

Evidence based strategies in RIF.

Novel approaches



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What is RIF

- **How many attempts?**
- How many embryos?
- Age?
- Embryo quality?
- Failure in the presence of potential obstacles?

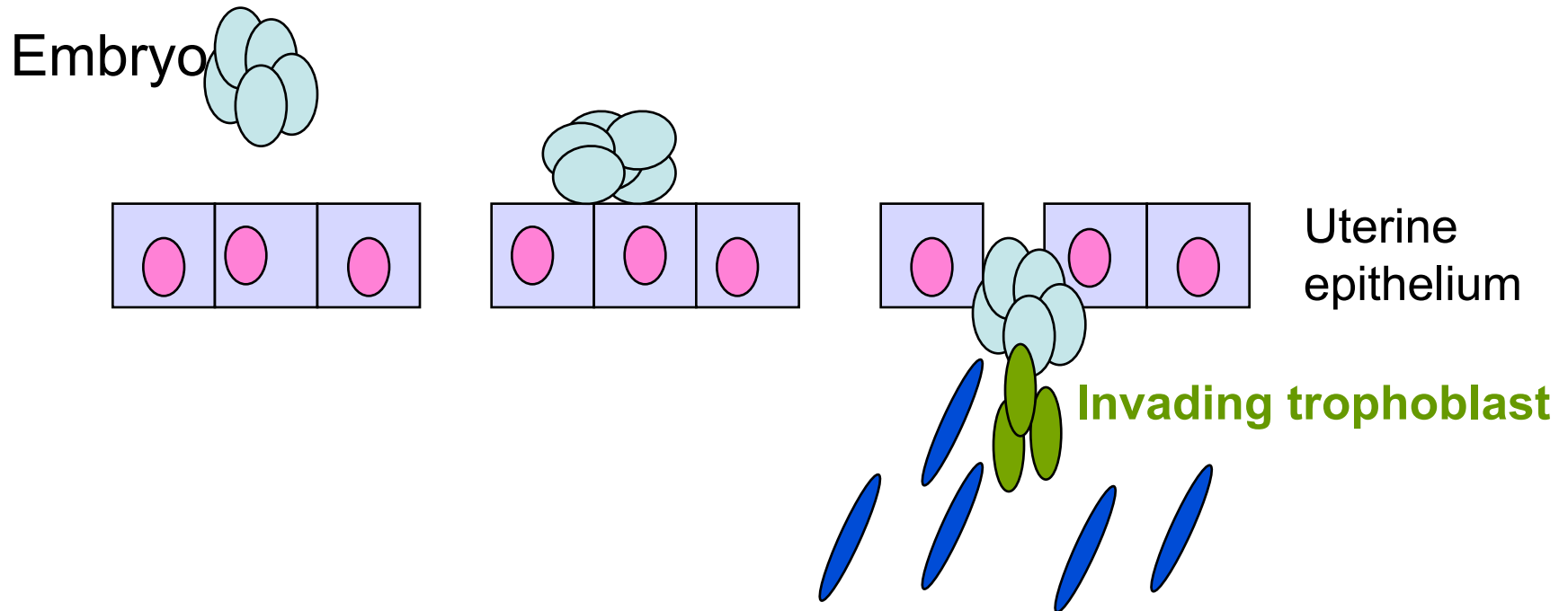
RECURRENT IMPLANTATION FAILURE

- Failure to achieve a pregnancy after 3 completed fresh IVF-ET cycles (Tan et al 2005)
- Failure of ≥ 10 embryos to implant
- In the era of SET/DET should the definition of RIF be revised

WHY SHOULD
IMPLANTATION FAIL
TO TAKE PLACE ?

