune cells. Upon binding of progesterone to PRs on lymphocytes, a downstream mediator called **progesterone-induced blocking factor (PIBF)** is produced. The full-length PIBF is a 90 kDa protein; however, because of alternative splicing, several smaller isoforms are also produced. While the 90 kDa molecule plays a role in cell cycle regulation, the small isoforms are localized in the cytoplasm, and after secretion, they bind to their receptors on other cells and act in a cytokine-like manner. **The communication between the embryo and the maternal immune system is established through PIBF-containing extracellular vesicles. PIBF induces an increased production of Th2 cytokines and inhibits degranulation of NK cells, and by regulating the maternal immune response, it contributes to successful implantation and maintenance of pregnancy.**



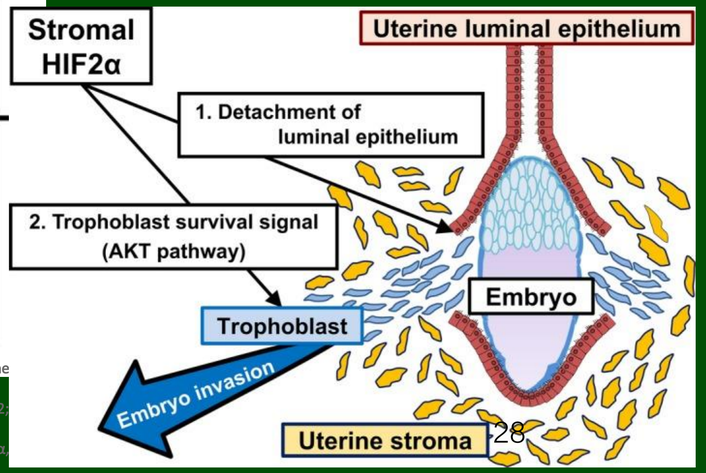


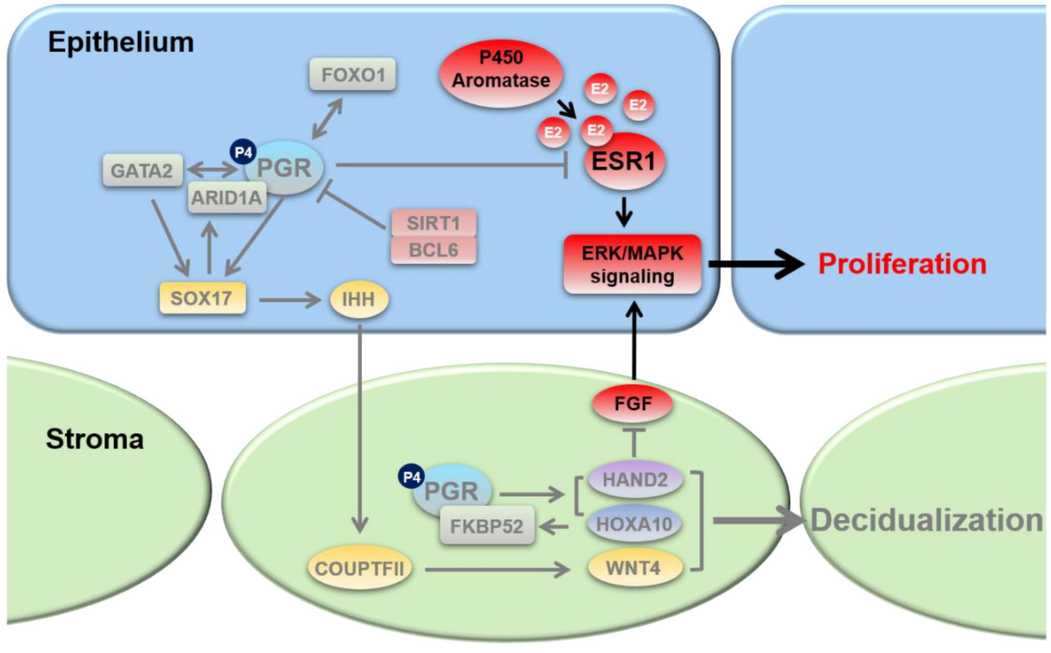


Review Progesterone and Estrogen Signaling in the Endometrium: What Goes Wrong in Endometriosis? Ryan M. Marquardt jeongj@msu.edu; Tel.: +1-616-234-0987 , 2019 In the healthy endometrium, progesterone and estrogen signaling coordinate in a tightly regulated, dynamic interplay to drive a normal menstrual cycle and promote an embryo-receptive state to allow implantation It is well-established that progesterone and estrogen act primarily through their cognate receptors to set off cascades of signaling pathways and enact large-scale gene expression programs. In endometriosis, when endometrial tissue grows outside the uterine cavity, progesterone and estrogen signaling are disrupted, commonly resulting in progesterone resistance and estrogen dominance. This hormone imbalance leads to heightened inflammation and may also increase the pelvic pain of the disease and decrease endometrial receptivity to embryo implantation. **This review focuses on the molecular mechanisms governing progesterone and estrogen signaling supporting endometrial function and how they become dysregulated in endometriosis**. Understanding how these mechanisms contribute to the pelvic pain and infertility associated with endometriosis will open new avenues of targeted medical therapies to give relief to the millions of women suffering its effects. Keywords: progesterone; estrogen; endometrium; infertility; endometriosis; progesterone resistance



Key signals and pathways in the multistep processes of embryo implantation. Progesterone, P4; progesterone receptor, PR; proliferation-differentiation switching, PDS; planar cell polarity, PCP; forkhead box protein A2, FOXA2; leukemia inhibitory factor, LIF; signal transducer and activator of transcription 3, STAT3; hypoxia-inducible factor 2α,



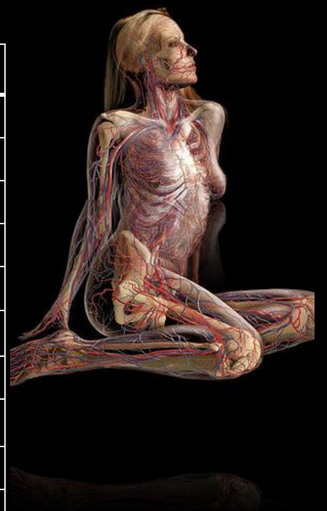


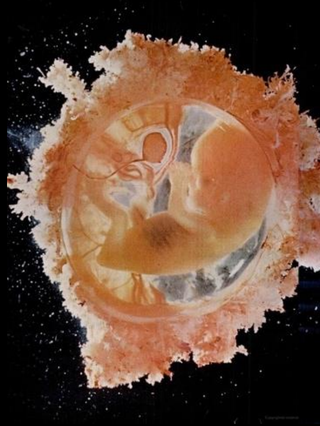
Diagnostic and Therapeutic Algorythms Usefull in The Diagnosis and Treatment of Infertile Couples

