REPRODUCTION
REVIEW

Immunomodulation for unexplained recurrent implantation failure: where are we now?

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Abstract

In brief: Immune dysfunction may contribute to or cause recurrent implantation failure. This article summarizes normal and pathologic immune responses at implantation and critically appraises currently used immunomodulatory therapies.

Abstract: Recurrent implantation failure (RIF) may be defined as the absence of pregnancy despite the transfer of ≥3 good-quality blastocysts and is unexplained in up to 50% of cases. There are currently no effective treatments for patients with unexplained RIF. Since the maternal immune system is intricately involved in mediating endometrial receptivity and embryo implantation, both insufficient and excessive endometrial inflammatory responses during the window of implantation are proposed to lead to implantation failure. Recent strategies to improve conception rates in RIF patients have focused on modulating maternal immune responses at implantation, through either promoting or suppressing inflammation. Unfortunately, there are no validated, readily available diagnostic tests to confirm immune-mediated RIF. As such, immune therapies are often started empirically without robust evidence as to their efficacy. Like other chronic diseases, patient selection for immunomodulatory therapy is crucial, and personalized medicine for RIF patients is emerging. As the literature on the subject is heterogenous and rapidly evolving, we aim to summarize the potential efficacy, mechanisms of actions and side effects of select therapies for the practicing clinician.

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Introduction

Recurrent implantation failure (RIF) is often defined as the absence of pregnancy despite the transfer of ≥3 good-quality blastocysts (Shaulov *et al.* 2020); however, there is no standard or universally recognized definition. While the definition of RIF varies widely and depends upon maternal age, embryo quality, the presence or absence of aneuploidy screening and the number of embryos transferred, the incidence of RIF is estimated to be 10% of couples undergoing *in vitro* fertilization (IVF) (Bellver & Simon 2018). Like recurrent pregnancy loss (RPL), up to 50% of patients will have unexplained RIF (uRIF) despite extensive investigation (Bashiri *et al.* 2018). In a recent retrospective cohort review of 118 women <39 years with uRIF, the probability of live birth per embryo transferred was 12% (Koot *et al.* 2019) compared to the 25–35% reported for unselected women <40 years undergoing IVF (2019 CARTR report). While embryo aneuploidy explains a large proportion of RIF (Pirtea *et al.* 2021), endometrial factors cannot be ignored especially for patients with recurrent euploid blastocyst transfer failures or young patients with multiple good-quality blastocyst transfer failures. uRIF is associated with substantial physical, emotional, and financial distress as well as high health-care resource

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utilization (Coomarasamy *et al.* 2016), and there are no clearly effective therapies to improve conception rates.

Because the immune system is thought to be intricately involved in mediating endometrial receptivity and facilitating implantation (Fig. 1), a dysfunctional immune response during the window of implantation (WOI) has long been suspected to explain RIF in certain patients (Liu *et al.* 2017). Both insufficient (Dekel *et al.* 2010) and overactive endometrial inflammatory response (Negishi *et al.* 2021) are hypothesized to lead to implantation failure through various mechanisms (Figs. 2a and b). However, because of the inherent difficulty and ethical challenges imposed by studying the human endometrium during implantation, most of the data acquired to date is extrapolated from inbred murine studies, human peripheral blood or decidual tissue from miscarried pregnancies. This poses several issues. First, murine models may not reflect human endometrial physiology (Prabhudas *et al.* 2015, Fitzgerald *et al.* 2021). The mechanisms of decidualization appear relatively well conserved in both mice and humans, but the murine decidual reaction occurs after embryo implantation, while in humans, decidualization occurs cyclically after ovulation (Ramathal *et al.* 2010). Mice and humans both exhibit hemochorial placentation where the trophoblast is in direct contact with maternal blood. However, the human trophoblast invades deeper into the myometrium and gestates for much longer, implicating the need for more complex mechanisms to ensure maternal tolerance (Schmidt *et al.* 2015). Second, many studies have noted differences in peripheral blood inflammatory biomarkers between patients with RIF and fertile controls; peripheral blood immune testing for natural killer (NK) cell number and function, pro-inflammatory cytokine to immunoregulatory cytokine levels and T helper cell 1 (Th1) to T helper cell 2 (Th2) ratios are used as markers of immune dysfunction. However, these tests lack diagnostic validity (Moffett & Shreeve 2015), have not been shown to correlate with reproductive outcomes (Thum *et al.* 2005, Donoghue *et al.* 2019, Zhang *et al.* 2020) or reflect local endometrial immune events involved in implantation (Harrity *et al.* 2019). Last, some of our knowledge on the immune network involved in trophoblast tolerance stems from the analysis of spontaneously aborted decidua compared to decidua isolated from voluntary pregnancy terminations (Guo *et al.* 2021). Yet a miscarriage is an inflammatory event (Ticconi *et al.* 2019), and it is unclear if miscarried decidual immune cell phenotypes are the result or the cause of the pregnancy loss and if these results can be extrapolated to immune events occurring during implantation.

