EFFICACY OF THERAPIES AND INTERVENTIONS FOR REPEATED EMBRYO IMPLANTATION FAILURE

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INTRODUCTION

- Repeated embryo implantation failure (RIF) is an extremely **frustrating** condition for both patients and clinicians and its treatment constitutes one of the most difficult **challenges** in the field of in vitro fertilization (IVF).

Possible Causes

- 1- **known**: wrong lifestyle habits (i.e. smoking and obesity), low quality of gametes [age], thrombophilia, uterine factors (i.e. congenital uterine anomalies, endometrial polyps, submucosal fibroids, intrauterine adhesions) and adnexal pathologies (i.e. hydrosalpinx).
- 2- **Unknown**: in the great majority of cases, the etiology remains unknown.

Sources of research

A systematic review and meta-analysis
Andrea Busnelli, Edgardo Somigliana, Federico Cirillo, Annamaria Baggiani & Paolo Emanuele Levi-Setti , *Scientific Reports*
volume 11, Article number: 1747 (2021)
Twenty-two RCTs and nineteen observational studies were included

Guideline of the Canadian Fertility and Andrology Society (CFAS) is to provide the most relevant evidence to date for the assessment and management of RIF (2020), using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) (2022)

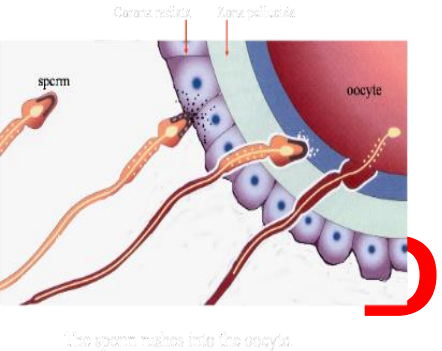
ESHRE & ASRM in particular 2021+2022

Table 1Definitions of recurrent implantation failure IN THE LITERATURE

Author, Publication	Study Topic	Definition
Polanski et al., 2014b	RIF review	Absence of implantation after two consecutive cycles of fresh or frozen IVF embryo transfers with a cumulative number of transferred embryos of four or more cleavage-stage embryos or two or more blastocysts, all of good quality
Coughlan et al., 2014	RIF review	Failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years
El-Toukhy et al., 2016	Hysteroscopy in RIF	Two to four previous fresh or frozen IVF treatment cycles ending in an embryo transfer but no pregnancy
Mariee et al., 2012	Endometrial immune profile in RIF	Failure of three fresh IVF cycles or two fresh IVF and two frozen embryos transfer cycles
Ledee et al., 2016	Endometrial immune profile in RIF	Failure to have an ongoing pregnancy >10 weeks after at least six embryos were transferred on day 3 or day 5 in women aged <43 years
Mitri et al., 2016	Embryo transfer technique	Failure to have a clinical pregnancy after four or more blastocysts (fresh or frozen) after ruling out malformed uterine cavity, hydrosalpinx, abnormal karyotype or persistently thin endometrium in women aged 38 year or younger
Kitaya et al., 2017	Chronic endometritis and RIF	Serial negative pregnancy tests following transfer of three or more morphologically good cleavage-stage embryos and/or blastocysts

Table 1Definitions of recurrent implantation failure IN THE LITERATURE

Author, year of publication	Study topic	Definition
Lensen et al., 2019	Endometrial injury	Two previous implantation failures, no precision on number of embryos
Olesen et al., 2019	Endometrial injury	Implantation failure despite top-quality embryo or blastocyst transfer(s)
Greco et al., 2014	PGT-A in RIF	Three to nine previous implantation failures after IVF (mean 4.9)
Huang et al., 2017	HCG infusion in RIF	Two or more failed transfer of good quality embryos
Makrigiannakis et al., 2015	PBMC infusion in RIF	Three or more failed IVF cycles with a cumulative transfer of six embryos or three blastocysts of good quality
Koot et al., 2019	Prognosis of RIF	Three failed IVF or ICSI treatments, each with at least one fresh good quality embryo per transfer, or failure to achieve pregnancy after transfer of 10 good quality embryos



Most common Diagnostic Criteria & Defined RIF

- proposed more stringent diagnostic criteria and defined RIF as the failure after the transfer of at least four good-quality embryos within minimum three fresh or frozen cycles under 40 years of age,
- The definition of good quality embryos is subjective and the authors often do not refer to shared classification criteria.
- In the present systematic review and meta-analysis, we defined RIF as the failure to obtain a clinical pregnancy after at **least three ET attempts.** By using this threshold, the risk of false positive diagnosis is significantly lower. Importantly, these diagnostic criteria also exclude elements of subjectivity and are therefore easily replicable in any clinical setting.