Endometriosis	**Presumptive diagnosis by questionnaire**	Chapron C, Lafay-Pillet MC, Santulli P, Bourdon M, Maignien C, Gaudet-Chardonnet A, Maitrot-Mantelet L, Borghese B, Marcellin L. A new validated screening method for endometriosis diagnosis based on patient questionnaires. EClinicalMedicine. 2022 Jan 10;44:101263. doi: 10.1016/j.eclinm.2021.101263. PMID: 35059616; PMCID: PMC8760436.
Sperm Evaluation according to WHO criteria : WHO Semen analysis 2021	**Sperm evaluation by WHO criteria, 2021**	Boitrelle F, Shah R, Saleh R, Henkel R, Kandil H, Chung E, Vogiatzi P, Zini A, Arafa M, Agarwal A. The Sixth Edition of the WHO Manual for Human Semen Analysis: A Critical Review and SWOT Analysis. Life (Basel). 2021 Dec 9;11(12):1368. doi: 10.3390/life11121368. PMID: 34947899; PMCID: PMC8706130.
Ovarian reservoir according Anti Mullerian Hormone (AMH)	**Classification of ovarian reservoir according to AMH and age**	• Wang X, Jin L, Mao Y-d, Shi J-z, Huang R, Jiang Y-n, Zhang C-l and Liang X-y (2021) Evaluation of Ovarian Reserve Tests and Age in the Prediction of Poor Ovarian Response to Controlled Ovarian Stimulation—A Real-World Data Analysis of 89,002 Patients. *Front. Endocrinol.* 12:702061. doi: 10.3389/fendo.2021.702061 • Esteves SC, Alviggi C, Humaidan P, Fischer R, Andersen CY, Conforti A, Bühler K, Sunkara SK, Polyzos NP, Galliano D, Grynberg M, Yarali H, Özbek IY, Roque M, Vuong LN, Banker M, Rienzi L, Vaiarelli A, Cimadomo D and Ubaldi FM (2019) The POSEIDON Criteria and Its Measure of Success Through the Eyes of Clinicians and Embryologists. *Front. Endocrinol.* 10:814. doi: 10.3389/fendo.2019.00814
Ovarian Reservoir According Antral Follicular Count (AFC)	**Classification of Ovarian Reservoir according to the Antral Follicular Count (AFC)**	https://advancedfertility.com/infertility-testing/antral-follicle-counts/
Starting Dose Gonadotropins	**Gonadotrohin starting dose calculator**	Michael Fanton , Veronica Nutting1 , Arielle Rothman1 , Paxton MaederYork , Eduardo Hariton , Oleksii Barash , Louis Weckstein , Denny Sakkas , Alan B. Copperman , Kevin Loewke , An interpretable machine learning model for individualized gonadotrophin starting dose selection during ovarian stimulation RBMO VOLUME 45 ISSUE 6 2022. https://doi.org/10.1016/j.rbmo.2022.07.010 1472-6483/© 2022
Trigger Time Calculation : Day/Hour	**To develop an interpretable machine learning model for optimizing the day of trigger in terms of mature oocytes (MII), fertilized oocytes (2PNs), and usable blastocysts**	Michael Fanton, Veronica Nutting, Funmi Solano, Paxton Maeder-York, Eduardo Hariton, Oleksii Barash, Louis Weckstein, Denny Sakkas, Alan B. Copperman, Kevin Loewke, An interpretable machine learning model for predicting the optimal day of trigger during ovarian stimulation, Fertility and Sterility, Volume 118, Issue 1, 2022,Pages 101-108, ISSN 0015-0282, https://doi.org/10.1016/j.fertnstert.2022.04.003. (https://www.sciencedirect.com/science/article/pii/S0015028222002448)
