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The role of Immunologic factors in RSA and RIF

Elham Amirchaghmaghi

MD, PhD in Medical Immunology

Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

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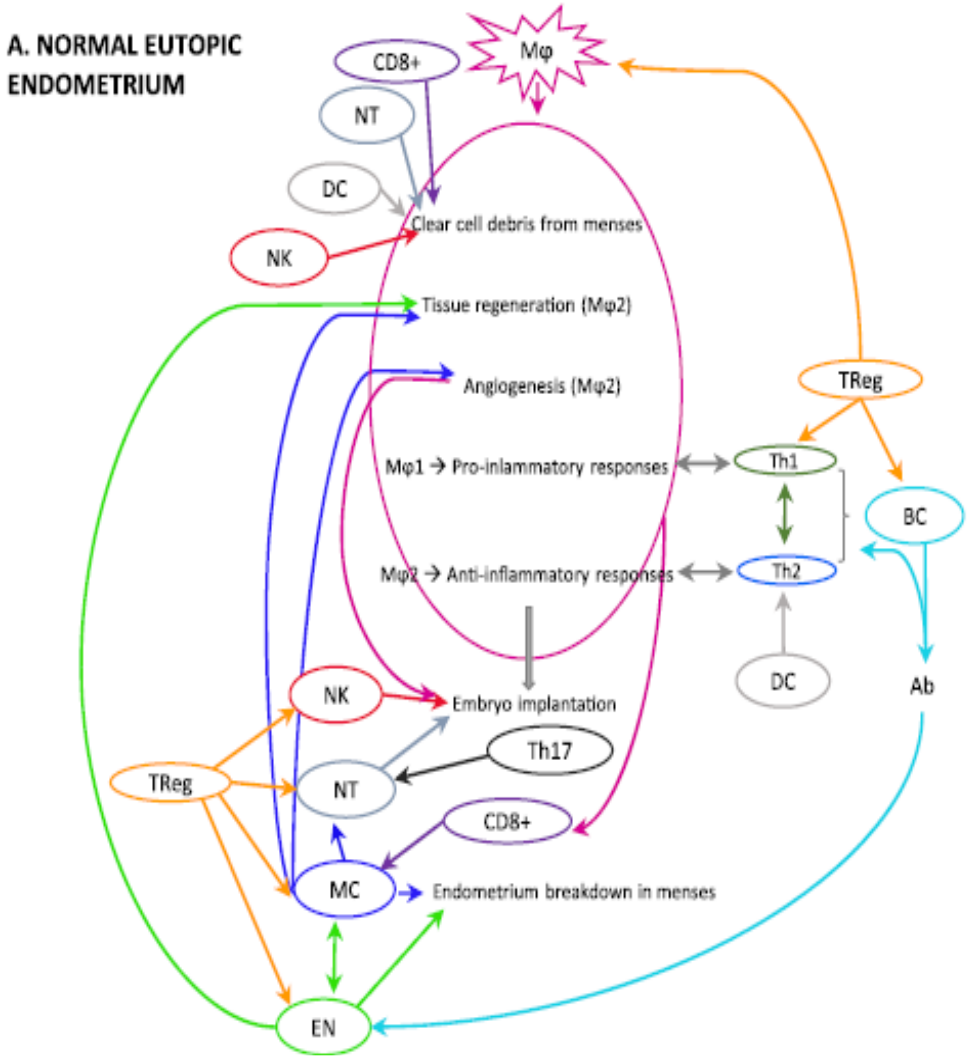
Immune cells in Endometrium



Vallve-Juanico et al. 2019

Immunology of Embryo Implantation

- The immune system plays a key role in
  - fetomaternal cross-talk, embryo development
  - normal implantation and placentation
  - at all phases contribution for pregnancy success.

Chaouat G, 2016

- At materno-fetal interface,
  - Most immune cells (70%) being CD56+ natural killer (NK) cells
  - 20% of monocytes
  - Between 10% to 15% of all cells found in the decidua are lymphocytes, mainly regulatory T cells (Treg).

Moffett-King A ,2002

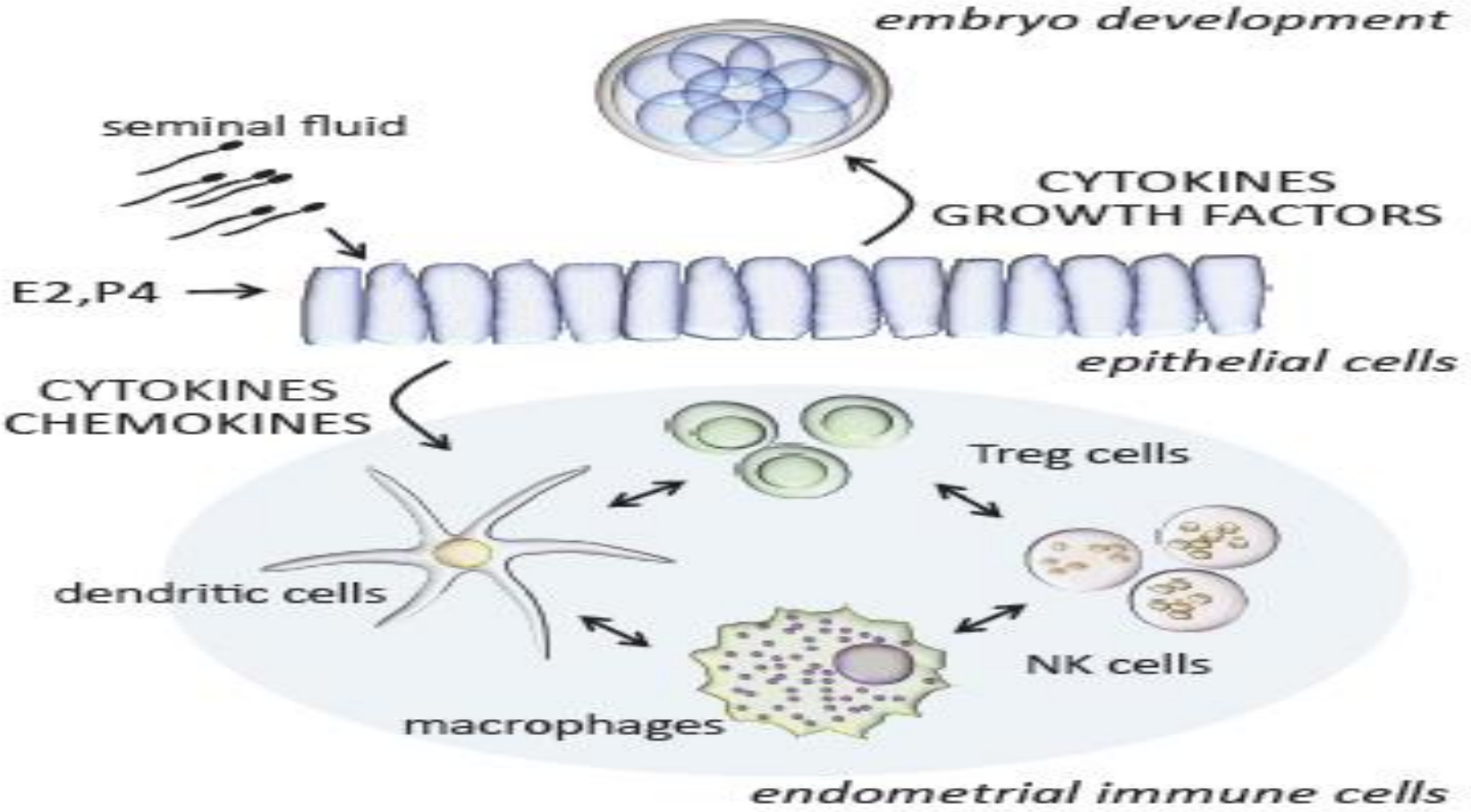


Fig. 1. Successful implantation depends on a dynamic and synchronized process in which both the embryo and the endometrium are adequately developed. *The immune system is involved in the leukocyte and cytokine networks required to generate endometrial receptivity, and also in the provision of cytokine signals to regulate development of the pre-implantation embryo. Critical endometrial immune cells are macrophages, dendritic cells, natural killer cells (NK cells) and regulatory T cells (Treg cells).*