Implantation - key event in the establishment of pregnancy

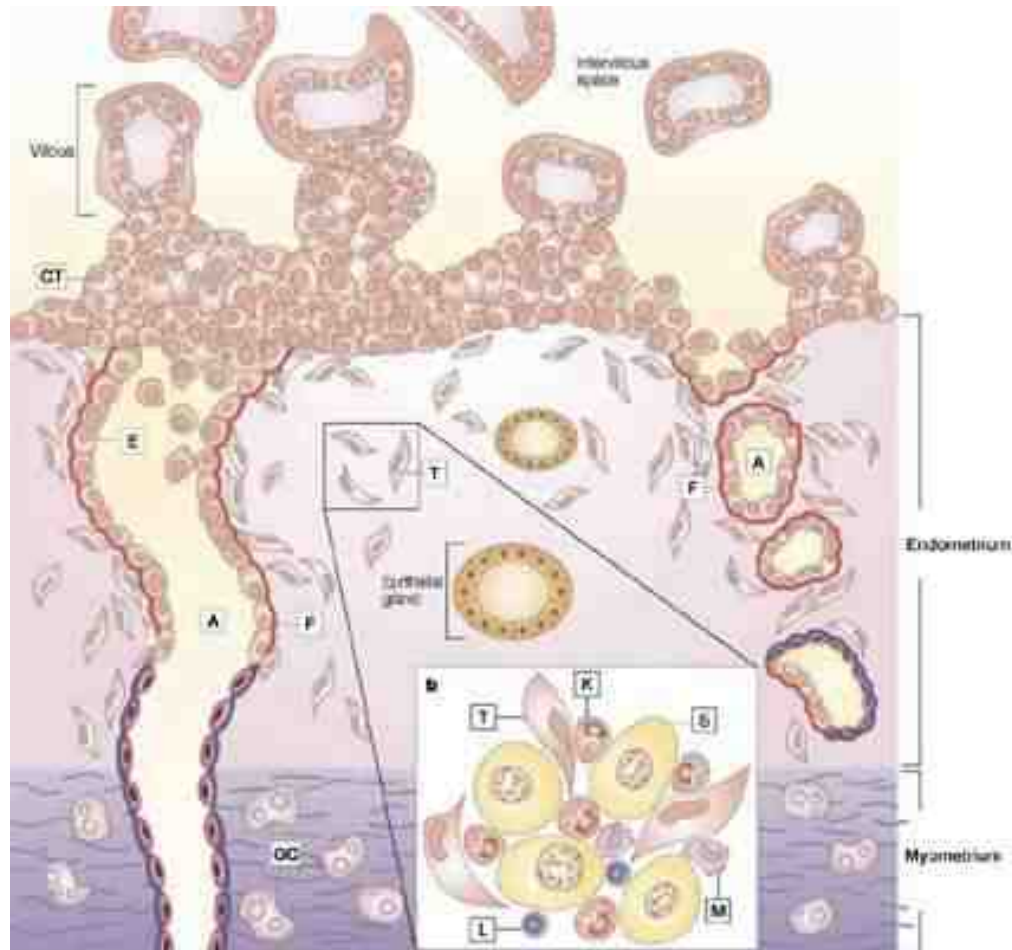
Apposition Adhesion Invasion



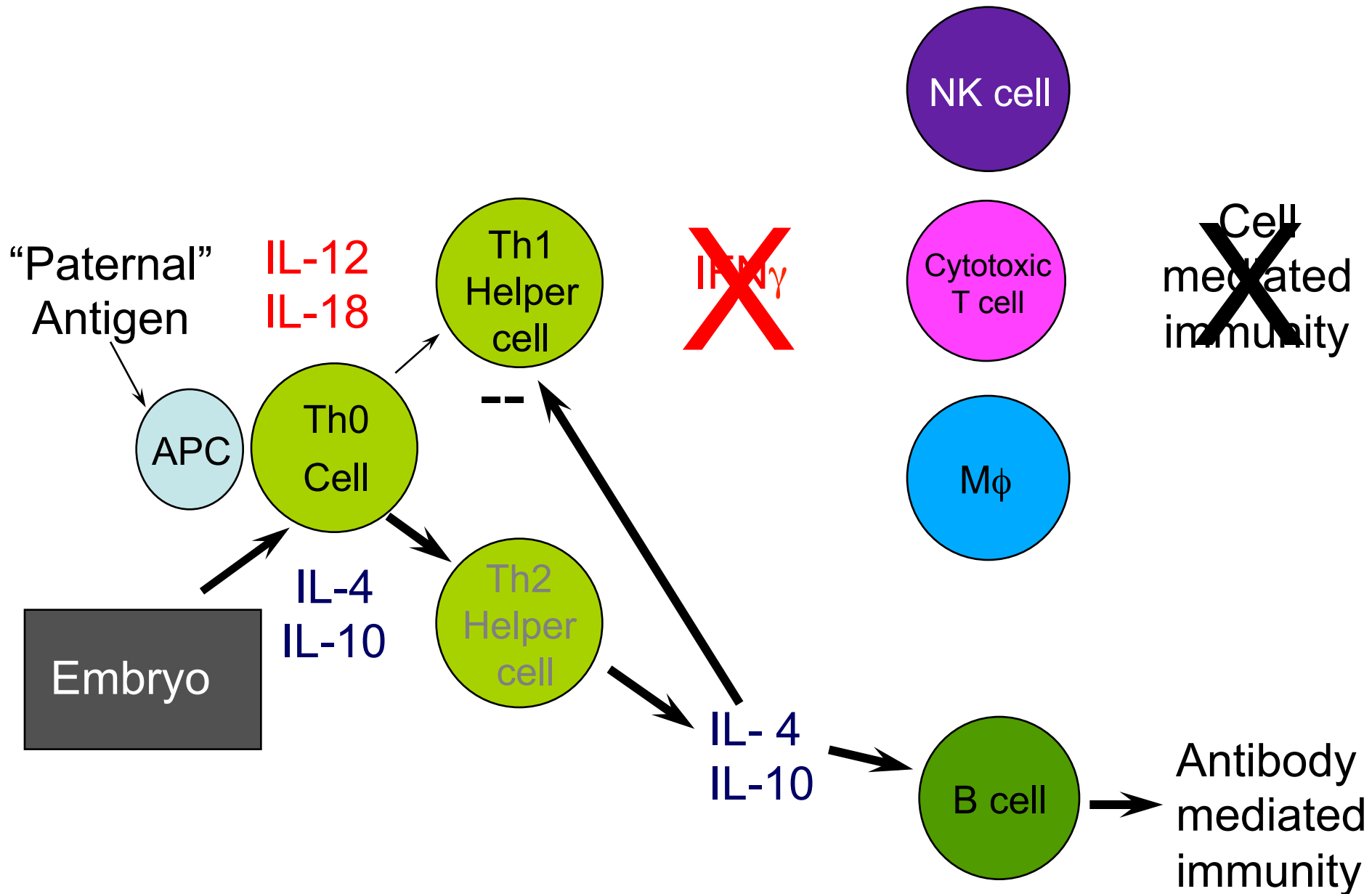
Continuous process from conception to 22 weeks gestation

Histopathology-Immunology of implantation site

T=interstitial EVT, E=intravascular EVT, K=uNK cells, M=macrophage, L=T cells



Th1/Th2 balance in Normal Invasion



TREATMENT STRATEGIES FOR RECURRENT IMPLANTATION FAILURE

- Embryo
- Endometrium

EMBRYO



- Preimplantation genetic diagnosis
- Blastocyst transfer
- Assisted hatching
- Co-culture of embryos with endometrium
- Other methods of embryo selection
- Donor oocyte/embryo

TREATMENTS OF PROVEN BENEFIT

embryo : assisted hatching

TREATMENT STRATEGIES FOR RECURRENT IMPLANTATION FAILURE

- Embryo
- **Endometrium**

ENDOMETRIUM



- **Hysteroscopy**
- Hydrosalpinges

IVIG ?



- The only properly conducted prospective RCT by Stephenson & Fluker (2000) involving 51 women with 2 or more IVF failures showed IVIG has no benefit

STEROIDS ?



- A meta-analysis of 13 RCTs by Boomsma et al (Cochrane database 2007) showed no evidence of benefit of routine use in women undergoing IVF +/- ICSI

ACA – anticardiolipin antibodies



- Two studies showed a higher prevalence of ACA in women with RIF (Kaider et al 1996, Qublan et al 2006)
- However, the only RCT on the use of heparin and aspirin in women with RIF tested + for ACA showed no benefit (Stern et al 2003)

Aspirin



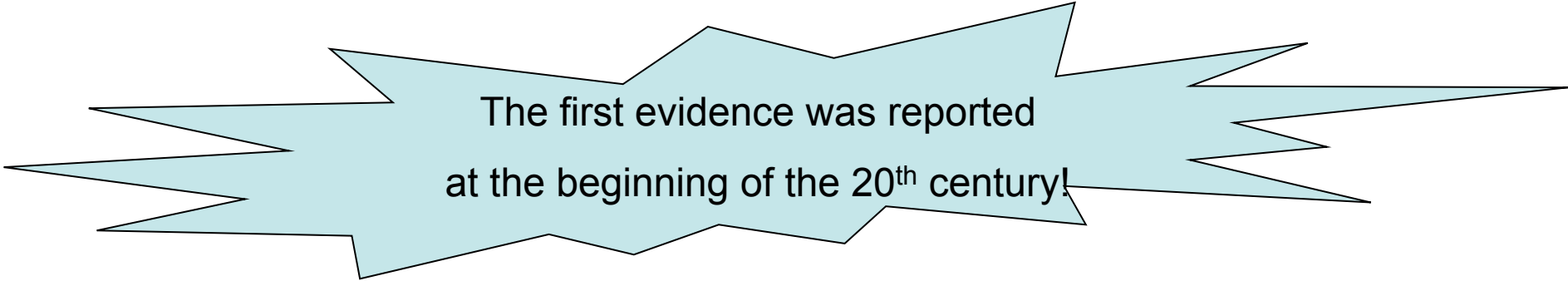
low-dose aspirin showed no benefit of its use in IVF programme (Gelbaya et al, Human Repro Update 2007)