Estimation of Oocyte Metaphase II to Obtain and Euploid Blastocyst	**Blastocyst presumptive calculation from Oocytes MII Recruited**	Esteves SC, Yarali H, Ubaldi FM, Carvalho JF, Bento FC, Vaiarelli A, Cimadomo D, Özbek İY, Polat M, Bozdag G, Rienzi L and Alviggi C (2020) Validation of ART Calculator for Predicting the Number of Metaphase II Oocytes Required for Obtaining at Least One Euploid Blastocyst for Transfer in Couples Undergoing *in vitro* Fertilization/Intracytoplasmic Sperm Injection. *Front. Endocrinol.* 10:917. doi: 10.3389/fendo.2019.00917
The Development of Nomograms to Predict Blastulation Rate Following Cycles of In Vitro Fertilization in Patients With Tubal Factor Infertility, Polycystic Ovary Syndrome, or Endometriosis	**Blastulation rate Prediction in specific infertile groups selection**	Jin H, Shen X, Song W, Liu Y, Qi L, Zhang F. The Development of Nomograms to Predict Blastulation Rate Following Cycles of *In Vitro* Fertilization in Patients With Tubal Factor Infertility, Polycystic Ovary Syndrome, or Endometriosis. Front Endocrinol (Lausanne). 2021 Nov 3;12:751373. doi: 10.3389/fendo.2021.751373. PMID: 34803917; PMCID: PMC8595301.
Implantation rate estimation according ATA et al, 2021 modified	**Human Embryo Implantation Estimation According to Age and Aneuploidy Establishment**	*A new definition of recurrent implantation failure on the basis of anticipated blastocyst aneuploidy rates across female age Baris Ata, M.D., M.Sc., Erkan Kalafat, M.D., M.Sc., Edgardo Somigliana, M.D., Ph.D. Fertility and Sterility* Volume 116 Issue 5 Pages 1320-1327 (November 2021) DOI: 10.1016/j.fertnstert.2021.06.045
Endometrial Receptivity Score	**Endometrial Scoring for implantation prediction**	Narendra Malhotra, Jaideep Malhotra, Neharika Malhotra, JP Rao, Neelam Mishra Endometrial Receptivity and Scoring for Prediction of Implantation and Newer Markers Donald School Journal of Ultrasound in Obstetrics and Gynecology, October-December 2010;4(4):439-446 DOI:10.5005/jp-journals-10009-1164
Your Chance to have a baby	**Presumptive successful IVF treatment stratified for the infertile couples features**	• Y E M Koot, M Hviid Saxtorph, M Goddijn, S de Bever, M J C Eijkemans, M v Wely, F van der Veen, B C J M Fauser, N S Macklon, What is the prognosis for a live birth after unexplained recurrent implantation failure following IVF/ICSI?, *Human Reproduction*, Volume 34, Issue 10, October 2019, Pages 2044–2052, https://doi.org/10.1093/humrep/dez120 • MacLernon DJRaja Edwin-Amairaj, Toner James P….Lin Paul C, Bhattacharya A, Van Voorhis Bradley J Predicitng personalized cumulative live birth following in vitro fertilization Fertil Steril Vol 117,issue 2, p326-338, February 1 , 2022 https://doi.org/10.1016/j.fertnstert.2021.09.015 • https://w3.abdn.ac.uk/clsm/SARTIVF/home/toolintro
The working group achieved consensus on a list of Key Performance Indicators(KPIs) , Performance Indicators(PIs) and recommendation indicators (RIs) useful for internal and external controls of ART treatments	**KPIs, PIs and RIs to ascertain good medical practice in ART**	Vaiarelli A et al, Clinical and Laboratory Key Performance Indicators in IVF setting: A comprehensive Italian Consensus on behalf of the Italian Society of Fertility and Sterility and Reproductive Medicine (SIFES-MR). 2023 Submitted for Publication

