Evidence based strategies in RIF. Novel approaches

Antonis Makrigiannakis MD, PhD
Professor of Obstetrics and Gynecology
Medical School, University of Crete

What is RIF

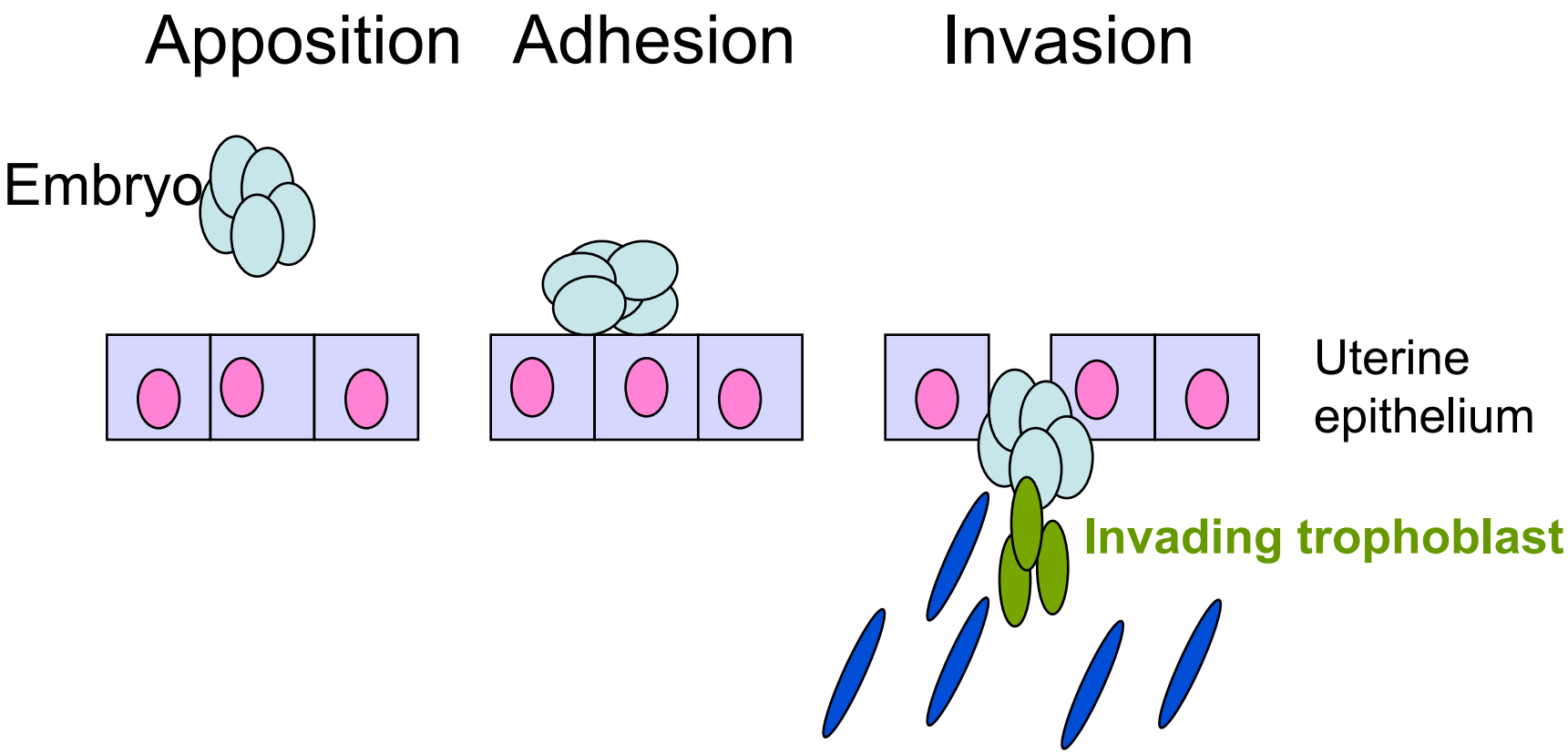
- **How many attempts?**
- How many embryos?
- Age?
- Embryo quality?
- Failure in the presence of potential obstacles?

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 ?