RIF

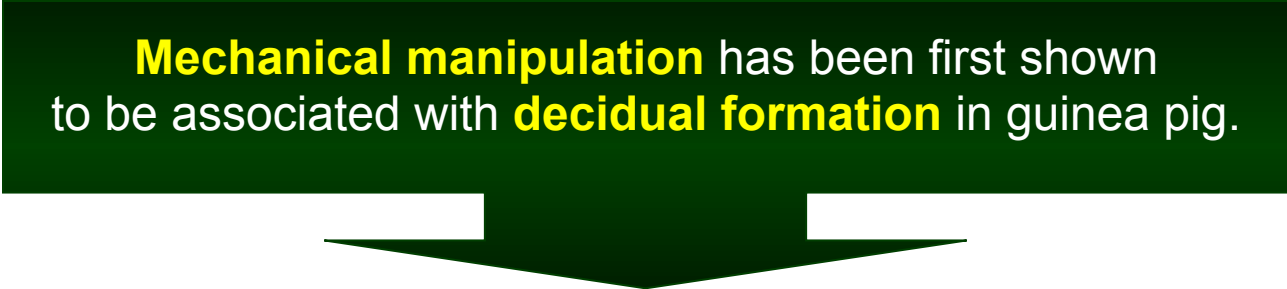


- **Novel approaches**


Does endometrial scratching promote
implantation and live birth rates
in patients with RIF?



The first evidence was reported
at the beginning of the 20th century!



Mechanical manipulation has been first shown
to be associated with **decidual formation** in guinea pig.



scratching the uterus during the progestational phase
of the estrous cycle provoked a rapid growth of decidual cells

Loeb L. Zentralblatt für allgemeine Pathologie und pathologische
Anatomie 18 563–565. 1907

Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization

Amihai Barash, M.D.,^a Nava Dekel, Ph.D.,^b Sheila Fieldust, Bs.C.,^a Ilana Segal, Bs.C.,^a Edna Schechtman, Ph.D.,^c and Irit Granot, Ph.D.^a

IVF Unit, Department of Obstetrics and Gynecology, The Kaplan Medical Center, Rehovot, Israel

Randomized control study

- 45 patients underwent endometrial biopsy
- 89 controls

Endometrial biopsy: days 8,12,21,26 during the cycle prior to IVF

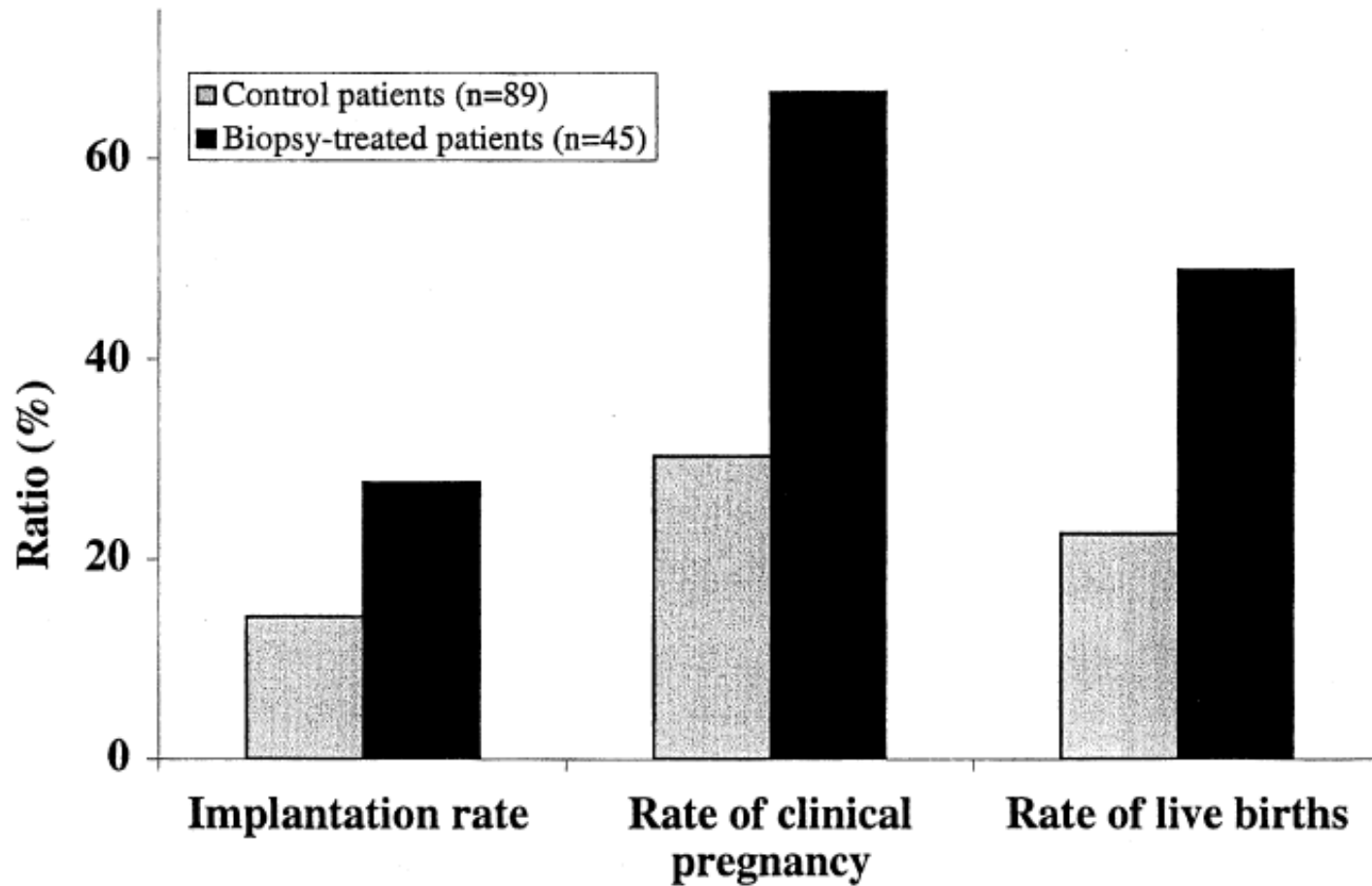
FERTILITY AND STERILITY®

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Significant increase in clinical pregnancy and live birth rate

Association between mode of embryo transfer and the incidence of pregnancy.

Mode of embryo transfer	Control patients (n = 89)		Biopsy-treated group (n = 45)	
	Pregnant (n = 27)	Nonpregnant (n = 62)	Pregnant (n = 30)	Nonpregnant (n = 15)
6-8 cells	2 (16.7%)	10 (83.3%)	4 (57.1%)	3 (42.9%)
Blastocysts	15 (28.9%)	37 (71.1%)	18 (69.2%)	8 (30.8%)
Double transfer	10 (40%)	15 (60%)	8 (66.7%)	4 (33.3%)
P values	.33		.83	

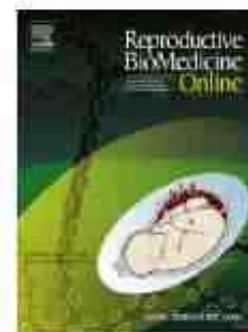
χ^2 test revealed no significant association mode of embryo transfer and incidence of pregnancy in both groups.