Endometrial adequacy check list

	Date	Parameter	Arbitrary weight 1-10
✓		Smoking	3
✓		Alcohol	2
✓		Obesity	2
✓		Gamete quality : oocytes	5
✓		Gamete quality: Spermatozoa	4
✓		Embryo quality. Morphology	4
✓		Embryo quality: euploidy	9
✓		Uterine factor: Uterine septum	6
✓		Uterine factor : Submucosal Fibroids	6
✓		Uterine factors: polyps	6
✓		Uterine factors : Intramural fibroids	4
✓		Uterine factors: Adhesions	6
✓		Uterine facotrs Adenomyosis	3
✓		Uterine factors: Hydosalpinx	7
✓		Uterine factors: Endometriosis	5
✓		Uterine factors : Difficult embryo transfer	5
✓		Endometrium: Thickness	6
✓		Endometrium: In phase/Out of phase	7
✓		Endometrium: omics	3
✓		Endometrium: Pinopodes	7
✓		Endometrium: endometritis	3
✓		Endometroium : Genomic assessment	1
✓		Systemic factors : trompbophilia	3
✓		Systemic factors: Immunological factors	2
✓		Systemic factors: Progesterone at the trigger	6
✓		Systemic factors: progesterone at mid secretive phase in fresh and/or FET	5
✓		Systemic factors: subclinical hypothyroidism	7





Conclusions

1. Implantation (EI) Mostly Depend On Embryo Euploidy
2. The possibility to transfer at least 3 euploid embryo leave only 5% to 15% couples without child in some estimations (Ata B, Busnelli A, Pirtea P). However
3. The possibility to obtain 3 or more euploid embryo in women aged as those undergo IVF program in west countries (>37) is quite rare
4. The endometrial and/or systemic factor influencing the adequate endometrial receptivity for an euploid embryo are approximately 40% to 50% and still
5. They represent the main forefront to explore the embryo's implantation efficiency because their specific weight is not fully clarified;
6. Unfortunately those factors are independent one each other and their conjunctural coordination is presently difficult to establish for a multifactorial cyclical process that depend for its own sequence on undeterminable variables (e.g. exercise or BT may change the MCR of estrogens and progesterone)
7. Two future promising prospective researches are under the attention of a broad front of scientists:
   a) the immunomodulation of the endometrial reaction to the attachment and invasion of the embryo;
   b) the new algorithms which include all the known factors influencing embryonic implantation and which can enhance their accuracy day after day by sharing a large data base relating to the temporal coordination of embryonic and endometrial differentiation as well as their respective potential successfull
8. The time lapse interval for adequate endometrial receptivity should be more deeply investigated in close association with the dormant embryo phenomenon observed in other species

Thanks

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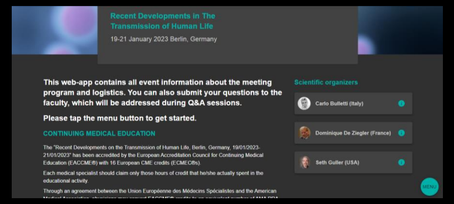

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