While the field of reproductive immunology had exploded since Sir Peter Medawar first described the 'immunological paradox of pregnancy' in 1953, our scientific community still has an incomplete comprehension of the immune events required for embryo attachment and implantation. Therefore, the evidence behind an aberrant immune response contributing to RIF is lacking and the ability to confirm immune-mediated RIF is limited (Bashiri *et al.* 2018). Clinical strategies to manipulate the immune system exist but are often started on clinical speculation, lack alternative explanation for RIF or are based on unvalidated testing (Harrity *et al.* 2019, Zhang *et al.* 2020); their use to improve reproductive outcomes in uRIF patients remains highly controversial (Hviid & Macklon 2017). These strategies act either by enhancing inflammation ('pro-inflammatory strategies') or by suppressing inflammation ('anti-inflammatory strategies') and include medicinal compounds (aspirin and low-molecular-weight heparin (LMWH)), hormones (glucocorticoids and human chorionic gonadotropin), growth factors (granulocyte colony-stimulating factor (G-CSF)), cell extracts (peripheral blood mononuclear cells (PBMCs) and platelet-rich plasma (PRP)), biological compounds (intralipid and intravenous immunoglobulin (IVIg) and medical procedures (endometrial scratching). The goal of this review is to provide readers with a comprehensive analysis and critical appraisal of the current literature, reviewing the proposed mechanisms and potential efficacy of currently used immune modulating therapies in patients with uRIF (referred herein as RIF).

Materials and methods

To capture enough articles for this review, we defined uRIF as the absence of pregnancy after ≥2 good-quality blastocyst transfers and the absence of identifiable causes of implantation failure. We chose to discuss the following IVF adjunctive therapies (endometrial scratch, PBMC therapy, low-dose aspirin (LDA), LMWH, G-CSF, human chorionic gonadotropin, glucocorticoids and intralipid and IVI) because they have hypothesized effects on the immune system and are frequently encountered in the clinical setting.

A PubMed and Embase search of the English literature using keywords 'recurrent implantation failure', 'unexplained infertility' AND 'immunomodulation', 'aspirin', 'heparin', 'low molecular weight heparin', 'corticosteroids', 'peripheral blood mononuclear cells', 'granulocyte colony stimulating factor', 'human chorionic gonadotropin', 'intralipid' or 'intravenous immunoglobulin' and 'endometrial scratch' (1950 to January 2022) was performed. For each intervention, randomized controlled trials (RCTs) that reported live birth rate (LBR) as the primary outcome and which recruited women with RIF were selected. When these criteria were not fulfilled for an intervention, controlled cohort studies and case studies reporting at least the clinical pregnancy rate (CPR) in women with RIF were included.

Pro-inflammatory strategies

In this section, we discuss strategies hypothesized to enhance endometrial inflammation and improve endometrial receptivity (Fig. 3a).