Investigation

practise makes perfect

1- Cavity assessment

- The incidence of abnormal hysteroscopic findings in women with RIF varies between 14% and 51% (Gao et al., 2015, Hosseini et al., 2014, Lambert et al., 2016, Pabuccu et al., 2016
- Polyps, intrauterine adhesions and submucosal fibroids represent the most commonly detected anomalies.
- **Recommendation:**
- *In RIF patients with a normal baseline ultrasound, the routine use of hysteroscopy is not recommended.*
- *Strength: strong.*
- *Quality of evidence: high.*
- *Justification: this was based on a well-conducted multicentre randomized controlled trial (RCT) comparing hysteroscopy with no hysteroscopy in women with RIF, which showed no difference in LBR.*

2- Thrombophilia testing

- A possible mechanism on recurrent pregnancy loss (RPL) among ***inherited*** thrombophilia carriers is thrombosis of the maternal vessels, which could reduce perfusion of the intervillous space, leading to placental failure. It has been suggested similar damage to implantation failure Ata and Urman, 2016
- ***Acquired*** thrombophilia, including antiphospholipid syndrome, has been shown to be relevant in recurrent early pregnancy loss, as increased coagulability can theoretically affect embryo implantation and early pregnancy development, possibly through vascular occlusion Ata and Urman, 2016
- Autoimmune factors may play an additional role in the thrombotic activity of invading trophoblasts.

Thrombophilia testing

- Several studies describe an incidence of inherited and acquired thrombophilia in RIF patients that varies between 4% and 62%. However, many of these studies were small and include findings that are not clinically relevant (e.g. heterozygous status for *MTHFR* mutation) Bellver et al., 2008, Qublan et al., 2006, Safdarian et al., 2014, Simur et al., 2009
- **Recommendation** 2 **:**
- Testing for inherited or acquired thrombophilia in patients with RIF is not recommended.
- Strength: strong.
- Quality of evidence: low.
- Justification: there is insufficient evidence that either inherited or acquired thrombophilias are increased in RIF patients.

3- Immunological testing

- Mechanism: decidualized stromal cells of the endometrium are able to regulate trophoblast invasion and to dampen the local maternal immune response (Coughlan et al., 2014). The failure to control that immune reaction may lead to implantation failure.
- **Recommendation** 3.
- Serological or endometrial immune testing in RIF patients should be limited to research settings.
- Strength: strong.
- Quality of evidence: low.
- Justification: observational studies with multiple immunological profiles were tested, with inconsistent results. Studies are heterogeneous and not yet applicable to clinical practice.

4- Parental karyotype analysis

- Couples with balanced translocations often produce gametes with chromosomal aberrations, which may in turn result in various forms of reproductive failures, notably RPL (Tharapel et al., 1985).
- detected chromosomal anomalies in 2.5% in ***RIF*** (Stern et al., 1999). In comparison, 4.7% of patients with a ***RPL***
- During that same period, 1.3% of infertile couples undergoing their first IVF had chromosomal anomalies, and 0.3% normal ***neonates*** had chromosomal anomalies (baseline rate).
- Among the RIF population, the most common anomalies were translocations (reciprocal and Robertsonian

Parental karyotype analysis

- **Recommendation 4:**
- Karyotype testing may be offered to couples with RIF.
- Strength: weak.
- Quality of evidence: low.
- Justification: observational studies suggest that couples with RIF may have a slightly higher rate of chromosomal anomalies than fertile couples.