Robertson SA 2014

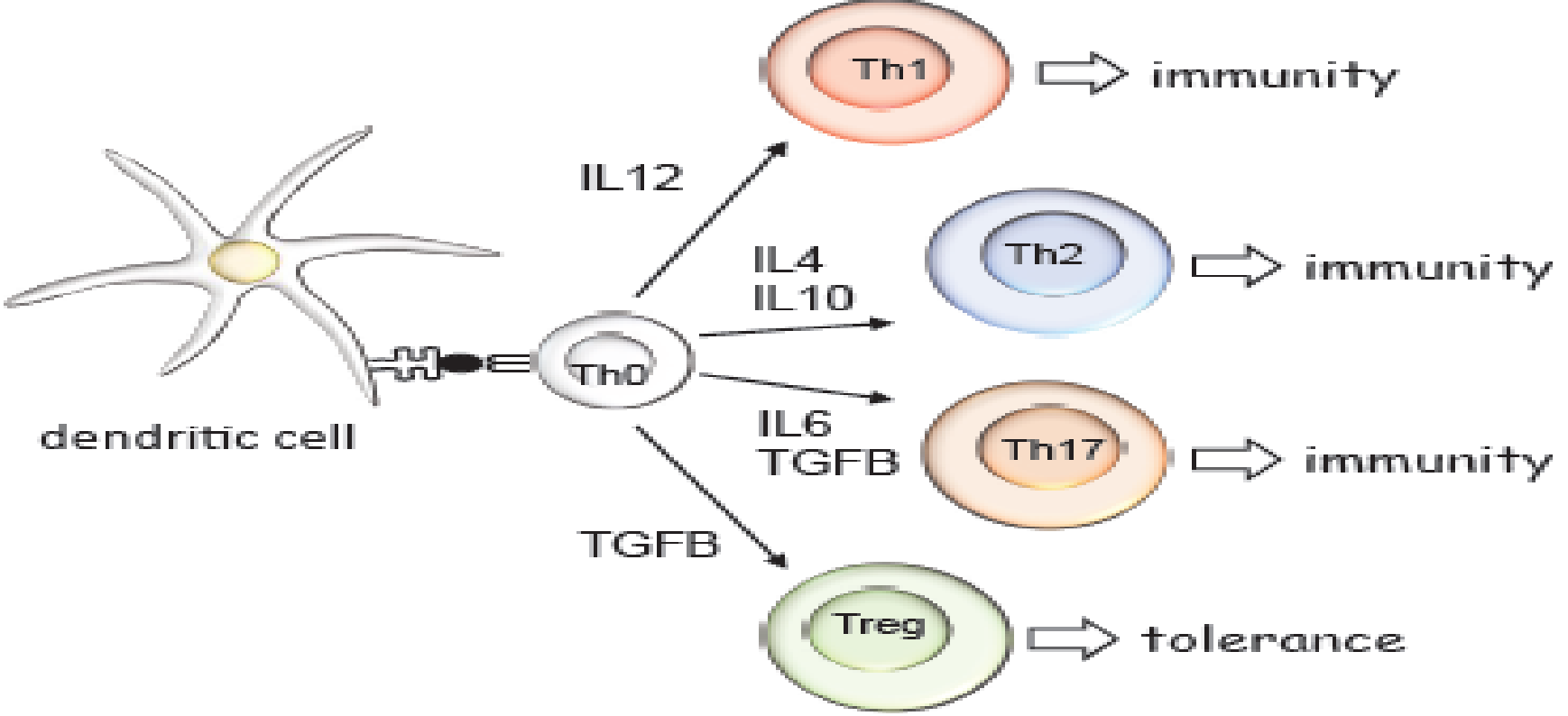


Fig. 3. The generation of Treg cells required for endometrial receptivity depends on the balance of cytokines and the activity of dendritic cells. *Cytokines are a key part of the microenvironmental context which determines whether CD4+ Th0 cells differentiate and develop into Treg cells as opposed to Th1, Th2 or Th17 cells. Treg cells confer immune tolerance and suppress inflammation while Th1, Th2 and Th17 cells mediate immunity and are linked with inflammation and rejection of the conceptus. Signals originating from the dendritic cell presenting antigen to the Th0 cell, as well as the relative concentrations of key cytokines in the immediate vicinity, are instrumental. IL, interleukin; Th1, Th2, Th17, T-helper type 1, type 2 and interleukin 17-producing Th cell; TGFB, transforming growth factor beta.*

Robertson SA 2014

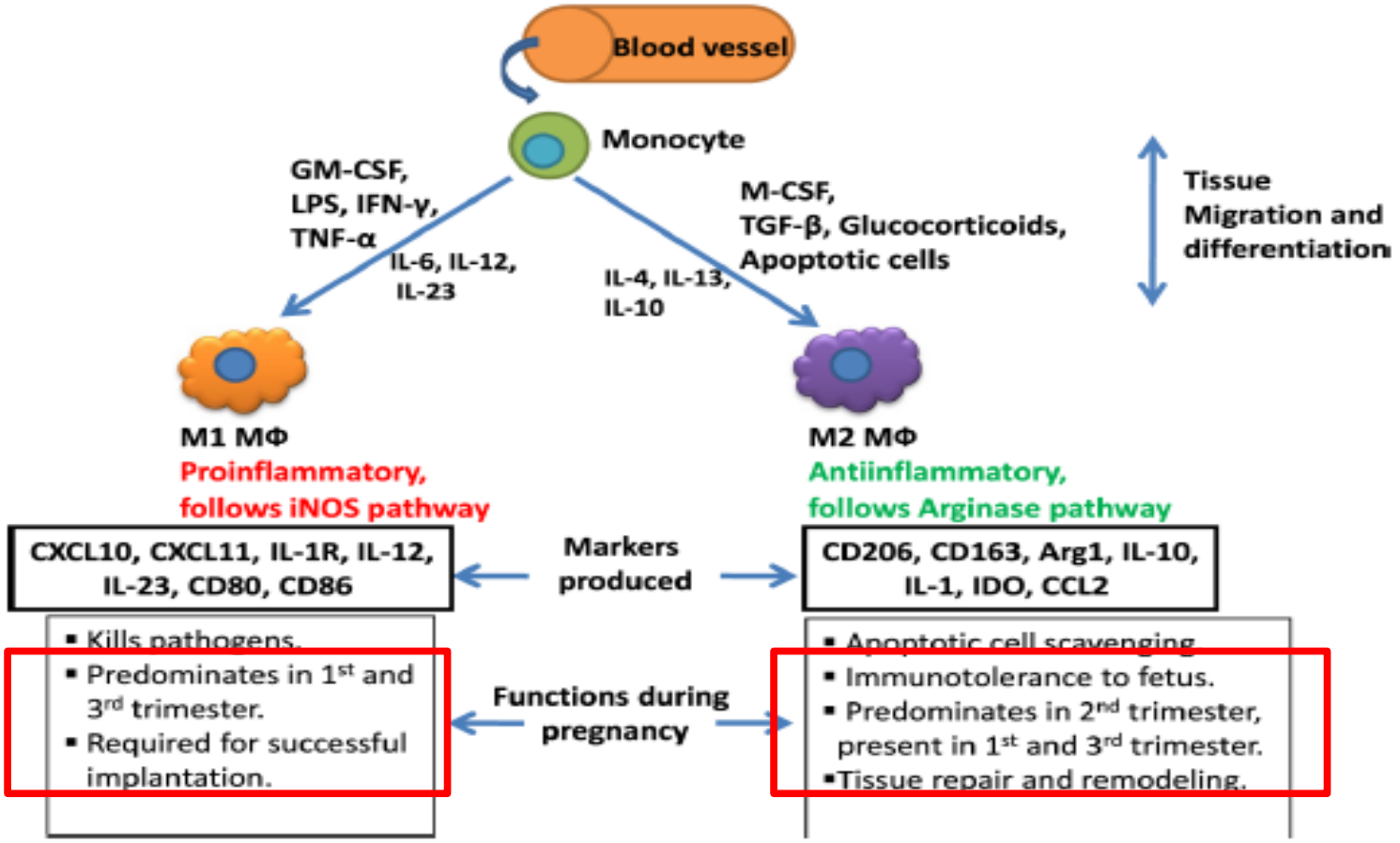


Fig. 1.
Macrophage differentiation and polarization in the decidua during pregnancy