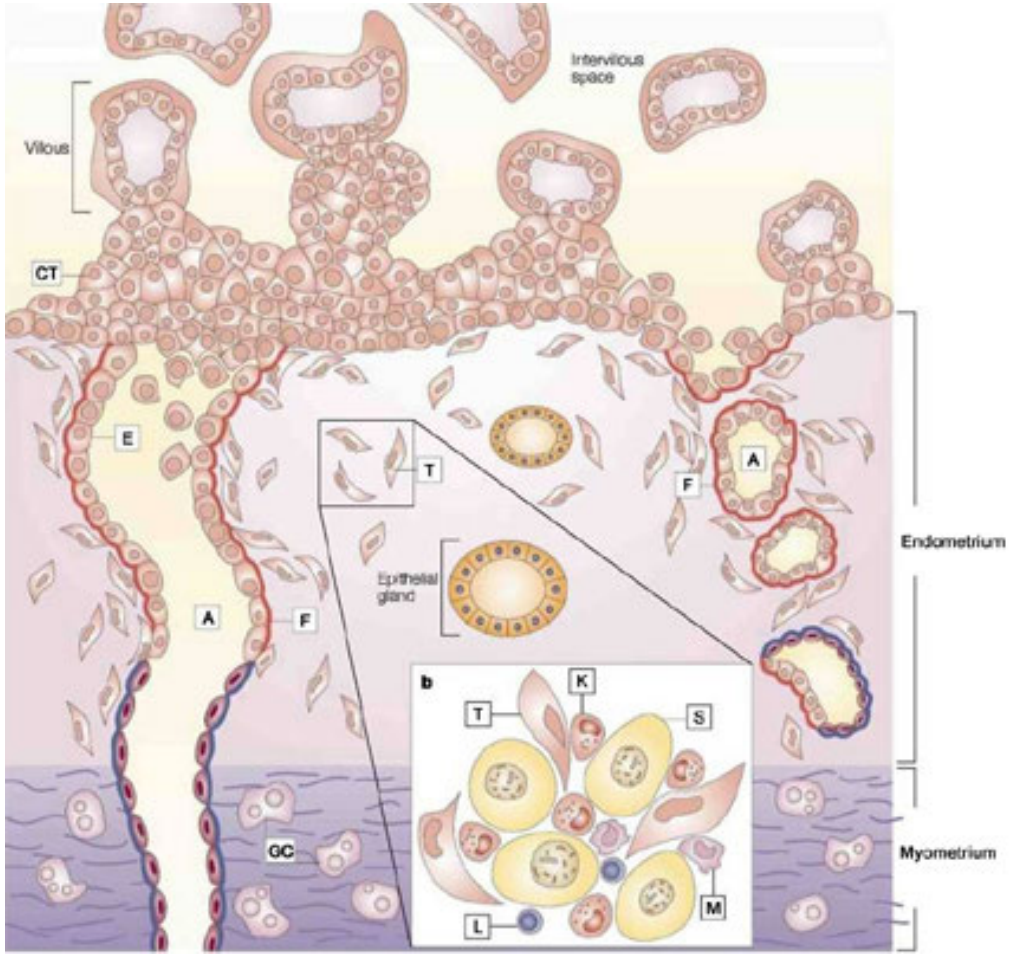
Implantation - key event in the establishment of pregnancy



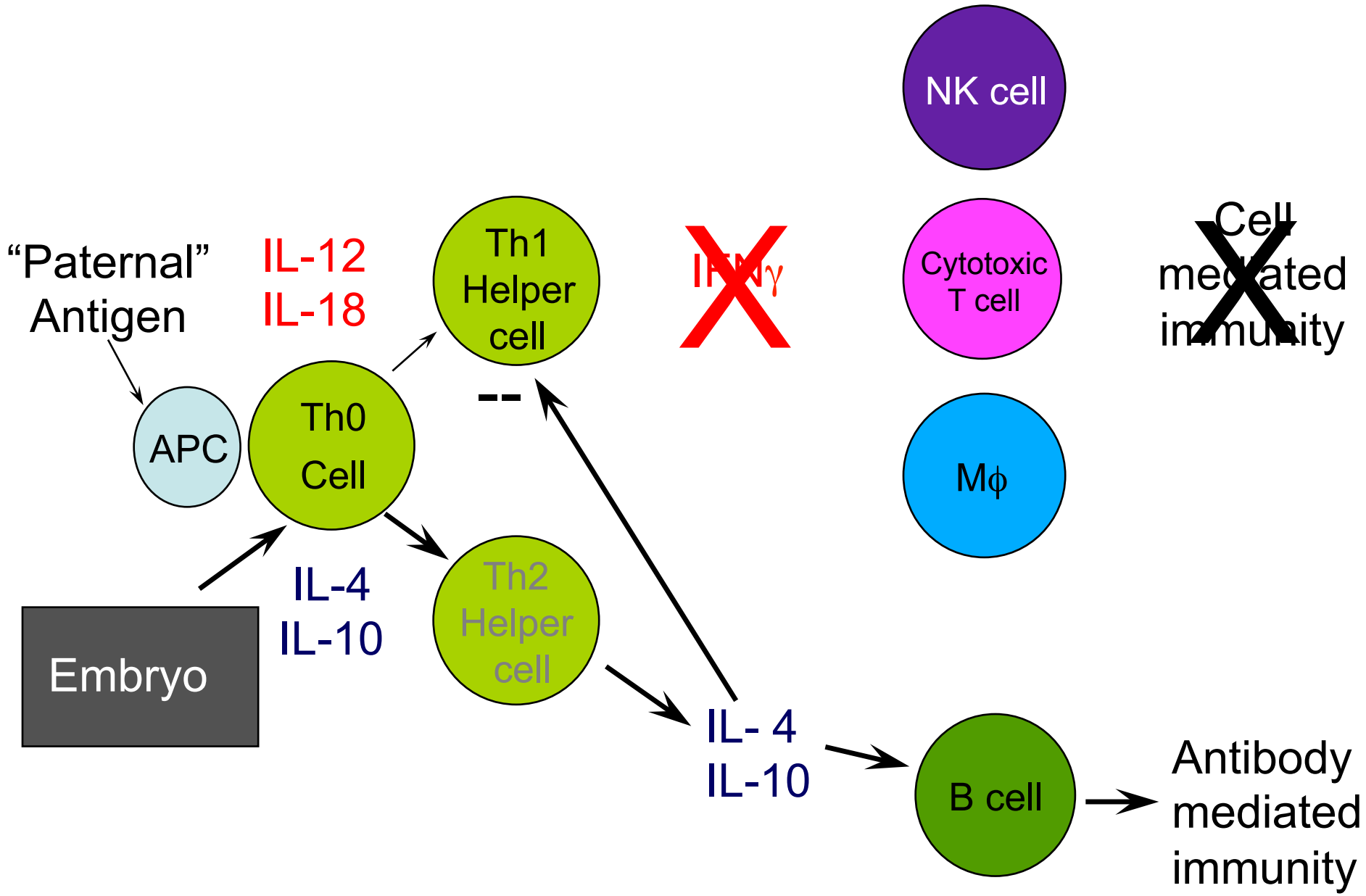
Continuous process from conception to 22 weeks gestation