5- Sperm DNA fragmentation testing

- Sperm DNA damage is associated with poor embryo development
- the American Society for Reproductive Medicine guideline on the clinical utility of sperm DNA integrity testing stated that there was insufficient evidence to recommend its routine use before IVF
- **Recommendation** 5:
- Sperm DFI testing should not be routinely offered in RIF.
- Strength: weak.
- Quality of evidence: low.
- Justification: small observational studies showed that high sperm DFI was not correlated to RIF.
- Simon et al., 2017, Bronet et al., 2012, Coughlan et al., 2015

6- Chronic Endometritis in women with RIF

- Most studies rely on immunohistochemical identification of CD138 cells as this has been shown to be a more sensitive diagnostic method Kitaya and Yasuo, 2013
- cut-off of CD138 cells used to diagnose chronic endometritis has yet to be established. a prevalence of 33.7% Kitaya et al., 2017, a ratio of the sum of stromal CD138+ cells per high-power field of 0.25 or more; this percentage ranges from 14% to 57.6%
- Hysteroscopic diagnosis has also been described, but has not been validated.
- There are no RCT
- Bouet et al., 2016, Cicinelli et al., 2015, Johnston-Macananny et al., 2010, Yang et al., 2014, Zargar et al., 2019, Zhang et al., 2019

Chronic Endometritis in women with RIF

- **Recommendation** 6:
- Screening for chronic endometritis should not be routinely offered in RIF.
- Strength: weak.
- Quality of evidence: low.
- Justification: this decision was based on small, low-quality heterogeneous observational studies and a lack of consensus diagnostic criteria for CE.

7- Endometrial receptivity array in women with RIF

- The endometrial receptivity array (ERA) is a tool used to detect a receptive endometrium by means of a specific transcriptomic gene signature and is a reproducible and more accurate method than receptivity assessed by histological evaluation (Diaz-Gimeno et al., 2013)
- There are no RCT, limited to four observational studies, Hashimoto et al., 2017, Mahajan, 2015, Patel et al., 2019, Ruiz-Alonso et al., 2013

- None of these trials had an appropriate control group to be able to draw conclusions regarding efficacy

Endometrial receptivity array in women with RIF

- **Recommendation** 7:
- The use of endometrial receptivity assay in RIF patients should be limited to research settings.
- Strength: strong.
- Quality of evidence: very low.
- Justification: there is currently no evidence that endometrial receptivity assay improves clinical outcomes in RIF.

8- Preimplantation genetic testing for aneuploidies in couples with RIF

- The use of fluorescence in-situ hybridization on cleavage-stage embryos to assess aneuploidy was associated with a high false-positive and false-negative rate, and therefore has created conflicting results with respect to the contribution of aneuploidy to implantation failure. Greco et al., 2014
- Next-generation sequencing (NGS) is the latest technological advancement in PGT, enabling whole-genome analysis with greater accuracy. (Ou et al., 2015). While further prospective data are needed, NGS technology may provide insight into previously unexplained cases of preimplantation failure in terms of the detection of segmental polymorphisms

Preimplantation genetic testing for aneuploidies in couples with RIF

- **Recommendation 8:**
- There are insufficient data to recommend for, or against, PGT-A for RIF.
- Strength: strong.
- Quality of evidence: low.
- Justification: the studies are few in number and small with respect to sample size. There are no RCT data available.

Treatment

Laboratory and procedural technologies and interventions

Definitions

- Primary outcomes:

1. (LBR) Live Birth Rate per patient "Live birth" was defined as the delivery of one or more living infants.
2. (CPR) Clinical Pregnancy Rate per patient was defined as the presence of one or more intrauterine gestational sacs on transvaginal ultrasound or other definitive clinical signs.

- Secondary outcomes:

1. (IR) implantation rate per embryo, defined as the number of gestational sacs on transvaginal ultrasound divided by the number of embryos transferred.
2. (MPR) multiple pregnancy rate per patient defined as the presence of two or more intrauterine embryos on transvaginal ultrasound.
3. (MR) miscarriage rate per patient, defined as fetal loss before 20 weeks' gestation.