Jena MK et al 2019

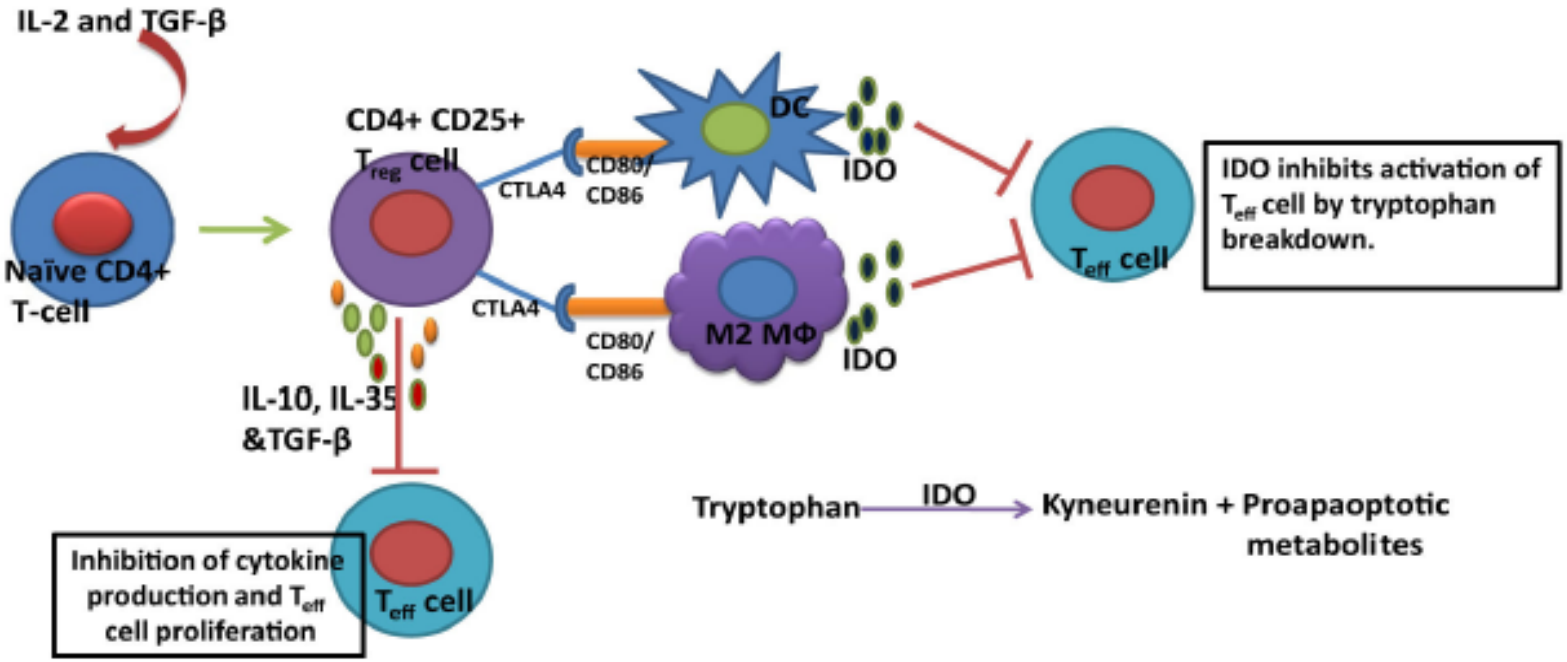


Fig. 2.
T_{reg} cells showing immunotolerance through M2 MΦs and dendritic cells (DCs). The naïve T cells are induced by IL-2 and TGF-β in the decidua, forming Treg cells. Treg cells express the surface molecule CTLA4 which interacts with CD80/CD86 of DCs and MΦs, thus inducing those cells for IDO production. IDO inhibits T_{eff} cell proliferation. The cytokines IL-10, IL-35, and TGF-β produced from Treg cells also inhibit T_{eff} cell proliferation. IDO = Indoleamine 2, 3-dioxygenase, T_{reg} = T regulatory, T_{eff} = T effector

Jena MK et al 2019

Clonal expansions of allospecific uterine and peripheral Treg together with the proliferation of uNKs and DCs are involved in the maintenance of immune tolerance to the fetus.

Ruocco MG, 2014, Chen T, 2013

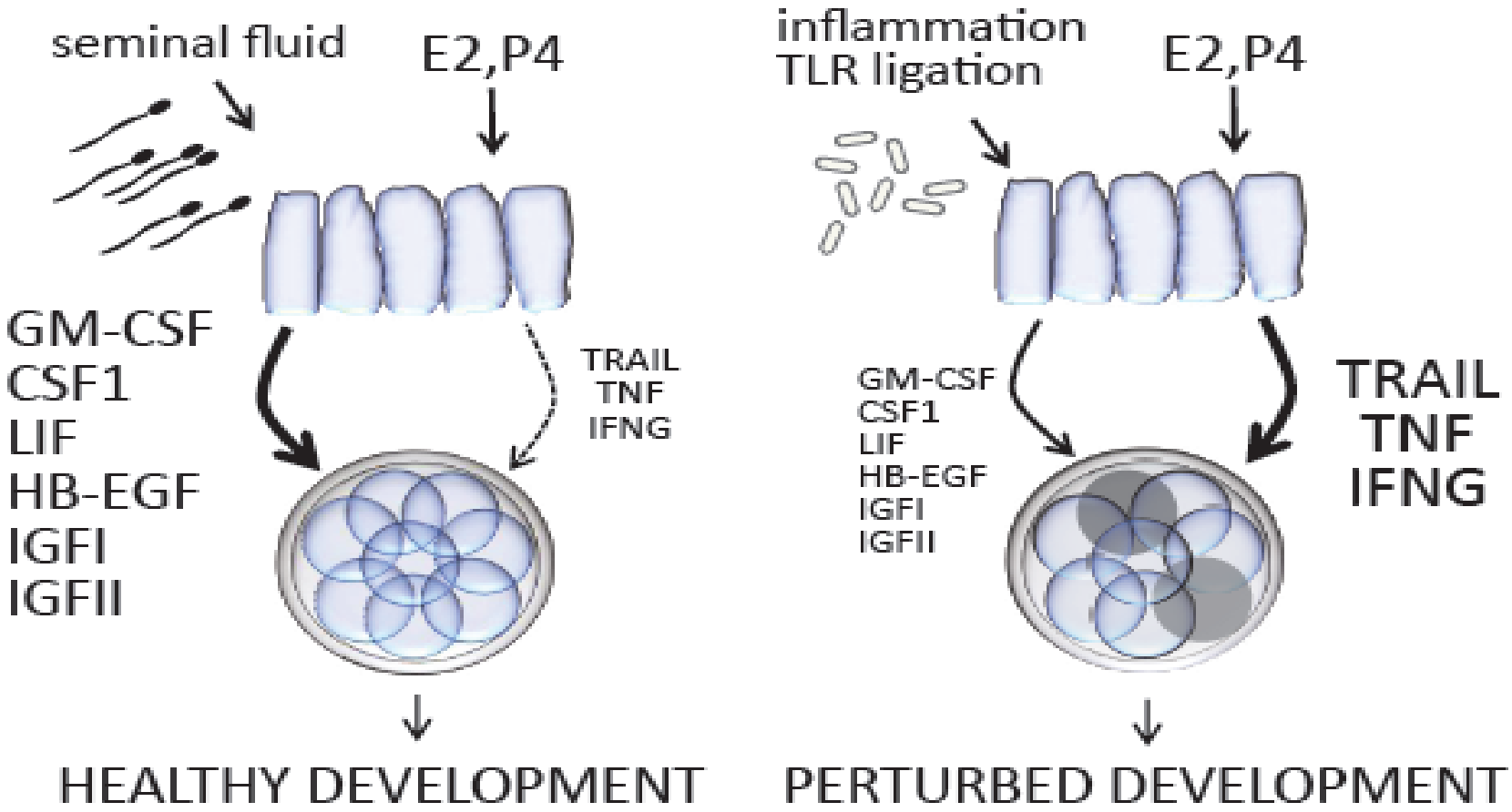


Fig. 2. Cytokines released from the oviduct and uterus, which signal via specific receptors on the embryo cell surface, exert effects on cell number and viability, gene expression and developmental competence in embryos. *Embryotrophic cytokines induced by seminal fluid modulate embryo metabolic function and gene expression, facilitate cell viability and sustain development, while embryotoxic cytokines induced by inflammatory challenges such as infection, act to induce apoptosis, inhibit embryo development potentially causing perturbed post-implantation development.*

Robertson SA 2014

Recurrent Abortion

- The guideline development group concludes to use the term Recurrent Pregnancy Loss (RPL).

- A diagnosis of Recurrent Pregnancy Loss (RPL) could be considered after the loss of **two** or more pregnancies.

ESHRE Guideline, 2017

- According to the guidelines of WHO , **recurrent miscarriage (RM)** : occurrence of **three** or more spontaneous and consecutive gestational losses in pregnancies of <20 weeks length.

- Recurrent miscarriage affects about 2%-5% of couples on their reproductive age.

Causes

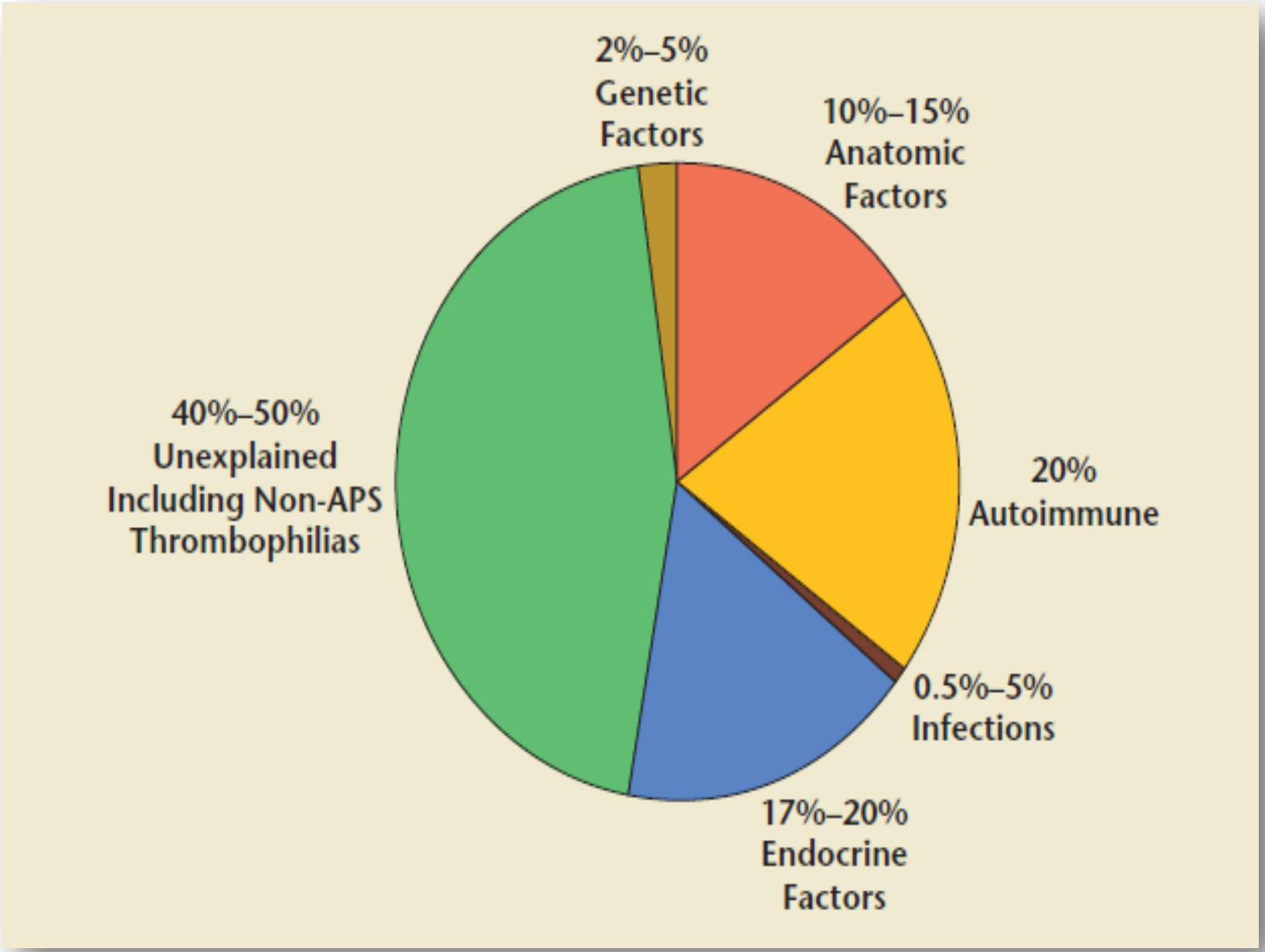
- Genetic abnormalities (Chromosomal aberrations , Balanced translocations)
- Uterine anomalies (Asherman syndrome, ..)
- Autoimmune diseases (SLE, Antiphospholipid syndrome)
- Blood clotting disorders (Hyperhomocystinemia or other types of thrombophilias)
- Infectious diseases
- Endocrinopathies (PCOS, Diabetes Mellitus, Hypothyroidism)
- **Immunologic factors**
- Sperm DNA fragmentation and meiotic alterations
- Idiopathic