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 fur 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]

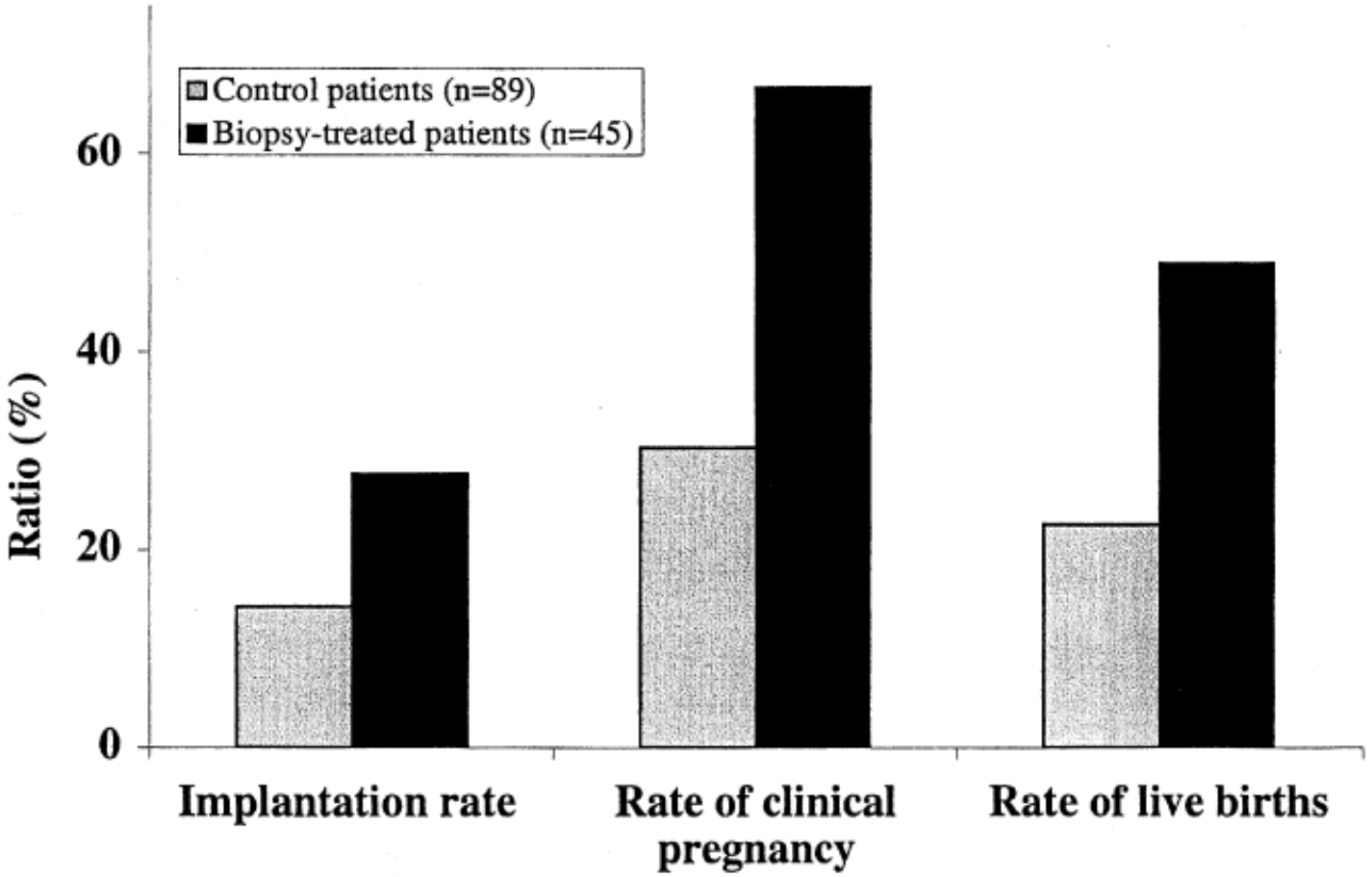
IVF Unit, Department of Obstetrics and Gynecology, The Kaplan Medical Center, Rehovot, Israel