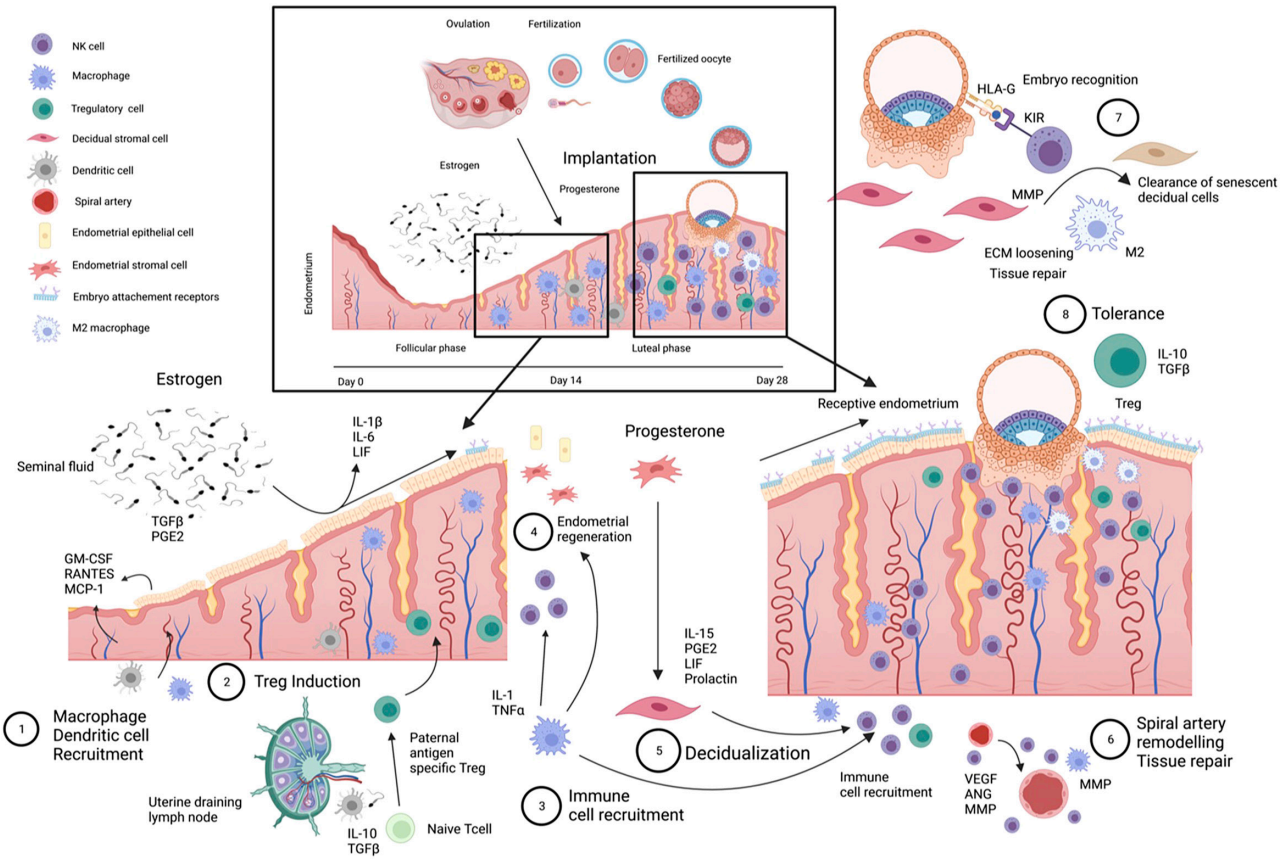

Figure 1 Immune contribution to endometrial function during the menstrual cycle. During the follicular phase, increasing ovarian production of estrogen acts on endometrial epithelial and stromal cells to induce the production of granulocyte macrophage colony-stimulating factor (GM-CSF), monocyte chemo-attractant protein-1 (MCP-1) and RANTES (CCL5) (Hornung *et al.* 1997, Robertson *et al.* 1997, Shuya *et al.* 2011). These chemokines initiate progressive macrophage and dendritic cell (DC) recruitment (Fig. 1-1). Endometrial DCs are phenotypically distinct and are thought to instigate paternal alloantigen tolerance prior to implantation. They phagocytose seminal fluid proteins and present antigens to naïve maternal T cells in the uterine draining lymph nodes, inducing a specific T-regulatory (T_{reg}) cells which home back to the endometrium upon antigen re-exposure (Robertson *et al.* 2018). Endometrial DCs also secrete immunomodulatory cytokines (IL-10, TGFβ, IL-6 and IL-8), thus modulating the effector phenotype of other endometrial immune cells (Fig. 1-2) (Liu *et al.* 2018). Macrophages constitute 10% of the endometrial leukocyte population by the mid-luteal phase (Russell *et al.* 2011). Initially thought to be skewed toward an immunoregulatory M2 phenotype, they are a heterogenous population, capable of both pro- and anti-inflammatory cytokine secretion (Chambers *et al.* 2020). In the follicular phase, transitory pro-inflammatory macrophage phenotype predominates (producing IL-1, TNFα and expressing high levels of MHCII), presumably required to support further immune cell recruitment and their phenotypic differentiation as well as to promote endometrial regeneration and proliferation (Fig. 1-3) (Thiruchelvam *et al.* 2013). This phenotype is further promoted by exposure to seminal fluid (Schjenken & Robertson 2020), which leads to endometrial epithelial cell production of pro-inflammatory cytokines IL-1β, IL-6 and LIF (Gutsche *et al.* 2003); inflammatory changes in the endometrium are necessary to further modulate endometrial immune cell function and upregulate receptors required for embryo attachment (Fig. 1-4) (Robertson *et al.