Gupta et al. 2007

Recurrent miscarriage may be due to one of **three** major causes:

1. Embryonic factor: There may be an embryo which is inherently incompatable with life, such as chromosome trisomy on triploidy.
2. Maternal factors such as uterine anomalies or antiphospholipid antibodies
3. The embryo may be made abnormal by maternal factors.

Carp H 2004



Ford et al. 2009

Recurrent Implantation Failure

- There is a wide variability in the definition of RIF.

- Three generic definitions can be considered according to:
  - The number of unsuccessful ART cycles
  - The number of embryos transferred
  - A combination of both factors

Polanski LT, 2014

RIF is generally defined as

- Failure to achieve clinical pregnancy: transfer of two good quality embryos, in at least three fresh or frozen IVF cycles/ETs (6 embryos in total) or in at least two egg donations (EG, i.e. 4 embryos in total).

  Margalioth EJ,2006

- Failure to achieve pregnancy :repeated IVF cycles (2 to 6 IVF cycles), in which at least 10 high-grade embryos were transferred

  Shufaru Y ,2011

Considering that embryo quality is closely related to maternal age, some definitions willonly consider failed IVF transfer cycle in women younger than 40 years:

- The failure of clinical pregnancy after 4 good quality embryo transfers, with at least three fresh or frozen IVF cycles in women under the age of 40.

  Coughlan C, 2014

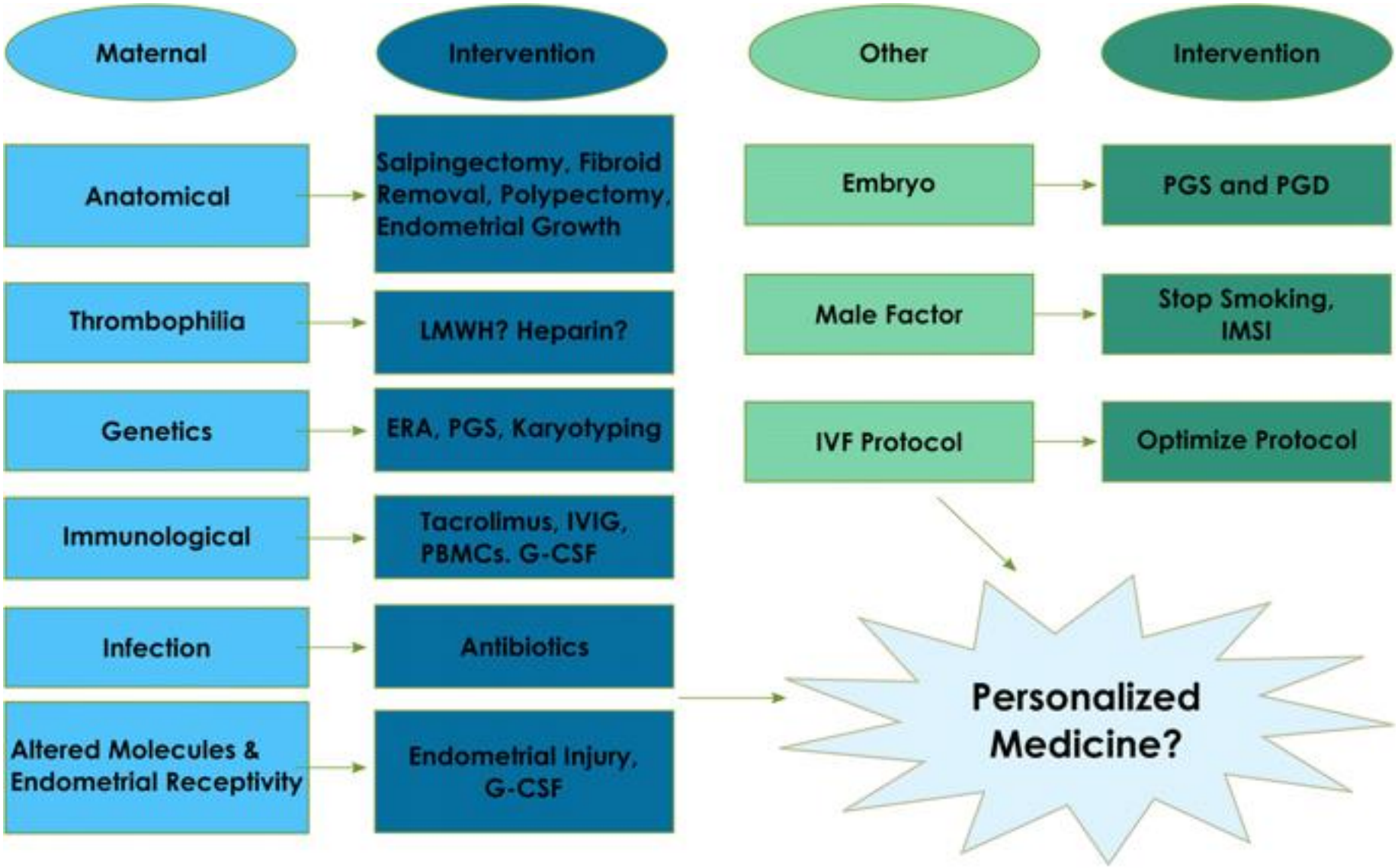
Causes

- Most common causes of RIF are classified as:
  - ❖ **Maternal factors:**
    - Uterine anatomical anomalies
    - Endometrium pathology resulting in altered endometrial receptivity;
    - States of hypercoagulability;
    - **Immunological factors (iRIF)**

Shufaru Y ,2011

  - ❖ **Embryological factors:**
    - It is important to note how good quality embryos are defined.
    - Routine assessment is usually done based on morphological factors but not on genetic embryo study.
    - Studies on pre-implantation genetic diagnosis (PGD) refer up to 67% of aneuploidy embryos in patients with RIF.

Pehlivan T 2003



Bashiri A 2018

Multiple factors may contribute to RIF

- Woman's age
- Oocyte quality
- Sperm quality
- Parental chromosomal anomalies
- Genetic or metabolic abnormalities of the embryo
- Poor uterine receptivity
- Immunological disturbances in the implantation site
- Gynological pathologies: endometriosis, uterine fibroids, hydrosalpinx ,endometrial polyps
- Process of preparation for ART
- Lifestyle: smoking, alcohol consumption, obesity

Cakmak and Taylor 2011; Coughlan et al. 2014; Das and Holzer 2012; Koot et al. 2012; Penzias 2012 , Nowak I 2017

Review

iMedPub Journals
http://www.imedpub.com/

Reproductive Immunology: Open Access
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Evidence-based Update: Immunological Evaluation of Recurrent Implantation Failure

Alejandra Comins-Boo, Áurea García-Segovia, Natalia Núñez del Prado, Laura de la Fuente, Jorge Alonso and Silvia Sánchez-Ramón*

Table 1: Categorization of evidence and basis of recommendation and strength of recommendation.