Barash. Endometrial injury increases its receptivity. Fertil Steril 2003.

This finding was independent of the mode of embryo transfer



www.sciencedirect.com
www.rbmonline.com



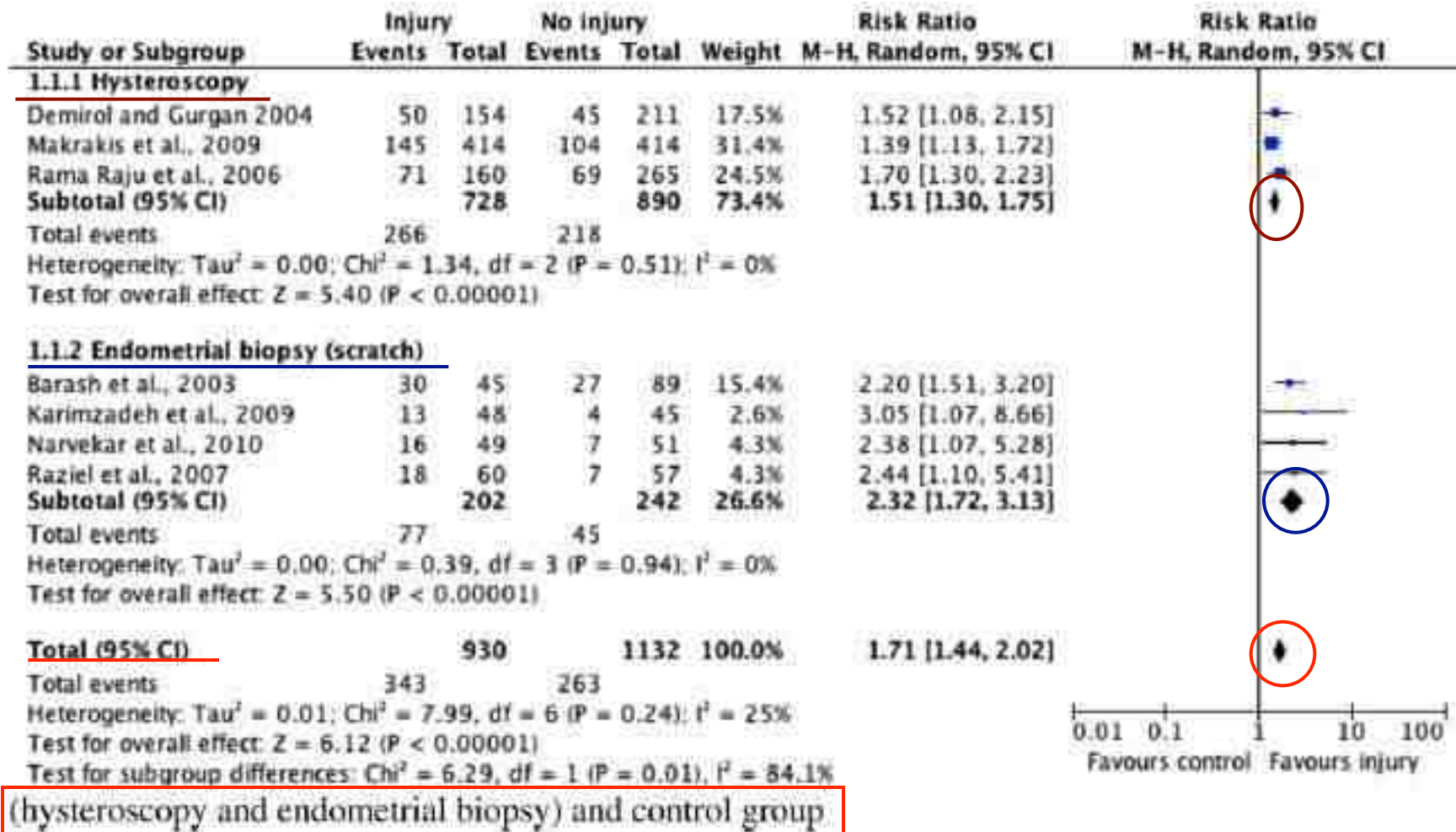
REVIEW

Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis

Neelam Potdar ^{a,*}, Tarek Gelbaya ^b, Luciano G Nardo ^c

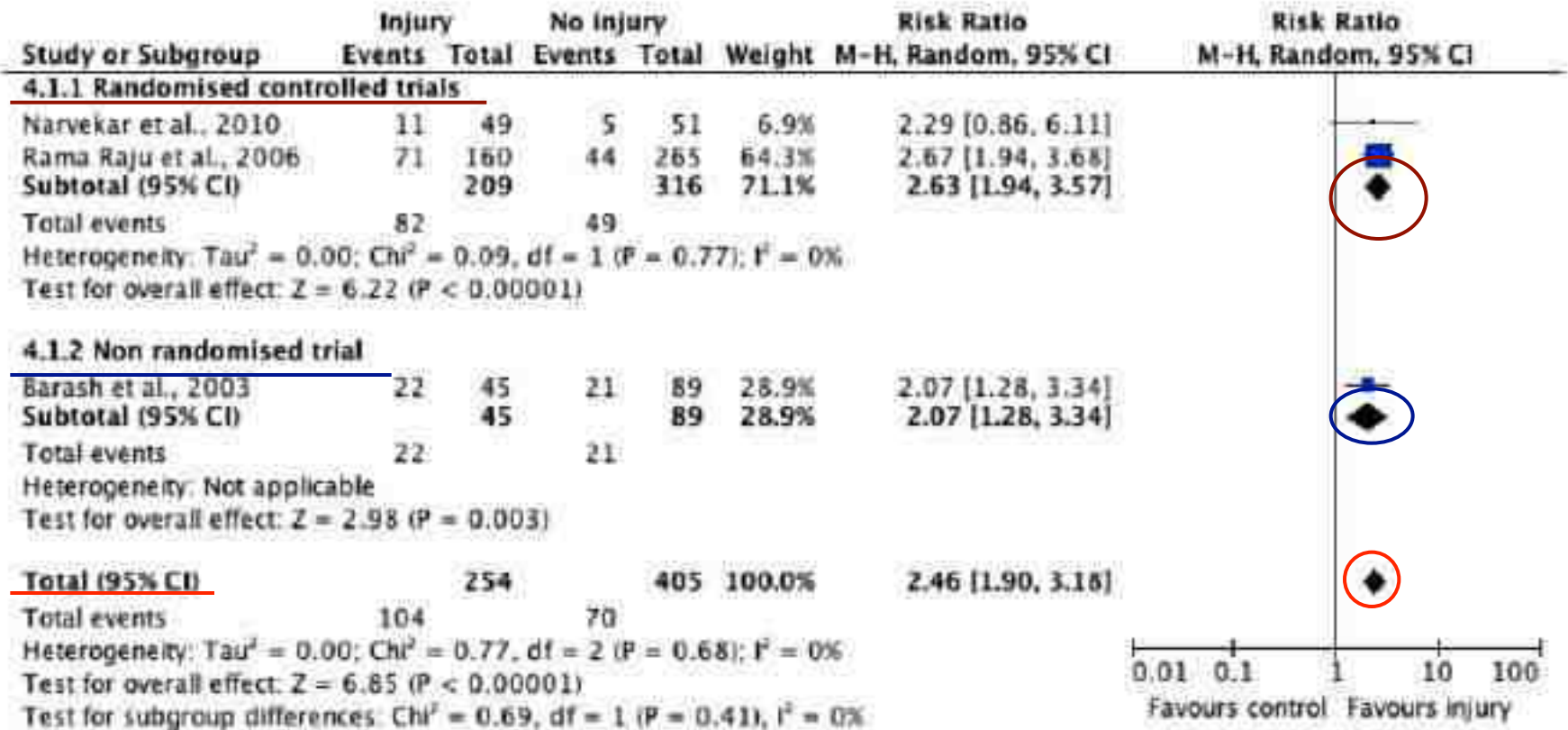
^a Leicester Fertility Centre, University of Leicester and University Hospitals of Leicester, Leicester, UK; ^b Leicester Fertility Centre, University Hospitals of Leicester, Leicester, UK; ^c Reproductive Medicine and Surgery Unit, Gynaehealth, Manchester, UK