1- Sequential ET

- One RCT and two observational studies compared sequential ET (cleavage stage ET followed by blastocyst ET) vs blastocyst stage ET in women with RIF.
- ***Primary outcomes*** Meta-analysis of observational studies showed an increased chance of clinical pregnancy (CPR) in women who underwent sequential ET (fixed effects model, OR 2.64; 95% CI 1.56–4.47; p = 0.0003; I^2 = 0%). On the contrary, Shahrokh Tehraninejad et al. failed to show a beneficial effect (RR 1.04; 95% CI 0.67–1.63; p = 0.85).
- ***Secondary outcomes*** Fang et al., observed a beneficial effect of sequential ET on implantation rate (IR)(OR 2.95; 95% CI 1.65–5.27; p = 0.0003) (Fang et al., 2013). Meta-analysis of observational studies and Shahrokh Tehraninejad et al. did not show an impact on MPR (fixed effects model, OR 2.38; 95% CI 0.87–6.47; p = 0.09; I^2 = 36% and RR 1.13; 95% CI 0.47–2.72; p = 0.79, respectively).

2- PGT-A
Preimplantation Genetic Testing for Aneuploidies

- Two RCTs and three observational studies investigated the potential role of PGT-A in improving IVF outcomes in women with RIF.
- ***Primary outcomes*** Meta-analysis of RCTs failed to show an improvement in both clinical pregnancy and live birth chances (random effects model, RR 1.07; 95% CI 0.36–3.15; $p = 0.90$; $I^2 = 89\%$ and RR 0.98; 95% CI 0.32–2.94; $p = 0.97$; $I^2 = 87\%$) in women who underwent PGT-A.
- Pooling of results of observational studies did not show a beneficial effect of PGT-A on both pregnancy (random effects model, OR 1.58; 95% CI 0.35–7.12; $p = 0.55$; $I^2 = 86\%$) and live birth chances (random effects model, OR 0.83; 95% CI 0.33–2.07; $p = 0.69$; $I^2 = 44\%$).
- ***Secondary outcomes*** Rubio et al. did not observe an impact of PGT-A on chances of embryo implantation and miscarriage in women who underwent PGT-A (RR 1.71; 95% CI 0.99–2.94; $p = 0.05$ and RR 3.58; 95% CI 0.42–30.83; $p = 0.25$, respectively).
- Notable: Aneuploidy rate of 53.8%.

Next-generation sequencing (NGS)

- Next-generation sequencing (NGS) is the latest technological advancement in PGT, enabling whole-genome analysis with greater accuracy. There has been one isolated case report describing the application of NGS in otherwise unexplained RIF and recurrent early miscarriage in two couples having undergone IVF with normal routine CGH-microarray results (Ou et al., 2015). While further prospective data are needed, NGS technology may provide insight into previously unexplained cases of preimplantation failure in terms of the detection of segmental polymorphisms.

Guideline of the Canadian Fertility and Andrology Society

- **Recommendation:**

There are insufficient data to recommend for, or against, PGT-A for RIF.

- Strength: strong.
- Quality of evidence: low.
- Justification: the studies are few in number and small with respect to sample size. There are no RCT data available.

3- Blastocyst-stage ET

- One RCT compared blastocyst-stage ET outcomes with day 2–3 ET outcomes in women who failed to conceive after three or more day 2–3 IVF/ET cycles.
- ***Primary outcomes*** Levitas et al. failed to show a benefit of this strategy on both CPR (RR 1.68; 95% CI 0.51–5.59; p = 0.39) and LBR (RR 1.35; 95% CI 0.30–6.08; p = 0.70).
- ***Secondary outcomes*** Authors observed a significantly increased chance of embryo implantation (IR) in treated women (RR 3.54; 95% CI 1.28–9.77; p = 0.01). MPR did not result significantly different between groups (RR 0.90; 95% CI 0.16–4.95; p = 0.90).