Ia	From meta-analysis of randomized controlled studies
Ib	From at least one randomized controlled study
IIa	From at least control trial without randomization
IIb	From at least one other type of quasi-experimental study
III	From non-experimental descriptive studies, such as comparative, correlation, or case-control studies
IV	From expert committees reports or opinion or clinical experience of respected authorities or both
A	Based on Category I evidence
B	Based on Category II evidence or extrapolated from Category I evidence
C	Based on Category III evidence or extrapolated from Category I or II evidence
D	Based on Category IV evidence or extrapolated from Category I, II or III evidence

Comins-Boo A et al. Reproductive Immunology 2016

Table 2: Evidence-based categorization and strength of recommendation assigned for the immunological tests proposed for the evaluation of recurrent implantation failure.

Immunological factor	Evidence Grade	Strength of Recommendation	Number of patients	Ref.
Antiphospholipid syndrome	IIb	B	7064	20, 22, 25, 28, 29
Thyroid autoimmunity	IIb	B	3016	63, 64, 66, 67, 68
Celiac disease	III	C	2078	71
pNK cell expansion	III	C	530	37, 38, 39, 40, 42, 44
uNK cells expansion	III	C	30	44, 53
Maternal KIR and HLA-C haplotype of the couple	III	C	420	54, 60
Blood pro-inflammatory dysbalance (ratio IFN-g/ IL-10; TNF-alpha/IL-10; Th1/Th2/Th17)	III	C	102	78, 79
Endometrial pro-inflammatory cytokine profile	III	C	394	80

Comins-Boo A et al. Reproductive Immunology 2016

Auto-antibodies

- Several autoimmune antibodies such as
  - ANA
  - Anti-cardiolipin antibodies (ACAs)
  - Anti phospholipid antibodies
- are involved in biochemical pregnancy loss.

Geva E,2000

- B2 glycoprotein 1 is the cofactor for anti cardiolipin.

- Although the mechanism is still not well understood, there is also a strong association between anti-β2 glycoprotein 1 and ANA and implantation failure.

Stern C, 1998

Antiphospholipid Syndrome (APS)

- The patient must have
  - vascular thrombosis
  - pregnancy morbiditiy
    - fetal death after 10 weeks, premature birth before 34 weeks, or three or more consecutive miscarriages before 10 weeks of gestation.

- Laboratory criteria can include either
  - Lupus anticoagulant (LA) measured in the plasma twice and 12 weeks apart
  - Anticardiolipin antibody in plasma, measured twice and 12 weeks apart
  - Anti-*β*2 glycoprotein-I antibody in plasma measured twice and 12 weeks apart.

- In order to be diagnosed with APS, **one clinical** and **one laboratory** criteria must be met.

Miyakis S,2006

- There is accumulating evidence of non-criteria clinical and laboratory manifestations of APS, of which one criterion is two or more unexplained failed IVF cycles.

- In addition, some studies have suggested that these women benefit from standard APS treatment.

Arachchillage DR 2015

Antinuclear Antibody (ANA)

- **ANA** positivity is a typical feature of several autoimmune diseases like lupus erythematosus systemicus (SLE)
- **low titers of ANA** can be detected in the serum of a relatively high proportion of apparently healthy individuals (about 30% and 10% at 1:40 and 1:80 dilution, respectively).

- Some studies have reported that ANAs are related to a decline in:
  - oocyte quality
  - impairment of embryo development,
- resulting in :
  - unexplained infertility
  - RSA
  - Endometriosis
  - Implantation failure and POF

➢ The possible role of ANA in RPL is still **strongly controversial**, :

➢ some studies found an increased frequency of ANA in women with RPL compared to normal control women,

➢ other investigations could not detect any difference in the prevalence of ANA between RPL and normal women.

Ticconi C.2010

RSA & ANA

Title	Journals and Year	Authors
Antinuclear Autoantibodies in Women with Recurrent Pregnancy Loss	Am J Reprod Immunol **2010**	Carlo Ticconi.et al
Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage	Iran J Reprod .**2014**	Morteza Molazadeh.et al
Antinuclear autoantibodies and pregnancy outcome in women with unexplained recurrent miscarriage	Am J Reprod Immunol **2016**	Carlo Ticconi.et al
Antinuclear antibodies and recurrent miscarriage: Systematic review and meta-analysis	Am J Reprod Immunol **2019**	Marcelo Borges Cavalcante.et al

560 women with unexplained RM and **560 healthy controls** accounted for this study over a period of 13 months.
ANAs were detected by **indirect immunofluorescence technique**.

ANAs were detected in :

- 74 of 560 **(13.21%)** patient with RM
- in only 5 of 560 (0.9%) controls ($p<0.001$).

ANA positivity was generally found with

- **Low-positive results (1.40-1.80) in about 38%** of positive cases,
- **Moderate titres (1.160-1.320) in about 46%**
- **High titres (>1.640) were seen in about 16% of cases**

ANAs are not uncommon in women with unexplained recurrent miscarriage, suggesting the possible role of an autoimmune disorder on abortion, at least in a subgroup of patients.

Molazadeh M. et al 2014

TABLE 1 Characteristics of the included studies

Study	Country	Design	Population
Harger, 1989	United States of America	CCS	Cases: 277 non-pregnant RM patients[a] Control: 119 non-pregnant and 299 pregnant controls
Xu, 1990	United States of America	CCS	Cases: 30 explained and 30 unexplained RM patients[b] Control: 61 non-pregnant and 61 pregnant controls
Kwak, 1992	United States of America	CCS	Cases: 153 unexplained RM patients[c] Control: 90 non-pregnant controls
Ruiz, 1995	Colombia	CCS	Cases: 68 non-pregnant and 25 pregnant unexplained RM patients[d] Control: 25 non-pregnant and 31 pregnant controls
Bustos, 2006	Argentina	CCS	Cases: 118 non-pregnant unexplained RM patients[d] Control: 125 non-pregnant controls
Ticconi, 2010	Italy	CCS	Cases: 194 non-pregnant RM patients[a] Control: 100 non-pregnant controls
Molazadeh, 2014	Iran	CCS	Cases: 560 non-pregnant unexplained RM patients[e] Control: 560 non-pregnant controls

This meta-analysis suggested that positive ANA might increase the risk of RM.

It was not possible to conclude which ANA pattern of immunofluorescence staining is more frequent in the RM group.

Borges Cavalcante.M et al. 2019

International Journal of *Molecular Sciences*

MDPI

Review

The Impact of Autoantibodies on IVF Treatment and Outcome: A Systematic Review

Mara Simopoulou [1,2,*], Konstantinos Sfakianoudis [3], Evangelos Maziotis [1], Sokratis Grigoriadis [1], Polina Giannelou [1,3], Anna Rapani [1], Petroula Tsioulou [1], Agni Pantou [3], Theodoros Kalampokas [4], Nikolaos Vlahos [2], Konstantinos Pantos [3] and Michael Koutsilieris [1]

- While the effects of autoantibodies have been researched for many decades their effect on IVF cycle outcomes cannot yet be fully delineated.
- aPLs, TAA, and ASA do not seem to exert a negative outcome on IVF cycles regarding LB/OP, CP, and BP rate.
- AEA and ANA present with lower CP rates, hence inducing a negative effect.
- aPL, TAA, and ANA are associated with higher miscarriage rates

Mara Simopoulou. et al 2019

HUMAN FERTILITY, 2017
http://dx.doi.org/10.1080/14647273.2017.1306657

Taylor & Francis
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COMMENTARY

Is there evidence to support serum antinuclear antibodies testing in women with recurrent implantation failure undergoing *in vitro* fertilization?

Yuuki Charlie Barke Na[a], Sonia Asif[b,c] and Nicholas J. Raine-Fenning[b,c]

[a]School of Medicine, Queen's Medical Centre, University of Nottingham, Nottingham, UK; [b]Nurture Fertility, East Midlands Fertility Centre, Nottingham, UK; [c]Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, Nottingham, UK

ABSTRACT

One of the most challenging aspects of reproductive medicine is the management of recurrent implantation failure. Various investigations, including antinuclear antibodies testing, are performed to seek an explanation and guide treatment. However, is there sufficient evidence or available therapeutic options to support antinuclear antibodies testing? We present a short review on the current literature and an attempt at a systematic review evaluating the association between antinuclear antibodies and recurrent implantation failure to address this question.

ARTICLE HISTORY
Received 30 March 2016
Accepted 13 September 2016

KEYWORDS
In vitro fertilization; antinuclear antibodies; recurrent implantation failure

- ✓ We attempted a detailed search evaluating the association, if any, between ANA at or above a 1:80 titer, and RIF, defined as a minimum of two failed IVF cycles.
- ✓ Of the 1275 citations identified, no studies met the inclusion criteria.
- ✓ Furthermore, of the 37 excluded articles, over half (n=20) were published a decade ago, demonstrating the paucity of good quality evidence to support such measurements in women undergoing IVF.
- ✓ However, there is little guidance to demarcate ANA testing between autoimmune diseases and RIF.