Endometrial Injury (hysteroscopy or scratching) vs control: Clinical pregnancy rate



Endometrial injury favors implantation

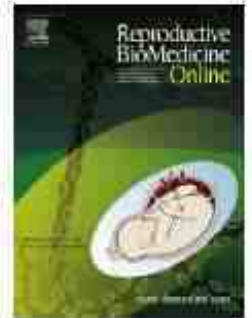
Randomized & non randomized trials for endometrial injury & control groups : Live birth rate



Endometrial injury improves live birth rate



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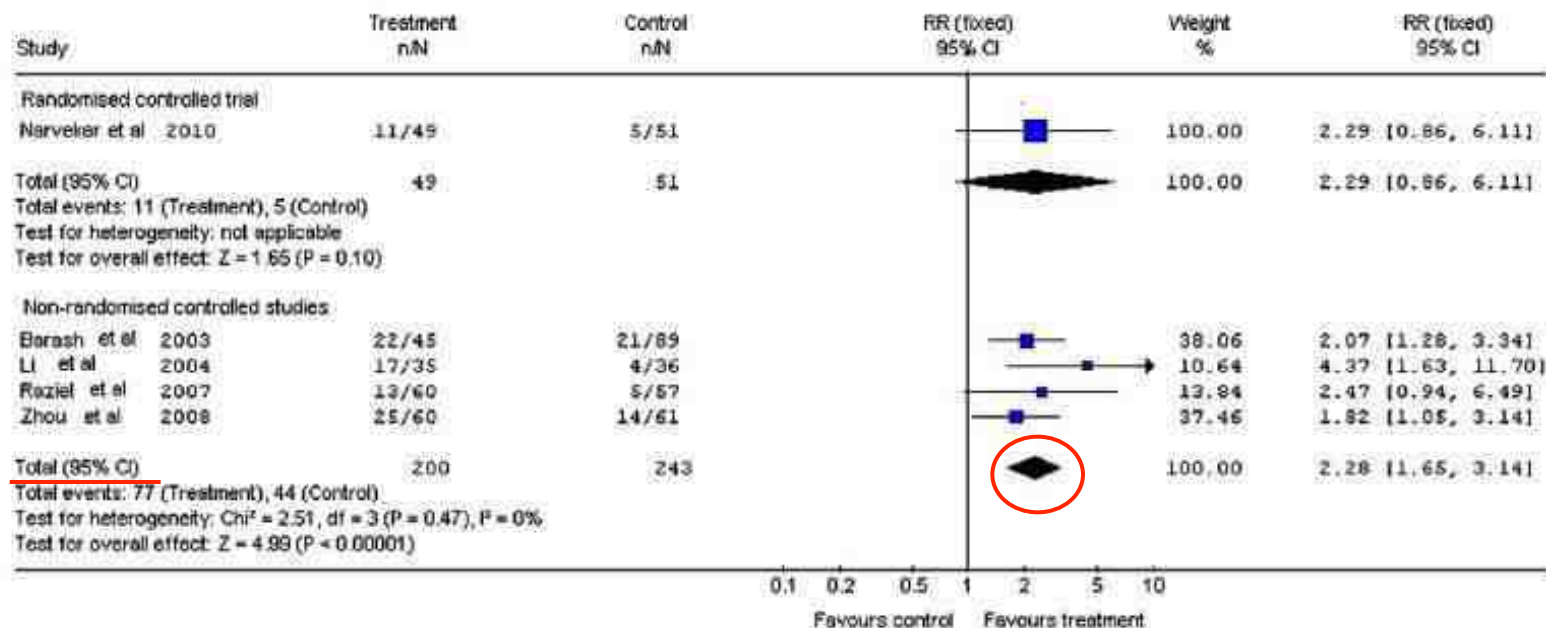


ARTICLE

Local endometrial injury and IVF outcome: a systematic review and meta-analysis

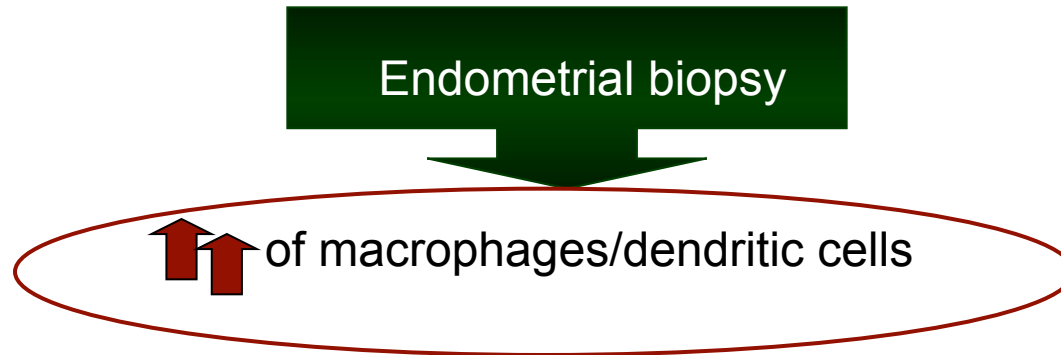
Tarek El-Toukhy *, SeshKamal Sunkara, Yakoub Khalaf

Summary of the live birth/ ongoing pregnancy rate for the 5 studies included in the systematic review

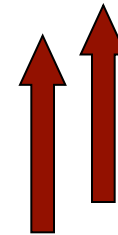


Improvement of live birth/ongoing pregnancy rate

Local injury of the endometrium induces an inflammatory response that promotes successful implantation



- tumor necrosis factor- α (TNF- α),
- growth-regulated oncogene- α (GRO α),
- interleukin-15 (IL-15),
- macrophage inflammatory protein 1B (MIP-1B),
- Osteopontin



A positive correlation was found between the levels of macrophages/dendritic cells, MIP-1B expression, and TNF- α expression & the pregnancy outcome.