4- ZIFT
zygote Intra-Fallopian tubal Transfer

- Three Meta-analysis observational studies investigated the possible beneficial effect of ZIFT in women with RIF.
- ***Primary outcomes*** Meta-analysis did not show increased chances of clinical pregnancy (random effects model, OR 2.40; 95% CI 0.52–11.05; $p = 0.26$; $I^2 = 87\%$) and live birth (random effects model, OR 3.43; 95% CI 0.03–43.80; $p = 0.62$; $I^2 = 91\%$) in women who underwent ZIFT.
- ***Secondary outcomes*** Pooling of results failed to show a benefit on embryo implantation chances (random effects model, OR 3.73; 95% CI 0.69–20.27; $p = 0.13$; $I^2 = 64\%$). MPR resulted significantly lower in women who underwent ZIFT (OR 0.26; 95% CI 0.07–0.91; $p = 0.04$). Shahrokh Tehraninejad et al. did not observe an impact on MR (OR 2.09; 95% CI 0.70–6.21; $p = 0.19$).

5- AH Assisted Hatching

- One RCT and one observational study investigated the effect of AH on IVF outcomes in women with RIF.
- ***Primary outcomes*** 156 did not observe an increased chance of clinical pregnancy in women who underwent AH (RR 0.78; 95% CI 0.48–1.27; p = 0.31).
- Primi et al., confirmed this finding (CPR, OR 1.42; 95% CI 0.45–4.48; p = 0.55) and failed to show a beneficial effect also on chances of live birth (OR 1.92; 95% CI 0.48–7.67; p = 0.36).
- ***Secondary outcomes*** Primi et al. did not observed any difference in MPR between groups (OR, 1.49; 95% CI 0.09–24.44; p = 0.78).

Clinical approach

1- Uterine interventions
A- Endometrial scratching

- Release of inflammatory factors favorable to implantation (cytokines, interleukins, growth factors, macrophages and dendritic cells) (Gnainsky et al., 2015), Nastri et al., 2015, Simon and Bellver, 2014

- A number of meta-analyses and a recent well-designed RCT show no benefit of endometrial injury on LBR in unselected women undergoing IVF (Nastri et al., 2015, Lensen et al., 2019)

Endometrial scratching

- **Recommendation**:
- Endometrial injury in the cycle preceding the embryo transfer should not be routinely offered in RIF.
- Strength: strong.
- Quality of evidence: moderate
- Justification: results from two well-designed RCT and pooled results from RCT reporting on LBR show no benefit of endometrial injury.

Endometrial scratching
2021 Analyzing the results

- ***Primary outcomes*** *(LBR + CPR)* Meta-analysis of RCTs did not show significantly increased chances
- ***Secondary outcomes*** (IR/MPR/MR): observed a slight benefit of endometrial injury on (IR) implantation rate (RR 1.70; 95% CI 1.01–2.84; p = 0.04). Meta-analysis of RCTs did not show any impact on MR (fixed effects model, RR 1.39; 95% CI 0.55–3.53; p = 0.48; I^2 = 0%)
- (no benefits were observed) In fact, authors reported a higher incidence of clinical miscarriages in the context of in-cycle scratching

B- Hysteroscopy

- One RCT investigated whether outpatient hysteroscopy in the month before starting IVF treatment cycle could improve the outcome in women with RIF.
- *Primary outcomes* 144 failed to show an increase in live birth chances (RR 0.96; 95% CI 0.69–1.32; p = 0.79).

2- Atosiban

- The decrease of the frequency and amplitude of uterine contractions obtained through the administration of Atosiban, has also been theorized as a method to enhance the probability of embryo implantation and pregnancy in women with RIF.
- One observational study examined the effect of Atosiban administered before transfer of frozen-thawed embryo to women with RIF.
- ***Primary outcomes*** Authors observed an increased (CPR) in treated women when compared to controls (OR 2.63; 95% CI 1.08–6.40; p = 0.03).
- ***Secondary outcomes*** 148 showed an effect on chances of embryo implantation increased (IR) (OR 3.12; 95% CI 1.54–6.28; p = 0.002) and did not find any impact of miscarriage risk (OR 1.66; 95% CI 0.43–6.35; p = 0.46) of Atosiban administration.
- (6.75mg IV 30min prior To ET, infusion 18mg/h for 1 h. then 6mg/h for 2h)