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®
VOL. 79, NO. 6, JUNE 2003



Significant increase in clinical pregnancy and live birth rate

Fertil & Steril 2003;79:1317

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

Fertil & Steril Vol. 79, No. 6, June 2003

Reproductive BioMedicine Online (2012) 25, 561–571

www.sciencedirect.com
www.rbmonline.com

REVIEW

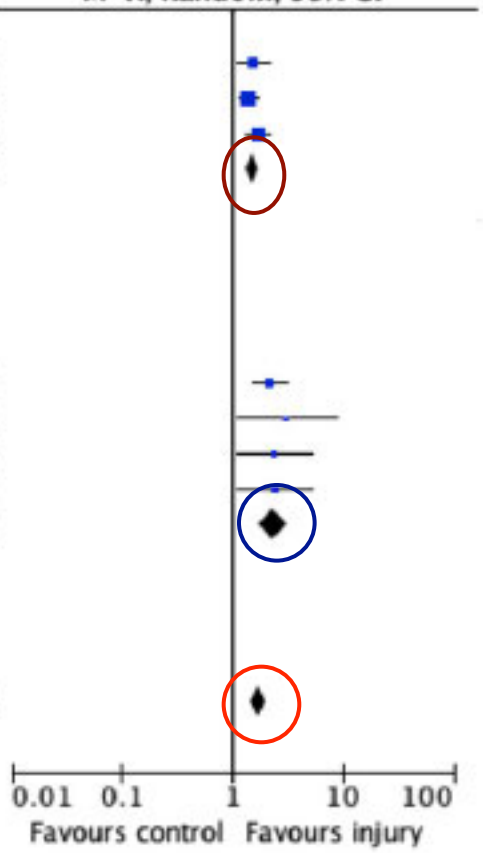
Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis

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, Gynehealth, Manchester, UK*

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

Study or Subgroup	Injury Events	Injury Total	No injury Events	No injury Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Hysteroscopy						
Demirol and Gurgan 2004	50	154	45	211	17.5%	1.52 [1.08, 2.15]
Makrakis et al., 2009	145	414	104	414	31.4%	1.39 [1.13, 1.72]
Rama Raju et al., 2006	71	160	69	265	24.5%	1.70 [1.30, 2.23]
Subtotal (95% CI)		**728**		**890**	**73.4%**	**1.51 [1.30, 1.75]**
Total events	266		218			
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.34$, df = 2 (P = 0.51); $I^2 = 0\%$						
Test for overall effect: Z = 5.40 (P < 0.00001)						
1.1.2 Endometrial biopsy (scratch)						
Barash et al., 2003	30	45	27	89	15.4%	2.20 [1.51, 3.20]
Karimzadeh et al., 2009	13	48	4	45	2.6%	3.05 [1.07, 8.66]
Narvekar et al., 2010	16	49	7	51	4.3%	2.38 [1.07, 5.28]
Raziel et al., 2007	18	60	7	57	4.3%	2.44 [1.10, 5.41]
Subtotal (95% CI)		**202**		**242**	**26.6%**	**2.32 [1.72, 3.13]**
Total events	77		45			
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.39$, df = 3 (P = 0.94); $I^2 = 0\%$						
Test for overall effect: Z = 5.50 (P < 0.00001)						
Total (95% CI)		**930**		**1132**	**100.0%**	**1.71 [1.44, 2.02]**
Total events	343		263			
Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 7.99$, df = 6 (P = 0.24); $I^2 = 25\%$						
Test for overall effect: Z = 6.12 (P < 0.00001)						
Test for subgroup differences: $Chi^2 = 6.29$, df = 1 (P = 0.01), $I^2 = 84.1\%$						