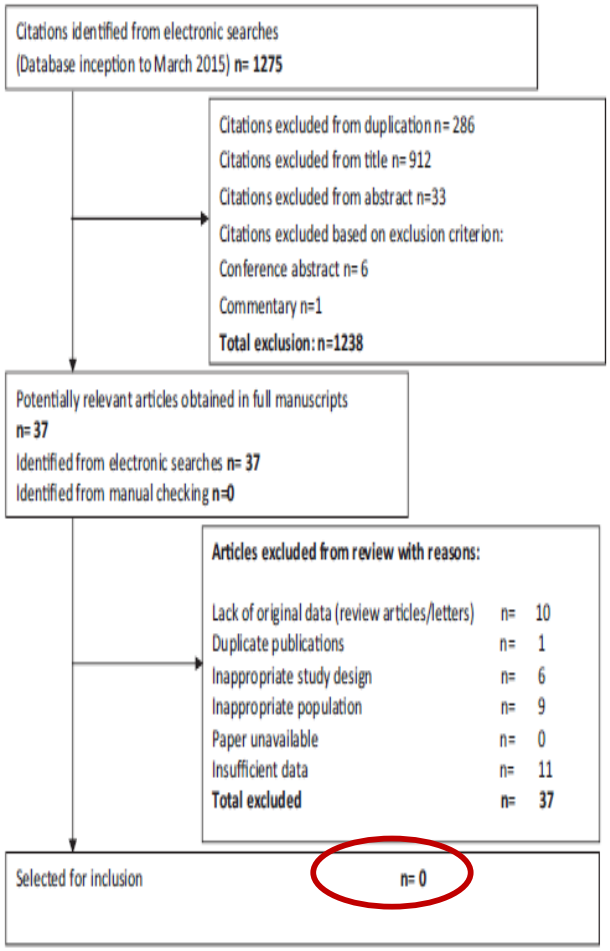

Figure 1. Flow diagram of study selection. For methodology and registration, please see PROSPERO (CRD42015017299) www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015017299).

Barke Na et al. 2017

Anti-double stranded DNA Antibody

- were cytotoxic to cells and induced apoptosis.
- It could lead to **abnormal development of oocyte and embryo.**
- There is little information on the clinical significance of anti-dsDNA for improvement of ART outcome.

Fan J 2017

Systematic Review and Meta-Analysis

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Glucocorticoid supplementation improves reproductive outcomes in infertile women with antithyroid autoimmunity undergoing ART

A meta-analysis

Guangqin Zhou, MD, Meiying Zhou, MD, Xuan Duan, MD, Weihong Li, MD*

Thyroid autoimmune disease :normal thyroid autoimmune function caused by the presence of **thyroid autoantibodies (ATA),** including thyroglobulin antibodies (Tg-Ab) and thyroid peroxidase antibodies (TPO-Ab).

Zhou et al. Medicine 2021

Systematic review and Meta-analysis

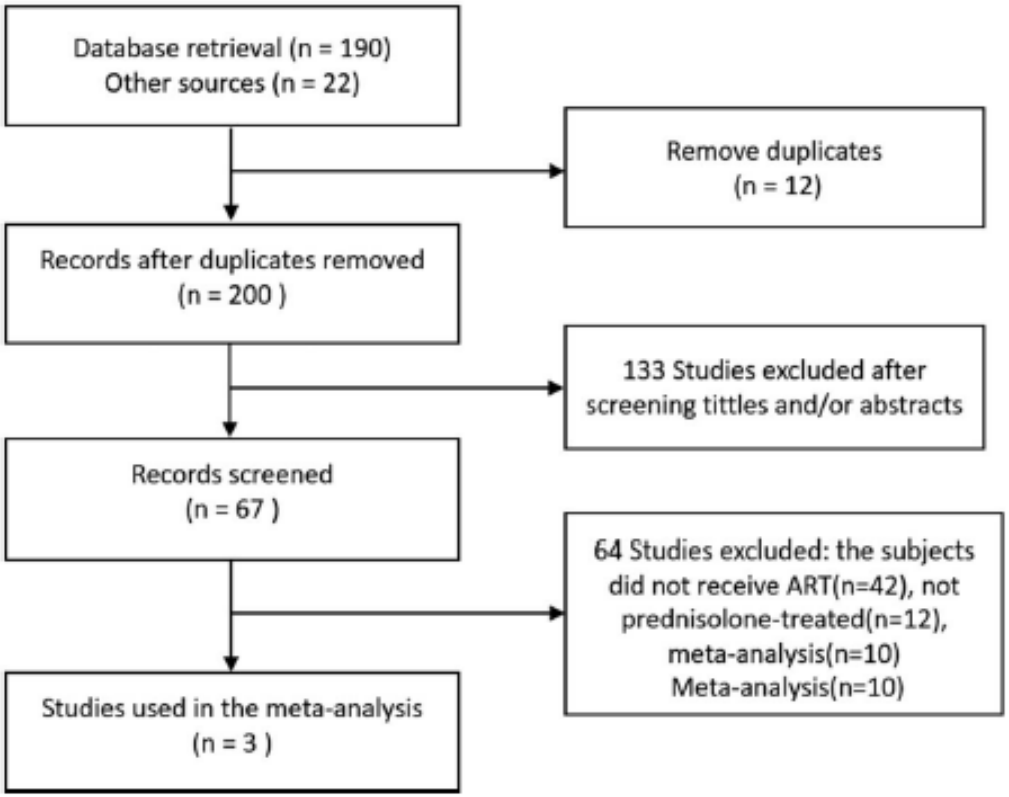

Figure 1. Flow chart of study selection.

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Table 1

Characteristics of the included studies.

First author (year), Country (reference)	Revelli et al (2009) Italy[17]	Turi et al (2010) Italy[18]	Litwicka et al (2015) Italy[19]
Period	Between February 2004 and May 2008	Between January 2006 and August 2008	Between January 2011 and April 2012
Patients	129 ATA+infertile women undergoing IVF, 36 received prednisone treatment and 38 did not	48 ATA+ infertile women undergoing IUI, 24 received prednisone treatment and 24 received placebo control	60 ATA+infertile women undergoing ICSI, 30 received prednisone treatment and 30 did not
Study design	Retrospective study	Prospective study	Prospective study
Causes of infertility	Pelvic endometriosis, reduced ovarian reserve, tubal disease, idiopathic infertility, male related infertility, PCO, hyperprolactinemia	Unclear	Tubal factor, male factor, idiopathic infertility
Conception method	IVF ("long" protocol)	IUI (ovulation induction)	ICSI ("long" protocol)
Assays used values for thyroid autoantibodies	Pharmacia Diagnostic commercial kits (Pharmacia, Sweden) using an immunofluorescence assay	TPO-Ab was determined using a radioimmunoassay kit (Brahms GmbH, Henningsdorf, Germany).	TPO and TG antibodies were assayed using enzyme linked immunoassay kits (Pharmacia; Upjohn Diagnostics, Freiburg, Germany)
Cut-off values for thyroid autoantibodies	TgAb: 0–40 UI/mL, TPOAb: 0–35 UI/mL	TPOAb 0–100 U/mL	TPOAb 0–18 UI/mL TGAb 0–40 UI/mL
Intervention	The treated group received 10 mg P (P was increased to 30 mg/d for 5 d starting the day of ET, and subsequently returned to 10 mg/d until the day of hCG test) until 10 wk of gestational age. After this time, the option to continue the treatment was left to doctors taking care of the pregnancy.	Prednisone was administered orally at 10 mg/d in the first week, 5 mg/d in the second week, 2.5 mg/d in the third week, and 2.5 mg/d 3 times per week in the fourth week before IUI. The control group received a placebo.	prednisolone was started from the day of oocyte retrieval and continued until the day of the pregnancy test. In the case of a positive test, this regimen was continued during the first pregnancy trimester
Outcomes	PR AR OPR NOR	PR AR LBR	NOR PR AR LBR

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- Glucocorticoid may improve the pregnancy outcomes of ART women with antithyroid antibody (ATA) positive.

- There is no significant reduction in the risk of miscarriage.

- Due to the limited enrolled references, glucocorticoid adjuvant therapy should be applied after more randomized controlled trials.

Zhou et al. Medicine 2021

frontiers in Immunology

ORIGINAL RESEARCH
published: 04 June 2020
doi: 10.3389/fimmu.2020.01032

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Endometrial Immune Profiling: A Method to Design Personalized Care in Assisted Reproductive Medicine

Nathalie Lédée[1,2], Marie Petitbarat[1], Laura Prat-Ellenberg[2], Géraldine Dray[2], Guy N. Cassuto[2,3], Lucie Chevrier[1], Alaa Kazhalawi[1], Katia Vezmar[1] and Gerard Chaouat[4]*

[1] MatriceLAB Innove, Pépinière Paris Santé Cochin, Hôpital Cochin, Paris, France, [2] Centre d'Assistance Médicale à la Procréation, Hôpital des Bluets, Paris, France, [3] Laboratoire Drouot, Paris, France, [4] INSERM U-976, St-Louis Hospital, Paris, France

- 1738 infertile patients had an immune profiling on a timed endometrial biopsy between 2012 and 2018.
- This test documented the absence or the presence of an endometrial immune dysregulation and identified its type.
- In case of dysregulation, a targeted personalized plan was suggested to the treating clinician aiming to supply the anomaly.
- One year after the test, the clinician was contacted to provide the outcome of the subsequent embryo transfer with the applied suggested plan.

Lédée et al. Frontiers in Immunology 2020

TABLE 1 | Summarize of clinical data of patients included in this study.