3- Anticoagulants in the management of RIF

A- LMWH

- The role of (LMWH) in implantation involved in endometrial receptivity and implantation (Potdar et al., 2013).
- It has been shown to decrease trophoblastic apoptosis, and promote angiogenesis and trophoblastic invasion (Hills et al., 2012) Shomer et al., 2016). Berker et al., 2011, Fawzy and El-Refaeey, 2014, Hamdi et al., 2015, Stern et al., 2003

LMWH

- Two RCTs and one observational study investigated the effect of subcutaneous LMWH administration.
- ***Primary outcomes*** Meta-analysis of RCTs failed to show a beneficial effect on both CPR (RR 1.39; 95% CI 0.87–2.23; p = 0.17; I^2 = 4%) and LBR (RR 1.38; 95% CI 0.64–2.96; p = 0.41). Berker et al.
- **Secondary outcomes** also did not observe a significant increase of pregnancy and live birth chances (OR 1.42, 95% CI 0.58–3.45; p = 0.44 and OR 1.50; 95% CI 0.59–3.82; p = 0.40, respectively)

LMWH

- **Recommendations** .
- Empirical LMWH for RIF patients should be limited to research settings.
- Strength: weak.
- Quality of evidence: low.
- Justification: studies are few, populations studied are heterogeneous, and study results are inconsistent.

Anticoagulants in the management of RIF

B- ASA

- Low-dose aspirin (acetylsalicylic acid [ASA]) is thought to have anti-inflammatory and antiplatelet properties that may enhance uterine perfusion and improve endometrial receptivity Siristatidis et al., 2016 Pakkila et al., 2005
- Low-dose ASA as an adjuvant treatment in RIF has not been studied on its own in any randomized trial

Anticoagulants in the management of RIF

ASA

- **Recommendations**.
- Aspirin should not be routinely offered in RIF.
- Strength: weak.
- Quality of evidence: low.
- Justification: there are no RCT evaluating aspirin alone, and only one RCT evaluating aspirin as a combination treatment.

4- Intrauterine hCG injection

- Intrauterine hCG infusion volumes of culture medium (1 ml and 0.2 ml) and doses of hCG (1000 UI and 500 UI).
- Two observational studies investigated the effect of intrauterine hCG injection in women with RIF.
- ***Primary outcomes*** Chances of clinical pregnancy (CPR) (fixed effects model, OR 1.81; 95% CI 1.23–2.65; p = 0.002; I^2 = 0%) and (LBR)(OR 1.78; 95% CI 1.02–3.09; p = 0.04) resulted significantly increased in treated women.
- ***Secondary ooutcomes*** Liu et al. showed a beneficial effect of intrauterine hCG injection on implantation rate (RI) (OR 1.71; 95% CI 1.08–2.71; p = 0.02).
- *Quality of the evidence* The quality of the evidence was downgraded by one level for risk of bias.

5- Immunomodulatory therapies

Immune therapy in women with RIF

A- (IVIG)

- Immune specifically a balance between T-helper 1 (Th1) and T-helper 2 (Th2) cytokines.
- Shifts toward Th1 lead to the production of pro-inflammatory cytokines (IFN-γ, IL-2 and Tumor Necrosis Factor-alpha) that mediate a cytotoxic cell-mediated immune response and increase phagocytosis and inflammation. Th2 cells produce an anti-inflammatory response (via production of interleukins that inhibit phagocytosis).
- The role of peripheral natural killer cells is questionable in implantation and early pregnancy; however, uterine natural killer cells are the dominant immune cells in the decidualized endometrium after ovulation, with increasing accumulation around the trophoblast cells, therefore playing an important role in the regulation of placentation and normal invasion of the Trophoblast (Hviid, 2017).
- Intravenous immunoglobulin (IVIG) has been considered to enhance the action of regulatory T cells and reduce Th1 cytotoxic reactions (Moraru et al., 2012
- Li et al., 2013, Madkour et al., 2016, Makrigiannakis et al., 2015, Okitsu et al., 2011, Yu et al., 2016

IVIG

- One observational study evaluated the efficacy of IVIG in women with RIF.
- ***Primary outcomes*** Chances of clinical pregnancy and live birth (LBR) resulted significantly increased in treated women (OR 2.08; 95% CI 1.28–3.36; p = 0.003 and OR 1.76; 95% CI 1.08–2.89; p = 0.02, respectively).
- ***Secondary outcomes*** Ho et al., observed an increased chance of embryo implantation (RI) (OR 1.43; 95% CI 1.06–1.94; p = 0.02) in treated subjects.