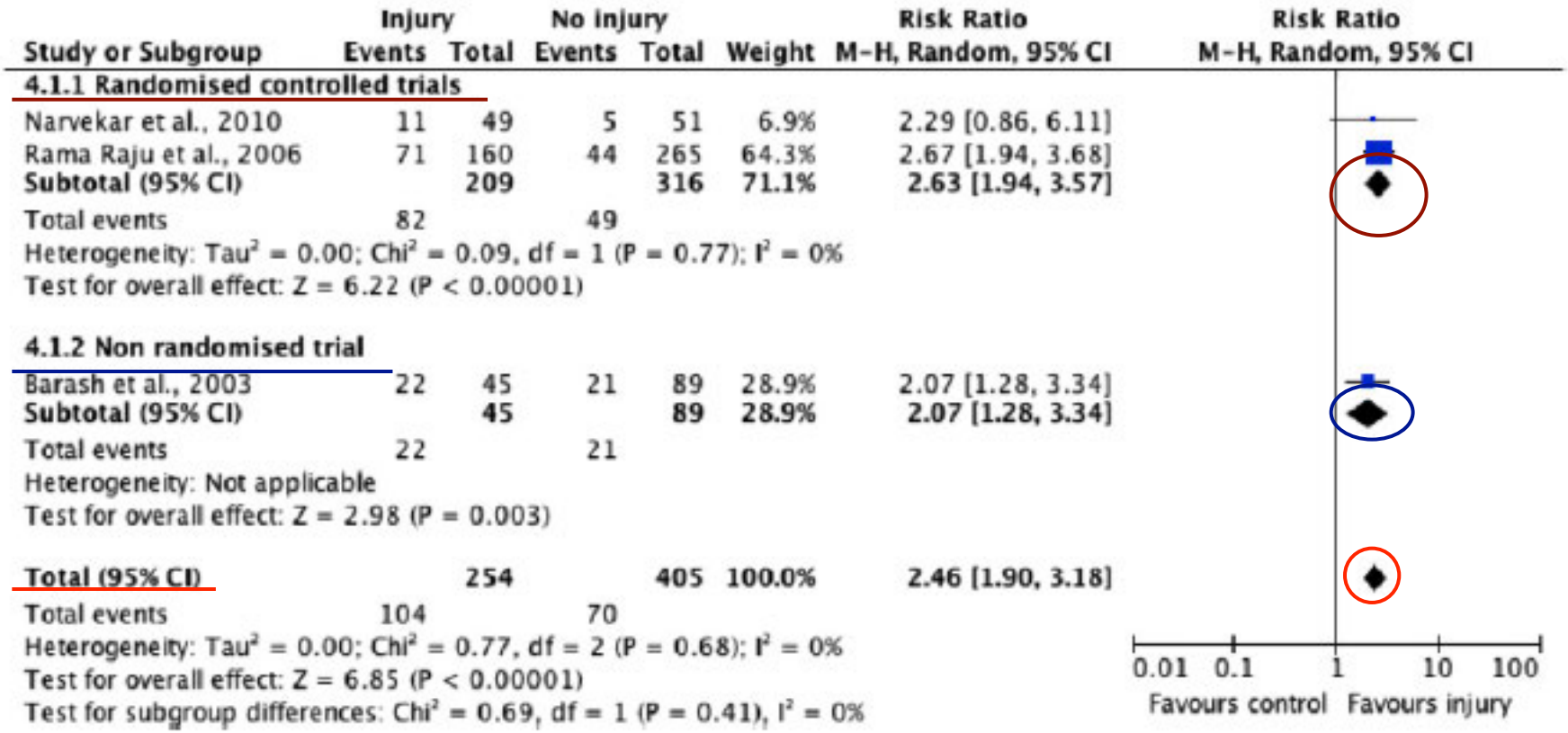


(hysteroscopy and endometrial biopsy) and control group

Endometrial injury favors implantation

Reproductive BioMedicine Online (2012) 25, 561– 571

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



Study or Subgroup	Injury Events	Injury Total	No injury Events	No injury Total	Weight	Risk Ratio M-H, Random, 95% CI
4.1.1 Randomised controlled trials						
Narvekar et al., 2010	11	49	5	51	6.9%	2.29 [0.86, 6.11]
Rama Raju et al., 2006	71	160	44	265	64.3%	2.67 [1.94, 3.68]
Subtotal (95% CI)		**209**		**316**	**71.1%**	**2.63 [1.94, 3.57]**
Total events	82		49			
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.09$, df = 1 (P = 0.77); $I^2 = 0\%$						
Test for overall effect: Z = 6.22 (P < 0.00001)						
4.1.2 Non randomised trial						
Barash et al., 2003	22	45	21	89	28.9%	2.07 [1.28, 3.34]
Subtotal (95% CI)		**45**		**89**	**28.9%**	**2.07 [1.28, 3.34]**
Total events	22		21			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.98 (P = 0.003)						
Total (95% CI)		**254**		**405**	**100.0%**	**2.46 [1.90, 3.18]**
Total events	104		70			
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.77$, df = 2 (P = 0.68); $I^2 = 0\%$						
Test for overall effect: Z = 6.85 (P < 0.00001)						
Test for subgroup differences: $Chi^2 = 0.69$, df = 1 (P = 0.41), $I^2 = 0\%$						