Clinical context	RIF	Good prognosis IVF	RM	OD	RIF-OD
Number of patients	1,145	210	164	91	128
Mean age (years) [min–max]	35.7 [22.3–42.8]	33.2 [24.9–38.8]	35.7 [24.8–42.7]	39.45 [25.6–47]	39.9 [27–50]
Range of oocytes collection	3.6 [2–9]	0.86 [0–1]	0.23 [0–2]	–	–
Cumulated number of embryo transferred [min–max]	9 [6–35]	3.1 [0–6]	0.55 [0–3]	0.56 [0–3]	7.2 [4–25]
Number of miscarriages	0.55 [0–5]	0.21 [0–2]	3.87 [3–9]	0.61 [0–4]	0.69 [0-4]

RIF, Repeated Implantation Miscarriages; IVF, In vitro Fertilization; RM, Recurrent Miscarriages; OD, Oocyte Donation.

Lédée et al. Frontiers in Immunology 2020

TABLE 2 | Summary of the suggested protocols according to the immune profile documented.

Suggestion of personalization/immune profile	No dysregulation	Low immune activation	Over immune activation	Mixed profile
Endometrial scratching	No	Yes	No	Yes
Exposure to high concentration of Estrogens in the proliferative phase	No impact	No	Yes	Yes
Immunotherapy	No	No	Yes (therapy test)	Yes (therapy test)
Hormonal adaptation of the luteal phase	No	No	Yes	Yes
Luteal hCG supplementation	No	Yes	No	Yes
Exposure to seminal plasma	No impact	Yes	No	No

TABLE 3 | Comparison of the repartition of the distinct immune profiles for each clinical context.

Endometrial immune profiling	Before OD	Before IVF	RIF-OD	RIF	RM	Average repartition
Number of patients	91	210	128	1145	164	–
No dysregulation	20.9% [19/91]	12.4% [26/210]	11.6% [15/128]	17.2% [197/1145]	19.5% [32/164]	289 (16.6%)
Immune under-activation	25.3% [23/91]	34.3% [72/210]	33.6% [43/128]	27.2% [311/1145]	23.2% [38/164]	487 (28%)
Immune over-activation	39.6% [36/91]	43.8% [92/210]	43.7% [56/128]	45.9% [525/1145]	45.1% [74/164]	783 (45.1%)
Mixed profile	14.3% [13/91]	9.5% [20/210]	10.9% [14/128]	9.8% [112/1145]	12.2% [20/164]	179 (10.3%)

RIF, Repeated Implantation Failures; IVF, In vitro Fertilization; RM, Recurrent Miscarriages; OD, Oocyte Donation.

Lédée et al. Frontiers in Immunology 2020

TABLE 4 | Outcome considering the presence or absence of dysregulation and clinical context.

Clinical context	Global PR	Immune profile	Number of patients	Miscarriage rate	No pregnancy	Pregnancy rate (PR)	*P*-value for the PR
OD	45%	Dysregulated	72	11%	46%	44%	0.64
		Not dysregulated	19	16%	31.6%	52.6%	
RIF-OD	42%	Dysregulated	113	14.90%	43.80%	41.6%	0.56
		Not dysregulated	15	0%	53.3%	46.70%	
Good prognosis IVF	56%	Dysregulated	184	7.60%	38%	55%	0.28
		Not dysregulated	25	4%	36%	57.6%	
RIF	36%	Dysregulated	948	8.40%	53.8%	38.4%*	0.002
		Not dysregulated	201	7.50%	65.6%	26.90%	
RM	50%	Dysregulated	132	12%	32%	57.6%*	0.001
		Not dysregulated	33	24%*	52%	25%	

PR, Pregnancy Rate; RIF, Repeated Implantation Miscarriages; IVF, In vitro Fertilization; RM, Recurrent Miscarriages; OD, Oocyte Donation.

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TABLE 5 | Outcome in deregulated immune profiles.

Clinical context	Immune endometrial profile	*n*	Ongoing Pregnancy rate	Miscarriages rate
Good prognosis IVF	Over-activation	92	51%	8%
	Under-activation	72	64%	5%
	Mixed	20	45%	10%
RIF	Over-activation	525	40%	8%
	Under-activation	311	38%	8%
	Mixed	112	31%	7%
OD	Over-activation	36	44%	8%
	Under-activation	23	43%	13%
	Mixed	13	46%	15%
RIF-OD	Over-activation	56	41%	14%
	Under-activation	43	42%	14%
	Mixed	14	43%	21%
RM	Over-activation	74	55%	15%
	Under-activation	38	66%	10%
	Mixed	20	50%	5%

RIF, Repeated Implantation Miscarriages; IVF, In vitro Fertilization; RM, Recurrent Miscarriages; OD, Oocyte Donation.

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TABLE 6 | Endometrial dysregulation and maternal age.

Maternal age	Endometrial profile	Number of patients using their own oocytes for ART	Mean PR	Number of patients using OD	Mean PR in OD
Less than 35 years old	Dysregulated	678	51%	51	55%
	Not dysregulated	132	40%	1	–
Between 36 and 39 years old	Dysregulated	423	37%	41	39%
	Not dysregulated	86	22%	7	71%
More than 40 years old	Dysregulated	163	24%	93	38%
	Not dysregulated	37	10%	26	46%

PR, Pregnancy Rate; ART, Assisted Reproductive Therapy; OD, Oocyte Donation.

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- 16.5% of the patients showed no endometrial immune dysregulation
- 28% had a local immune under-activation
- **45% had a local immune over-activation**
- 10.5% had a mixed endometrial immune profile.

- In patients with a history of RIF or RM:

the pregnancy rate was significantly higher if an endometrial dysregulation was found and the personalized plan applied, compared to the patients with an apparent balanced immune profile (respectively 37.7 and 56% vs. 26.9 and 24%, $p < 0.001$).

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- In good prognosis IVF subgroup and patients using donor eggs, this difference was not significant between dysregulated and balanced subgroups, but higher pregnancy rates were observed in absence of dysregulation.
- For patients with immune over-activation, pregnancy rates were significantly higher for patients who had a test of sensitivity, regarding the type of immunotherapy introduced, when compared to the ones who did not (51 vs. 39.9%, p = 0.012).

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Peripheral and Uterine NKs

- Sacks et al. found that women with RIF had significantly increased pNK by concentration and percentage of lymphocytes compared with controls.
- The sensitivity of this test was only 11% suggesting that women with RIF might have multiple other factors that contribute to their difficulty in achieving pregnancy.

Sacks G,2012

Poli A et al 2009

Table 1. Phenotypic comparison between $CD56^{bright}$ and $CD56^{dim}$ natural killer cells

	$CD56^{bright}$	$CD56^{dim}$
CD56	++	+
CD16	±	++
Inhibitory receptors		
KIR	−	+
ILT2	−	+
CD94/NKG2A	++	±
Activating receptors		
NKp46	++	+
CD117(c-kit)	++	−
Cytokine receptors		
IL2R$\alpha\beta\gamma$	+	−
IL2R$\beta\gamma$	++	+
IL1RI	++	+
IL18R	++	+
Chemokine receptors		
CCR7	++	−
CXCR3	++	±
CXCR1	−	++
CX3CR1	−	++
Adhesion molecules		
CD2	++	±
CD11c	++	±
CD44	++	+
CD49e	++	+
CD54	++	±
CD62L	++	±
CD11a	+	++
Other molecules		
CD57	−	+
CD160	−	++
CD55	++	+
CD59	++	+

- uNK cells are derived from the NK cell line due to their CD56+ marker, however they do not have the same ability to destroy cancerous cell lines and other HLA class 1 negative molecules.

- Therefore, they may not actually have a deleterious effect on an implanted embryo

Moffett A,2015

- Both pNK and uNK cells are elevated in patients with RIF.
- uNK cells were measured via endometrial biopsy were found in 53% of idiopathic RIF patients and only in 5% of controls.
- Though cut off values still require standardization, analysis of NK cells might eventually prove to be useful to women suffering from idiopathic RIF.

Santillan I,2015

- A recent meta-analysis set out to determine the role of both pNK and uNK cells in infertility and RM and found some conflicting data regarding their role.

Seshadri S,2014

- To date these studies are still investigative, and the role of NK cells in RIF and RSA is still controversial.
- NK cell level and activity is just one aspect of the immune system involved in women suffering from infertility, and we need more data in order to yield clinical value from this information.

Bashiri A ,2018

Th1/Th2 ratio and TNF-α levels

- There is a relative agreement :
  - Elevated levels of Th1 cells are associated with rejection of embryos
  - Elevated Th2 cell levels are associated with pregnancy.

- These T cells are measured by their cytokine production

Nakagawa K, 2015

- Cytokines produced by Th1 cells, such as TNF-α, suppress trophoblastic growth and promote inflammatory and thrombotic responses in maternal uterine blood vessels thus adversely affecting implantation.
- Cytokines produced by Th2 cells such as IL-4, IL-6 and IL-10 inhibit Th1 cell induced tissue factor by monocytes.
- Higher mean ratios of TNF-α/IL-4 and TNF-α/IL-10 in peripheral blood samples have been measured in women with RIF compared with mean ratios in controls

Kwak-Kim JYH 2003

The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure

Pei-Yan Liang [a,b,c], Liang-Hui Diao [b,c], Chun-Yu Huang [b,c], Ruo-Chun Lian [b,c], Xian Chen [b,c], Guan-Gui Li [b,c], Jin Zhao [a], Yu-Ye Li [b,c], Xue-bing He [b,c], Yong Zeng [b,c,*]

[a] *School of Chemical Biology and Biotechnology, Shenzhen Graduate School of Peking University, Shenzhen 518055, China;* [b] *Shenzhen Key Laboratory of Reproductive Immunology for Peri-implantation, Fertility Center, Shenzhen Zhongshan Urology Hospital, Shenzhen 518045, China;* [c] *Shenzhen Zhongshan Institute for Reproductive Medicine and Genetics, Shenzhen 518045, China*

* Corresponding author. *E-mail address:* zengyong1966@gmail.com (Y Zeng).