B- Intravenous Intralipid infusion

- Intralipid: A recent single-blinded RCT investigated the effect of administration of intravenous Intralipid on pregnancy outcome in women with previous implantation failure (Singh et al., 2019). While some benefit was found, the study population was not specific for RIF. A statistically significant increase was found in LBR.

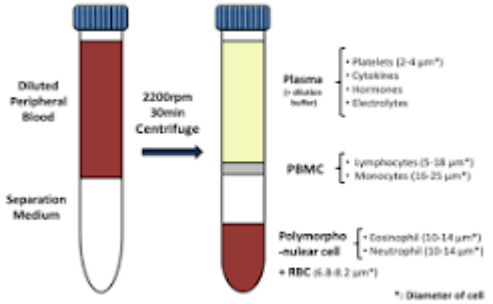
- One RCT investigated the effect of the intravenous infusion of intralipid.
- ***Primary outcomes*** Authors failed to show a benefit of the intravenous intralipid infusion on both the clinical pregnancy rate and the live birth rate (RR 1.30; 95% CI 0.80–2.10; p = 0.29 and 1.30; 95% CI 0.61–2.77, respectively).
- **Secondary outcomes** still

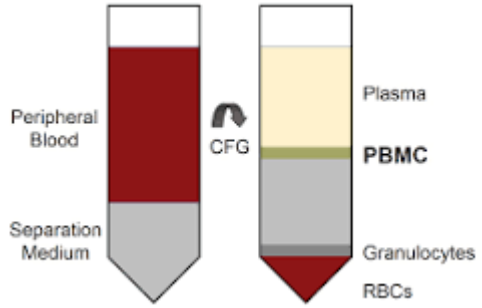
C- PBMC

- Infusion of peripheral blood mononuclear cells (PBMC) has been considered in the treatment of RIF.
- Based on the theory that local immune cells at the implantation site actively contribute to embryo implantation.
- There are several prospective studies that report on the treatment of women with RIF using intrauterine infusion of treated PBMC (Li et al., 2013, Madkour et al., 2016, Makrigiannakis et al., 2015, Okitsu et al., 2011, Yu et al., 2016.

PBMC peripheral blood mononuclear cells

- PBMC isolation is density gradient centrifugation





- most studies report an <u>increase in LBR</u> in the group receiving treatment compared with the placebo or control groups.

Intrauterine autologous PBMC infusion

- Three RCTs and three observational studies investigated the effect of intrauterine administration of autologous PBMC on IVF outcomes in women with RIF.
- ***Primary outcomes*** Meta-analysis of RCTs showed a significant increase in chances of clinical pregnancy (fixed effects model, RR 2.18; 95% CI 1.58–3.00; $p < 0.00001$; $I^2 = 0\%$) and (LBR) live birth (RR 2.41; 95% CI 1.40–4.16; $p = 0.002$) in treated women Pooling of results of observational studies confirmed the positive effect on both CPR (fixed effects model, OR 2.03; 95% CI 1.22–3.36; $p = 0.006$; $I^2 = 28\%$) and LBR (fixed effects model, OR 3.73; 95% CI 1.13–12.29; $p = 0.03$; $I^2 = 13\%$)
- ***Secondary outcomes*** Meta-analysis of observational studies showed an increased chance of RI embryo implantation in treated women (fixed effects model, OR 4.54; 95% CI 1.82–11.35; $p = 0.001$; $I^2 = 0\%$).

E- Glucocorticoids

- Glucocorticoids have Immunoregulatory properties and have been demonstrated to alter uterine natural killer cell activity (Polanski et al., 2014a); however, no prospective data exist evaluating their application in RIF.