Issues to be addressed

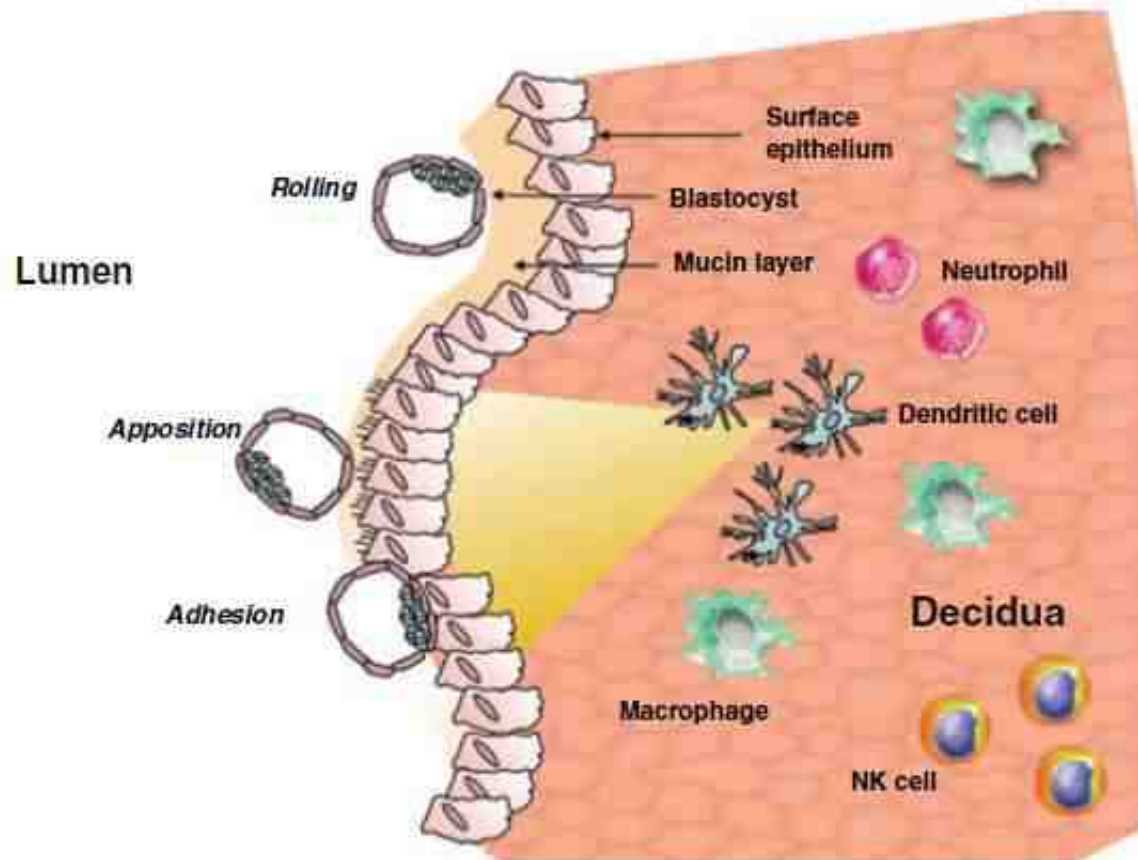
- Which is the best cycle?
 - Evidence support the performance of endometrial biopsy one cycle prior to IVF
 - It is advisable not to perform endometrial injury on the day of oocyte retrieval because it appears to significantly reduce clinical and ongoing pregnancy rates.

[Cochrane Database Syst Rev.](#) 2012 Jul 11;7:CD009517.

Issues to be addressed

- Which is the optimal day to perform endometrial biopsy?
 - Most studies support days 21-26 of the menstrual cycle
 - The evidence is weak

Up-regulation of dendritic cells, local inflammation and injury

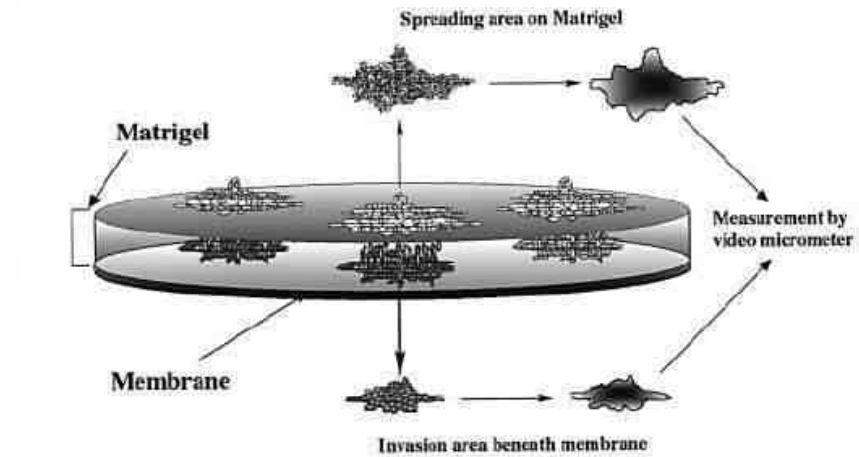
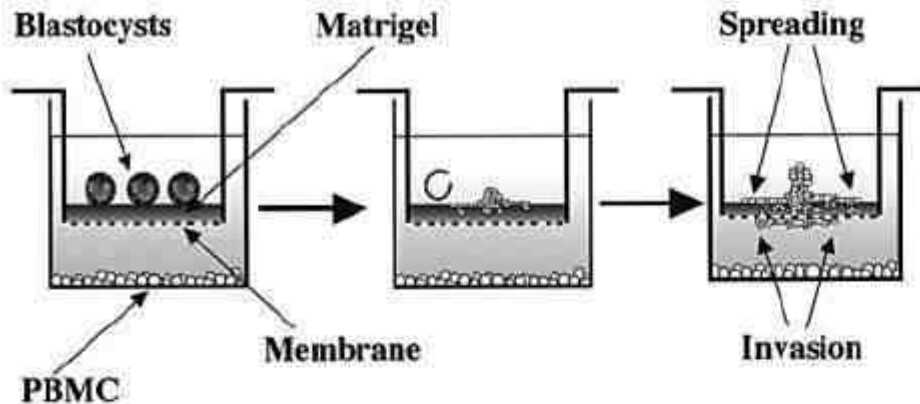


Dendritic cells & macrophages increase in local injury
& during the window of implantation

Dekel et al. Am J Reprod Immunol 2010;63:17

Does intrauterine administration of PBMCs
promote implantation & pregnancy rates
in patients with RIF?