Endometrial injury improves live birth rate

Reproductive BioMedicine Online (2012) 25, 561– 571

Reproductive BioMedicine Online (2012) **25**, 345–354

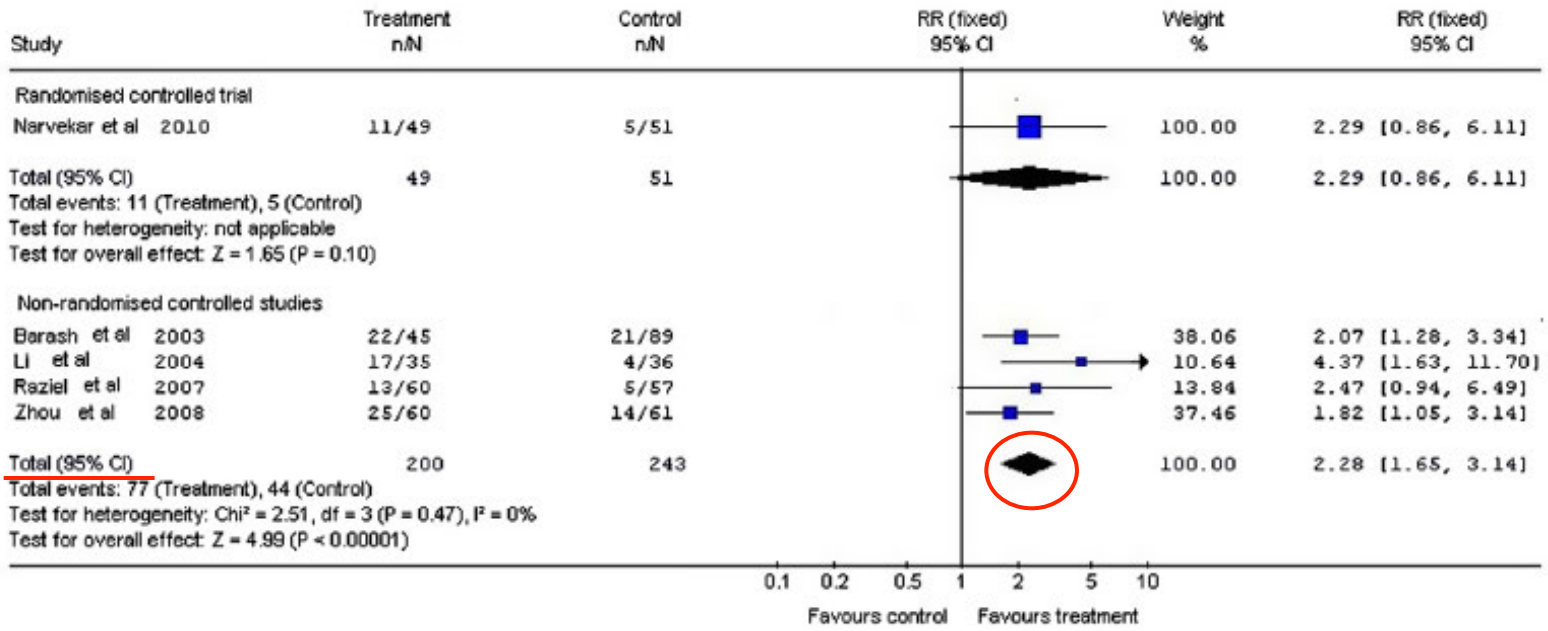
www.sciencedirect.com
www.rbmonline.com

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

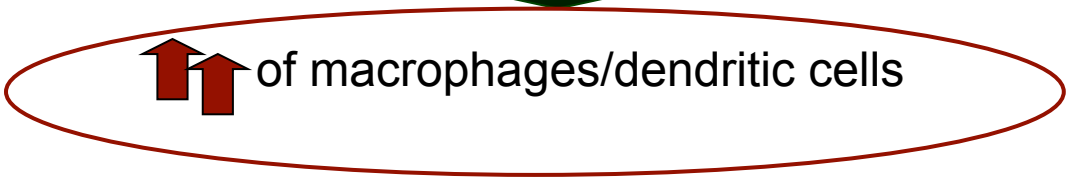


Improvement of live birth/ongoing pregnancy rate

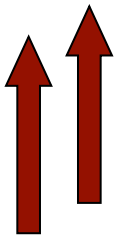
Reproductive BioMedicine Online (2012) 25, 345– 354

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

Endometrial biopsy



- tumor necrosis factor-a (TNF-a),
- growth-regulated oncogene-a (GROa),
- 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-a expression & the pregnancy outcome.

Gnainsky et al. Fertil & Steril 2010;94:2030

Issues to be addressed

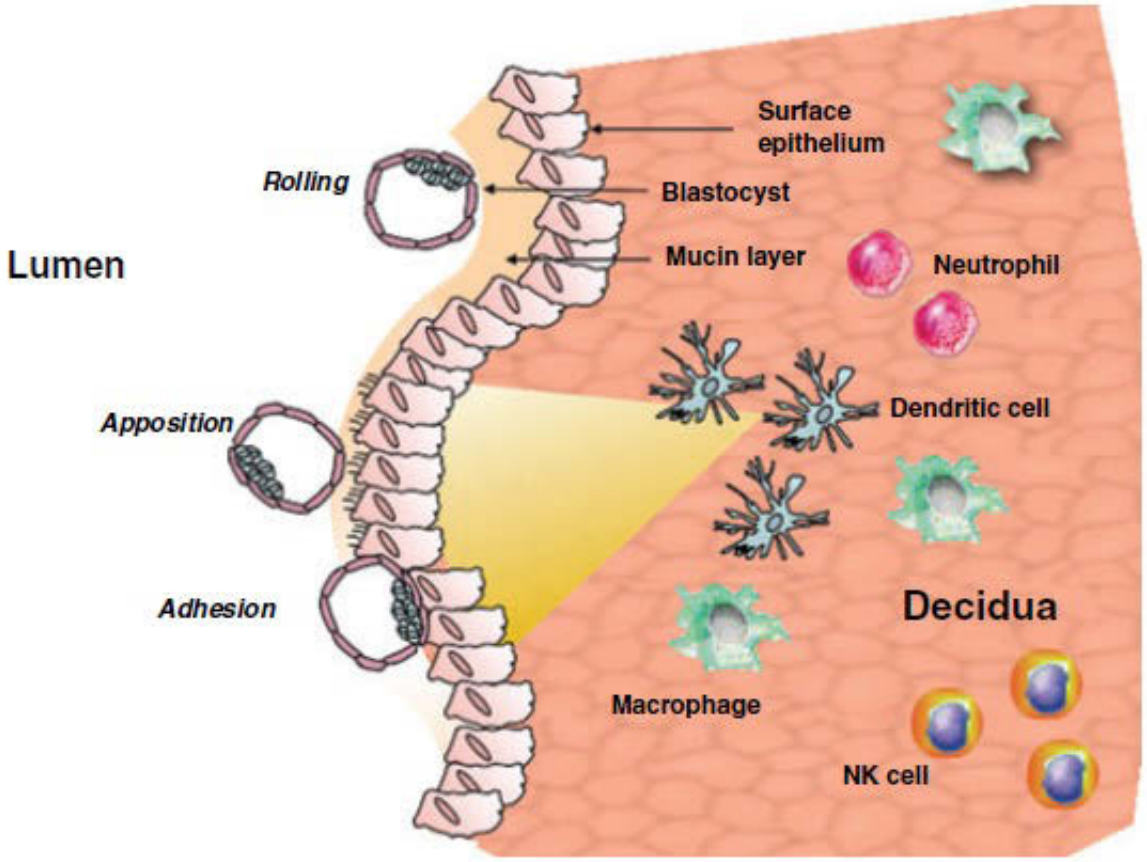
- Which is the best cycle?
  - Evidence support the performance of endometrial biopsy one cycle prior to IVF
  - It is advisable not to perform endometrial injury on the day of oocyte retrieval because it appears to significantly reduce clinical and ongoing pregnancy rates.