F- (G-CSF)

- Granulocyte Colony Stimulating Factor (G-CSF) is Glycoprotein, stimulates bone marrow to produce granulocytes and stem cells and release them into blood stream.
- It plays a role in the promotion of Neutrophilic granulocyte proliferation and differentiation, and has had clinical application in the treatment of myelosuppressive states such as aplastic anaemia and neutropenia in its recombinant form. It is expressed and produced by decidual cells and therefore its role in implantation has been considered.
- Filgrastim (recombinant) and Lenograstim (synthetic)
- Administration: 300pg /1ml administered on the day of EC or P4 admin

G-CSF administration

- Six RCTs evaluated the possible beneficial effect of the subcutaneous or intrauterine G-CSF administration.
- ***Primary outcomes*** Pooling of results from studies showed increased chances pregnancy CPR in treated subjects (fixed effects model, 1.94; 95% CI 1.47–2.55; $p < 0.00001$; $I^2 = 0\%$). Only one study investigated the impact of intrauterine G-CSF infusion on the chances of live birth and failed to show a benefit (RR 0.84; 95% CI 0.41–1.73; $p = 0.64$).
- ***Secondary outcomes*** Two trials reported implantation rate IR. Pooling of results showed a beneficial effect (fixed effects model, RR 2.41; 95% CI 1.38–4.22; $p = 0.002$; $I^2 = 0\%$). Kalem et al. did not observe any impact on MR (RR 3.20; 95% CI 0.69–14.93; $p = 0.14$).

Immune therapy in women with RIF Recommendation

- **Recommendation**:
- The use of immunotherapy, Intralipid, glucocorticoids and G-CSF in RIF patients should be limited to research settings.
- Strength: strong.
- Quality of evidence: low.
- Justification: the studies are few in number and small with respect to sample size. There are limited RCT data available, and the side effect profile is questionable and not documented, as well as there being a questionable use of resources.

6- Intrauterine PRP infusion

- Two RCTs investigated whether administration of intrauterine PRP could improve IVF outcomes in women with RIF.
- ***Primary outcomes*** Pooling of results showed a significantly increased chance of clinical pregnancy CPR in treated women (fixed effects model, RR 2.45; 95% CI 1.55–3.86; p = 0.0001; I^2 = 0%).
- **Secondary outcomes**: not yet
- *Quality of the evidence* The quality of the evidence was downgraded by one level for risk of bias and, considering the low number of events, by one level for imprecision.

May be offered	Limited to research settings only	Not recommended
Karyotype testing	Serological and endometrial immune testing	Hysteroscopy if baseline ultrasound is normal.
Preimplantation genetic testing for aneuploidies	Endometrial receptivity assays	Acquired and congenital thrombophilia workup.
	Empirical low molecular weight heparin	Sperm DNA fragmentation index.
	Immunotherapy, Intralipid, glucocorticoids, granulocyte colony-stimulating factor	Screening for chronic endometritis.
		Endometrial injury in the preceding menstrual cycle.
		Aspirin

Clinical treatment

		CPR	LBR	RI	MPR	MR
Uterine intervention	scratch	-	-	Slightly		
	hystero	-	-	-	-	-
Atosiban		Yes		yes		
Anticoagulant	LMWH					
	ASA	limit to research	limit to research	limit to research	limit to research	limit to research
IU hCG		Yes	Yes	Yes		
Immunomodulatory	IVIG	-	Yes	Yes	-	-
	Intralipid	no	no	no	no	No
	PBMC	-	Yes	Yes	-	-
	Glucoc	No study	No study	No study	No study	No study
	PBMC	Yes	Yes	Yes		
	G-CSF	Yes	-	Yes	--	-
IU PRP		Yes	-	-	-	-

CONCLUSIONS

- Pooling of results showed a beneficial effect of
- intrauterine **PBMC** infusion on both CPR and LBR
- subcutaneous **G-CSF** administration on CPR and IR
- intrauterine **PRP** infusion on CPR.
- positive effect of **IVIG** and intrauterine **hCG** infusion on both CPR and LBR.
- **Atosiban** on CPR and IR.
- **Intrauterine PBMC infusion and subcutaneous G-CSF administration** are the most promising therapeutic options for RIF.

Do not “Beck and call”

THANK YOU

practise makes perfect