Pei-Yan Liang, PhD, is a post-doctoral researcher at Shenzhen Graduate School, Peking University. She is a clinical doctor and researcher for the Department of Reproductive Immunology, Fertility Center, Shenzhen Zhongshan Urology Hospital. Her research interests focus on reproductive immunology, specializing in immunotherapy for recurrent miscarriage and recurrent implantation failure.

Abstract Limited information is available on the balance state of pro- and anti-inflammatory cytokines in patients with recurrent implantation failure (RIF). This study assessed the pro- and anti-inflammatory cytokines in plasma of 34 patients with RIF, compared with those of 25 women with a successful pregnancy in the first IVF/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI–ET) cycle. The IFN-γ, IL-1β, IL-6 and IL-4 concentrations were higher, whereas the TGF-β1 concentration was lower in the RIF group compared with the control group. Furthermore, the ratios of pro-inflammatory and anti-inflammatory cytokines IFN-γ/IL-4, IFN-γ/IL-10, IFN-γ/TGF-β1, IL-6/IL-10, IL-6/TGF-β1, IL-1β/TGF-β1 and TNF-α/TGF-β1 were higher in the RIF group (all $P < 0.01$). The results suggested a δ9shift toward a pro-inflammatory state in peripheral blood of the patients with RIF.

RBM Online 2015

The IFN-γ, IL-1β, IL-6 and IL-4 concentrations were higher, whereas the TGF-β1 concentration was lower in the RIF group compared with the control group.

Table 1 The pro-inflammatory and anti-inflammatory cytokine concentrations and ratios in the RIF and Control groups.

Cytokine	*RIF group (n = 34)*	*Control group (n = 25)*
Cytokine (pg/ml)		
IFN-γ	1323.20 ± 154.41***	352.58 ± 60.53
IL-6	7959.80 ± 385.79***	4243.90 ± 427.31
IL-1β	3411.00 ± 360.57**	1924.00 ± 357.51
TNF-α	4074.10 ± 385.96	3393.5 ± 486.56
IL-4	58.55 ± 5.97***	29.43 ± 4.19
IL-10	58.07 ± 6.18	51.60 ± 6.84
TGF-β1	1608.95 ± 89.90***	2334.28 ± 105.20
Cytokine ratio		
IFN-γ/IL-4	26.43 ± 3.00**	12.82 ± 2.15
IFN-γ/IL-10	30.11 ± 4.52***	7.00 ± 1.11
IFN-γ/TGF-β1	0.88 ± 0.11***	0.16 ± 0.03
IL-6/IL-4	189.50 ± 23.04	244.76 ± 66.21
IL-6/IL-10	202.65 ± 29.42*	113.18 ± 16.60
IL-6/TGF-β1	5.35 ± 0.34***	1.80 ± 0.16
IL-1β/IL-4	78.83 ± 9.87	102.38 ± 30.57
IL-1β/IL-10	78.48 ± 9.78	48.75 ± 12.03
IL-1β/TGF-β1	2.26 ± 0.24***	0.82 ± 0.15
TNF-α/IL-4	196.65 ± 12.60	180.60 ± 45.09
TNF-α/IL-10	94.83 ± 13.45	88.70 ± 17.13
TNF-α/TGF-β1	2.74 ± 0.28**	1.44 ± 0.21

Values are mean ± SE
RIF = recurrent implantation failure.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus Control group.

Furthermore, the ratios of pro-inflammatory and anti-inflammatory cytokines

- IFN-γ/IL-4
- IFN-γ/IL-10
- IFN-γ/TGF-β1
- IL-6/IL-10
- IL-6/TGF-β1
- IL-1β/TGF-β1
- TNF-α/TGF-β1

were higher in the RIF group (all $P < 0.01$).

The results suggested a shift toward a pro-inflammatory state in peripheral blood of the patients with RIF.

Liang et al. 2015

Other Molecules

- Leukaemia inhibitory factor (LIF)
- Cellular adhesion molecules (CAMs)
- Prostaglandins (PG)
  - Phospholipase A2 (PLA2)
  - Cyclooxygenase enzyme 2 (COX-2)

The role of immunotherapy in in vitro fertilization: a guideline

Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

Adjuvant immunotherapy treatments in in vitro fertilization (IVF) aim to improve the outcome of assisted reproductive technology (ART) in both the general ART population as well as subgroups such as patients with recurrent miscarriage or implantation failure. The purpose of this guideline is to evaluate the role of immunomodulating therapy in ART. Unfortunately, many of the evaluated therapies lack robust evidence from well-designed adequately powered randomized controlled trials to support their use. Immunotherapies reviewed in the present document are either not associated with improved live-birth outcome in IVF or have been insufficiently studied to make definitive recommendations. (Fertil Steril® 2018;110:387–400.

The role of immunotherapy in in vitro fertilization and recurrent pregnancy loss: a systematic review and meta-analysis

Chiara Achilli, M.D., Montserrat Duran-Retamal, M.D., Wael Saab, M.R.C.O.G, Paul Serhal, M.R.C.O.G, and Srividya Seshadri, M.D.

Centre for Reproductive and Genetic Health, London, United Kingdom

Objective: To study the current evidence on the role of immunotherapy in IVF and in the management of recurrent pregnancy loss (RPL).
Design: Systematic review and meta-analysis.
Setting: A literature search was performed using MEDLINE, PUBMED, CINAHL, and EMBASE until May 2017. Only randomized controlled trials were included, and a meta-analysis was carried out where appropriate.
Patient(s): Women undergoing IVF treatment with or without a history of recurrent implantation failure and women with idiopathic RPL.
Intervention(s): Assessment of the efficacy of commonly used immunomodulators such as IV use of [1] immunoglobulin, [2] lymphocyte immunotherapy and [3] intralipid; intrauterine infusion of [4] granulocyte colony-stimulating factor and [5] peripheral blood mononuclear cells; subcutaneous administration of [6] TNF-alpha inhibitors, [7] leukaemia inhibitory factor; and oral administration of [8] glucocorticoids.
Main Outcome Measure(s): The primary outcomes were live birth rate and miscarriage rate; secondary outcome was clinical pregnancy rate.
Result(s): Of the 7,226 publications identified, 53 were selected during the initial screening; 30 satisfied the selection criteria and were included in this review.
Conclusion(s): The available medical literature shows controversial results about the role of immunotherapy when used for improving reproductive outcomes. This study did not show a role for immunotherapy in improving the live birth rate in women undergoing IVF treatment or in the prevention of idiopathic RPL. Currently, immunotherapy should be used in the context of research and should not be used in routine clinical practice to improve reproductive outcomes. (Fertil Steril® 2018;110:1089–100. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Immunotherapy, IVF, repeated implantation failure, recurrent pregnancy loss, immunomodulation

Meta Analysis Conclusion

- No role for immunotherapy was reported

  in improving the LBR in women undergoing IVF treatment, with or without history of RIF or in the prevention of idiopathic RPL.

- Better-designed RCTs with better patient selection are strongly needed to finally address the role of the immunomodulators currently available.

Achilli , 2018

Conclusion

- Pregnancy is considered as a semi-allograft as fetus expresses paternal antigens.
- Immunological dysregulation is considered as one of the important factors involved in RSA and RIF.
- Different immunological factors and immune cells such as
  - Cytokines
  - Growth factors
  - T helper cells
  - Dendritic cells
  - Macrophages
  - Peripheral and uterine NK

have been considered important in pathophysiology of RSA and RIF.

Conclusion

- Different therapeutics agents including
  - Immunosuppressive drugs
  - IVIG
  - Aspirin
  - Hydroxychloroquine
  - Intrauterine infusion of different agents such as PRP, PBMC, G-CSF and hCG

are suggested in treatment of RIF.

Conclusion

- Reproductive immunology principles have been applied to the clinical management of RPL, RIF, and failed IVF cycles.

- A significant proportion of physicians recommend :
  - thrombophilia workups (86%)
  - parental genetic study (79%),
  - immunologic evaluations (69%) to IVF candidates who have a history of RPL or chemical pregnancy losses.

- IVF physicians consider an immunologic workup when patients have two (30%) or three (21%) failed IVF cycles.

Kwak kim et al. 2013

Conclusion

Assays for :

- anticardiolipin antibody
- lupus anticoagulant
- thyroid peroxidase antibody,
- antinuclear antibody

are the four most commonly ordered immunologic tests for RPL (88, 84, 50, 47% each) and RIF (68, 63, 38, 38% each).

Cellular immune evaluations, such as NK assay, human leukocyte antigen study, Th1/Th2 study or immunophenotype assay, are less commonly ordered.

A significant proportion of IVF physicians acknowledge the importance of immunologic alterations with reproductive outcomes.

Kwak kim et al. 2013

Conclusion

In the absence of well-designed adequately powered RCTs and Universal guidelines, patients must be informed of uncertain benefits and risks associated with immunotherapy.


Royan institute
Thank you for
Your Attention