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

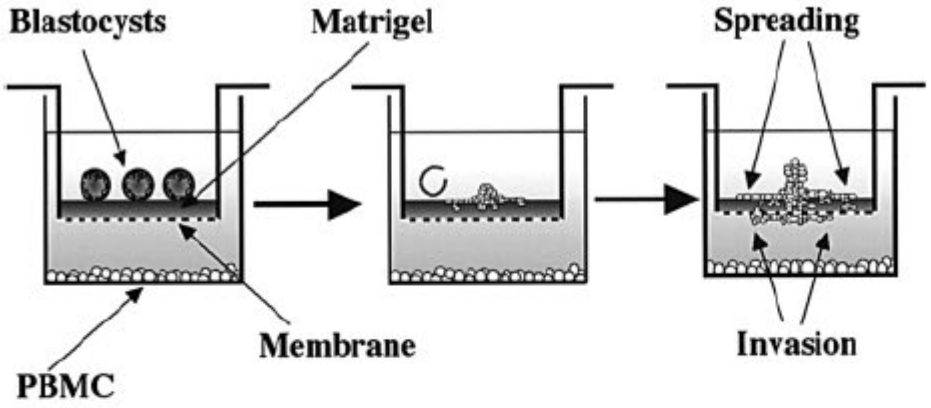


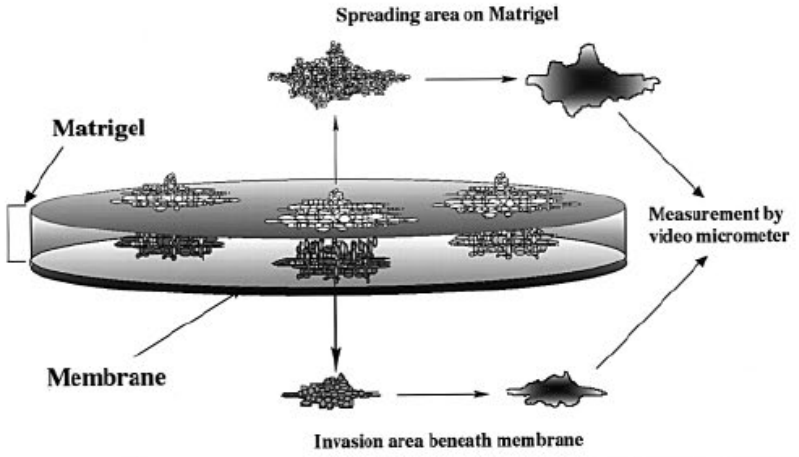
Dendritic cells & macrophages increase in local injury & during the window of implantation

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

Nakayama et al, Hum Reprod 2002

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%

Yoshioka et al. Hum Reprod 2006;21:3290

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**

Okitsu et al. Journal of Reproductive Immunology 92 (2011) 82– 87

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.**

Okitsu et al. Journal of Reproductive Immunology 92 (2011) 82– 87

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. Coutifaris[5], A. N. Margioris[1], G. Coukos[5], K. C. Rice[6], A. Gravanis[1,*] and G. P. Chrousos[4,*]

nature immunology • volume 2 no 11 • november 2001

DOI: 10.1111/eci.12417

ORIGINAL ARTICLE

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 (FSH< 12 mIU/ml)

IVF PROCEDURE

EMBRYO CULTURE

After fertilization was confirmed the day after fettilization (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

Table 1 Clinical characteristics of RIF women included in the study

		Clinical pregnancy		
	Overall (n = 45) Mean ± SEM	Yes (n = 20) Mean ± SEM	No (n = 25) Mean ± SEM	P
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			