The biological basis of the PBMC administration



PBMCs treated with HCG significantly increased murine embryo invasion.
Embryo outgrowth was not affected by HCG alone

Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in patients with repeated failure of IVF–embryo transfer

Intrauterine administration of autologous PBMCs promote **clinical pregnancy, implantation and live birth rates** in patients with repeated failure of IVF-embryo transfer

41.2% vs 11.1% and 23.4% vs 4.1% and 35.3% vs 5.5%

Intrauterine administration of autologous peripheral blood mononuclear cells increases clinical pregnancy rates in frozen/thawed embryo transfer cycles of patients with repeated implantation failure

- 253 cycles were studied
- All women received frozen/thawed embryos.
- **PBMCs were not treated with HCG**

Table 3

Characteristics and clinical outcomes of patients in the PBMC-treated and non-treated groups with three or more implantation failures.

	PBMC-treated	Non-treated	
I Characteristics of the patients:			
Age	37.4 ± 5.33	38.3 ± 4.20	n.s.
No. of IVF-ET previous attempts	4.47 ± 3.42	4.31 ± 1.73	n.s.
No. of embryos transferred	1.79 ± 0.79	1.78 ± 0.59	n.s.
II Clinical outcome			
Clinical pregnancy rate	42.1% (8/19)	16.7% (6/36)	↑ (p = 0.039)
Implantation rate	25.0% (8/32)	9.38% (6/64)	(p = 0.041)
Live birth delivery rate			
Per embryo transfer cycle	21.2% (4/19)	11.1% (4/36)	n.s.
Per transferred embryos	12.5% (4/32)	6.3% (4/64)	n.s.

PBMCs significantly improved implantation & clinical pregnancy rate when used in women with 3 or more implantation failures.

Proposed mechanisms for PBMC actions within the uterine cavity

- Activated PBMC that are administered into the uterine cavity can induce adequate **endometrial differentiation** for embryo implantation.
- PBMC can evoke favorable **inflammatory reactions** in the uterine cavity, for example, secreting proteases that may effectively change the function or structure of surface molecules expressed on the endometrial luminal epithelial cells.
- PBMC may move from the uterine cavity toward the endometrial stromal tissue, **creating a leading pathway** for subsequent embryo attachment and invasion

J. Mamm. Ova Res. 26, 122–128, 2009

J. Reprod. Immunol. 81, 1–8., 2009

Issues to be addressed

- What is the biological impact?
- When is the appropriate time to administer the PBMCs?
- Should PBMCs be pre-treated with HCG?
- Apart from RIF?
- Differential(Better) Activation of PBMCs?

**DOES INTRAUTERINE ADMINISTRATION
OF PBMCs PRETREATED WITH CRH
PROMOTE IMPLANTATION RATES IN
PATIENTS WITH RIF?**

Background

- Implantation sites in rat uterus contains increased CRH concentrations.

Makrigiannakis et al, JCEM 1995



Corticotropin-releasing hormone promotes blastocyst implantation and early maternal tolerance

A. Makrigiannakis^{1,2,3}, E. Zoumakis^{1,4}, S. Kalantaridou^{3,4}, C. Coutifaris⁵, A. N. Margioris¹, G. Coukos⁵, K. C. Rice⁶, A. Gravanis^{1,*} and G. P. Chrousos^{4,*}

Repeated implantation failure: a new potential treatment option

Antonis Makrigiannakis*, Moncef BenKhalifa[†], Thomas Vrekoussis[‡], Sami Mahjub[§], Sophia N. Kalantaridou[‡] and Timur Gurgan[¶]

*Department of Obstetrics and Gynecology, Medical School, University of Crete, Heraklion, Greece, [†]Reproductive Medicine & Medical Cytogenetics, Regional University Hospital & Medical School, Picardie University Jules Verne, Amiens, France,

[‡]Department of Obstetrics and Gynecology, Medical School, University of Ioannina, Ioannina, Greece, [§]Elyssa IVF Center, Tunis, Tunisia, [¶]Department of Obstetrics and Gynecology, Medical School, Hacettepe University, Ankara, Turkey

Eur J Clin Invest 2015; 45 (4): 380–384

SUBJECTS

97 cycles in 106 patients

All patients had experienced 3 or > failures of IVF-embryo transfer therapy without poor ovarian reserve ($\text{FSH} < 12 \text{ mIU/ml}$)



IVF PROCEDURE

pure FSH (Serono)



Day
of hCG



FSH until dominant follicle diameter > 18mm

GnRHa

EMBRYO CULTURE

After fertilization was confirmed the day after fertilization (day 1), the zygotes were cultured for another 2 days. For blastocyst transfer, embryos were further cultured in Blastocyst Medium with 10% of SPS. 2 or 3 blastocysts were transferred to the uterine cavity on day 5

REPEATED IMPLANTATION FAILURE: A NEW APPROACH

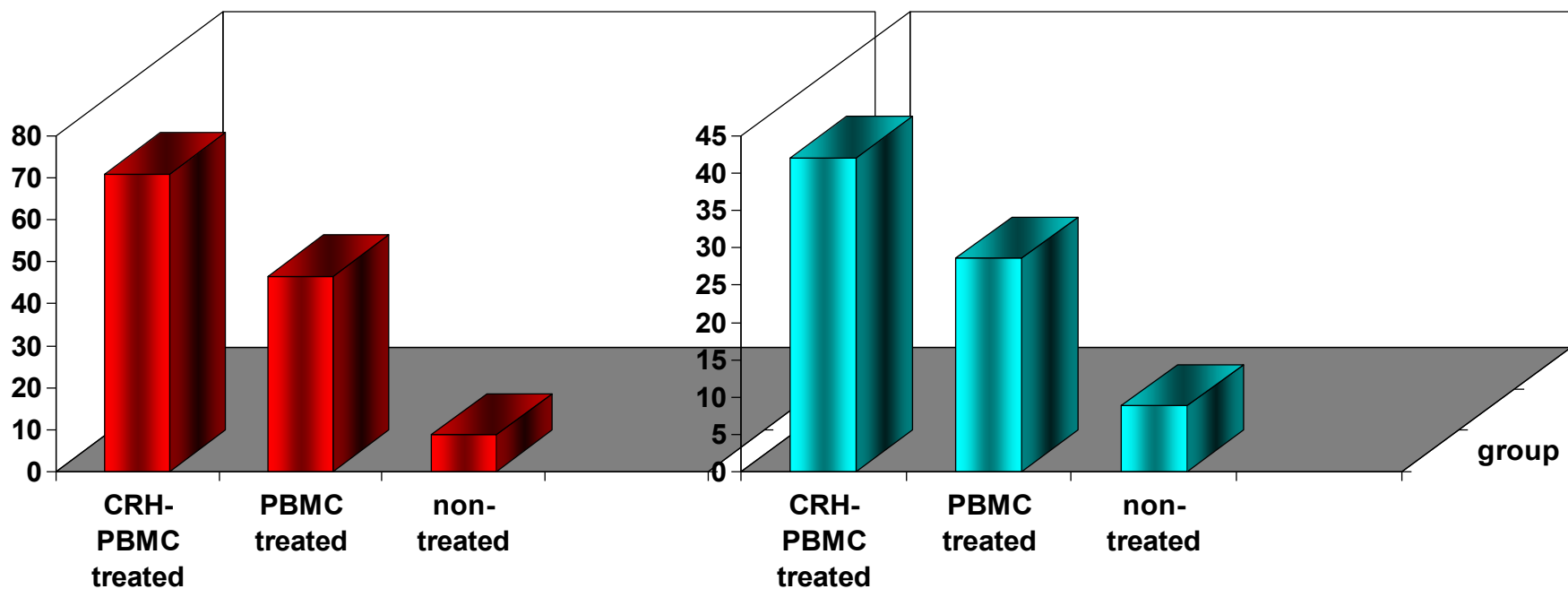
Table 1 Clinical characteristics of RIF women included in the study

	Overall (<i>n</i> = 45) Mean ± SEM	Clinical pregnancy		<i>P</i>
		Yes (<i>n</i> = 20) Mean ± SEM	No (<i>n</i> = 25) Mean ± SEM	
Age (years)	33.00 ± 0.57	33.85 ± 0.86	32.32 ± 0.75	NS
Previous failed IVF cycles	3.26 ± 0.09	3.30 ± 0.12	3.24 ± 0.13	NS
Endometrium thickness at the day of oocyte retrieval (mm)	11.04 ± 0.27	10.65 ± 0.34	11.36 ± 0.40	NS
Blastocysts transferred	2.37 ± 0.07	2.55 ± 0.11	2.24 ± 0.10	NS

NS, not significant.

Clinical outcome of the patients under 38

	CRH-PBMC treated	PBMC treated	Non-treated
Clinical pregnancy rate	44,8	22,3	2,5
Implantation rate	21,4	12,4	1,6
Live birth rate			



REPEATED IMPLANTATION FAILURE: A NEW APPROACH

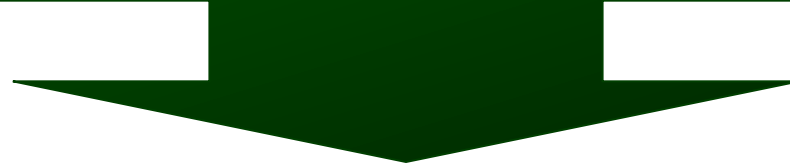
Table 2 Overview of the studies using PBMC for improving clinical pregnancy rate after blastocyst transfer in women with RIF

	Embryo type	Intervention	Participants (n)	Clinical pregnancy rate	Outcome significance
Yoshioka <i>et al.</i> , [9]	Blastocyst	HCG-treated PBMC	17	42.1% (7/17)	Significant
Okitsu <i>et al.</i> , [15]	Blastocyst	Untreated PBMC	13	46.1% (6/13)	Not significant
Current study	Blastocyst	CRH-treated PBMC	45	44.4% (20/45) *	Significant

$*P < 0.001$



**DOES INTRAUTERINE ADMINISTRATION OF
PBMCs PRETREATED WITH CRH & hCG
PROMOTE IMPLANTATION RATES IN
PATIENTS WITH RIF?**



YES!

POSSIBLE EXPLANATIONS?

Intrauterine PBMCs administration & IVF outcome in RIF patients

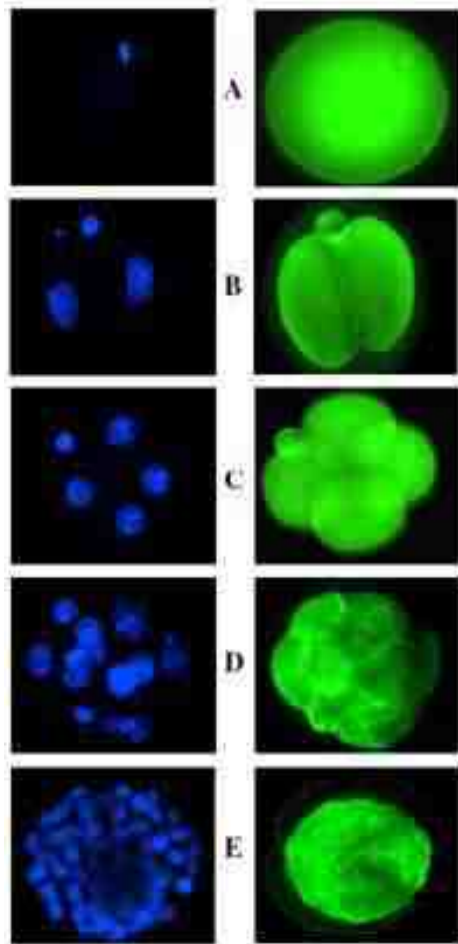
CRH & cytokine production



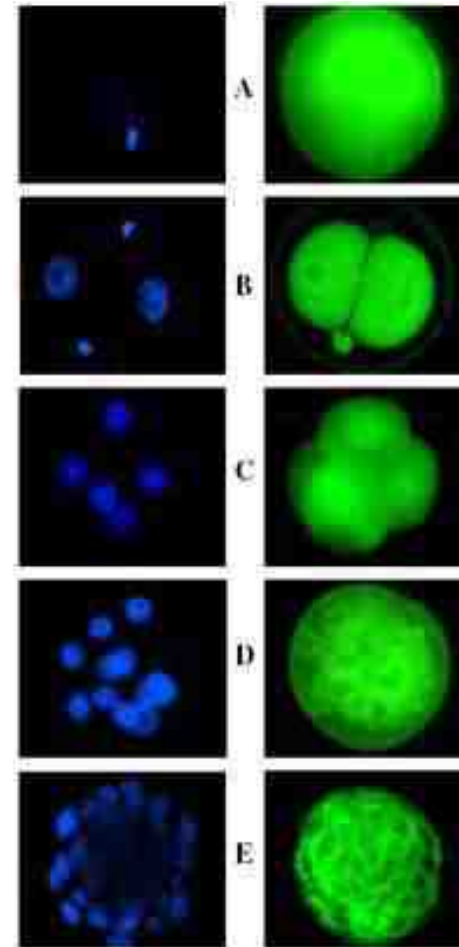
CRH added to primary cultures of PBMCs significantly increased IL-6 (Th2-type immunity) release and decreased IFN- γ (Th1-type immunity) levels in a dose dependent manner

Makrigiannakis et al, EJCI, 2015

CRH & development of embryos



CRH expression in embryo



CRHR1 expression in embryo

Intrauterine PBMCs administration & IVF outcome in RIF patients

CRH & endometrium



- CRH induces stromal decidualization and potentiates the decidualizing effect of progesterone
- CRH regulates local modulators of the **decidualization** process; it inhibits the enhancer PGE2, induces the inhibitor interleukin (IL) 1 and stimulates the inducer IL-6.

Makrigiannakis et al, MHR 1999

Zoumakis et al, 2000; Makrigiannakis et al 1999

Conclusions



- Local endometrial injury, PBMC and PBMC & CRH use may improve pregnancy outcomes in women with unexplained RIF

- ?? How it works: not entirely known (**Inflammation**)

- Use under approved clinical trials with appropriate patient consent

- Need for appropriate randomized trials comparing **standardized research interventions** with **no intervention** in a **well-defined RIF patient population**



Thank you for your attention