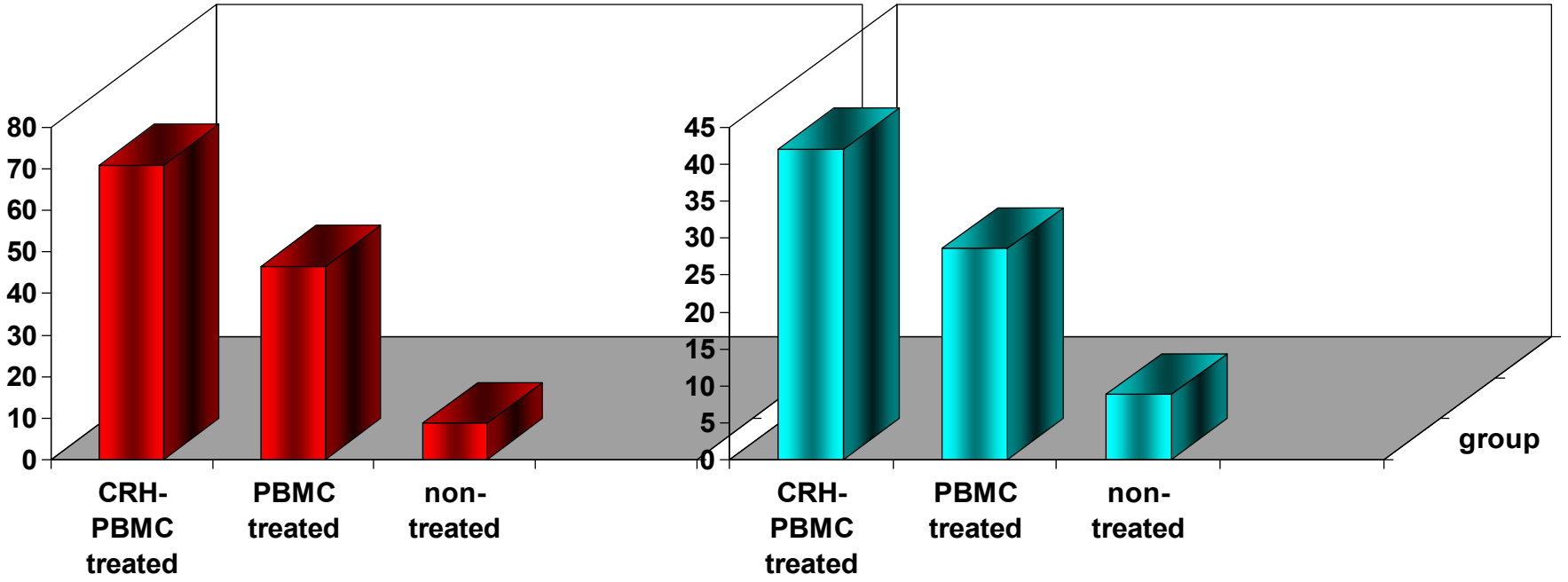


Table 2 Overview of the studies using PBMC for improving clinical pregnancy rate after blastocyst transfer in women with RIF

	Embryo type	Intervention	Participants (*n*)	Clinical pregnancy rate	Outcome significance
Yoshioka *et al.*, [9]	Blastocyst	HCG-treated PBMC	17	42·1% (7/17)	Significant
Okitsu *et al.*, [15]	Blastocyst	Untreated PBMC	13	46·1% (6/13)	Not significant
Current study	Blastocyst	CRH-treated PBMC	45	44·4% (20/45) *	Significant

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

YES!

POSSIBLE EXPLANATIONS?

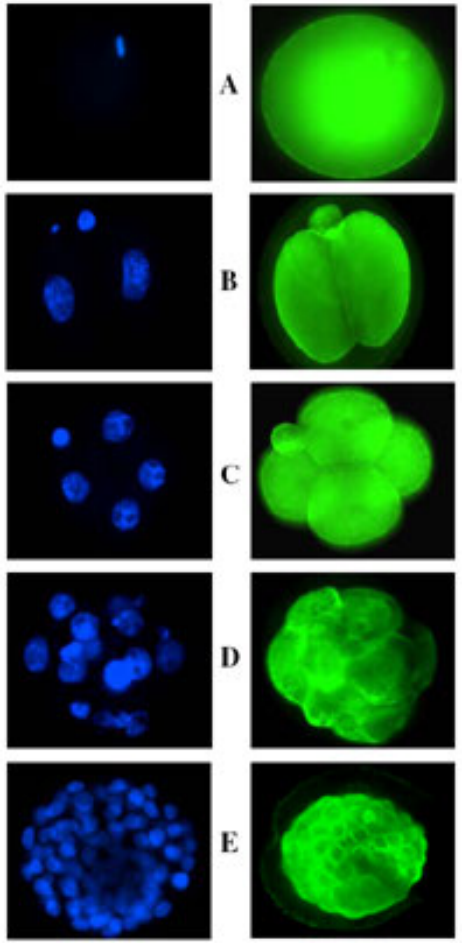
Intrauterine PBMCs administration & IVF outcome in RIF patients

CRH & cytokine production

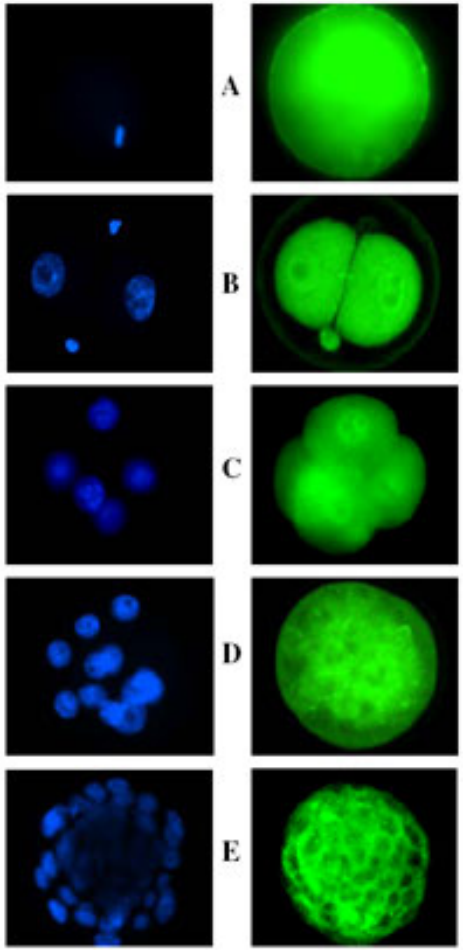
CRH added to primary cultures of PBMCs significantly increased IL-6 (Th2-type immunity) release and decreased IFN-γ (Th1-type immunity) levels in a dose dependent manner

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

Makrigiannakis et al,MHR 1999

- 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.

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