* 2018). After ovulation, ovarian production of progesterone predominates, inducing endometrial stromal cell transformation into decidual stromal cells (DSC) and the production of prostaglandin E2 (PGE2), monocyte chemoattractant proteins (MCP), prolactin, IL-15 and leukemia inhibitory factor (LIF), amongst others (Thiruchelvam *et al.* 2013, Niringiyumukiza *et al.* 2018, Chambers *et al.* 2020, Makrigiannakis *et al.* 2021). These cytokines and chemokines lead to further decidual macrophage accumulation but also recruit a growing population of NK cells which represent up to 70% of decidual leukocytes by the mid-luteal phase (Fig. 1-5) (Russell *et al.* 2011). Unlike inflammatory infiltrates in other tissues, neutrophils are not recruited to the normal decidualizing endometrium (Wang *et al.* 2021b). Decidual NK cells, contrary to peripheral NK cells, differentiate into weakly cytotoxic $CD56^{bright}CD16^{lo}$ heterogenous subset (Zhang & Wei 2021) and have many putative functions in the decidualizing endometrium. They have been shown to contribute to spiral artery remodeling through the secretion of pro-angiogenic factors (angiopoietins, vascular endothelial growth factor (VEGF)) and cooperation with macrophages to produce matrix metalloproteases (MMP) required to break down the vascular smooth muscle cell extracellular matrix (ECM) (Fig. 1-6) (Zhang *et al.* 2016). MMP production leads to loosening of the decidual ECM to permit trophoblast invasion (Smith *et al.* 2009), while NK cell-mediated clearance of senescent decidual stromal cells ensures a functional endometrium into which the embryo can implant (Brighton *et al.* 2017). Decidual NK cells may also play a role in embryo selection (Kong *et al.* 2021) and control trophoblast invasion during implantation (Diaz-Hernandez *et al.* 2021). Decidual M2 macrophages are thought to contribute to tissue repair around the site of implantation, thus limiting inflammatory spread (Fig. 1-7) (Zenclussen & Hammerling 2015). T_{reg} cells home to the decidualizing endometrium and are also thought to help resolve inflammation at the implantation site while promoting early maternal tolerance to the implanting embryo (Fig. 1-8) (Robertson *et al.* 2018).

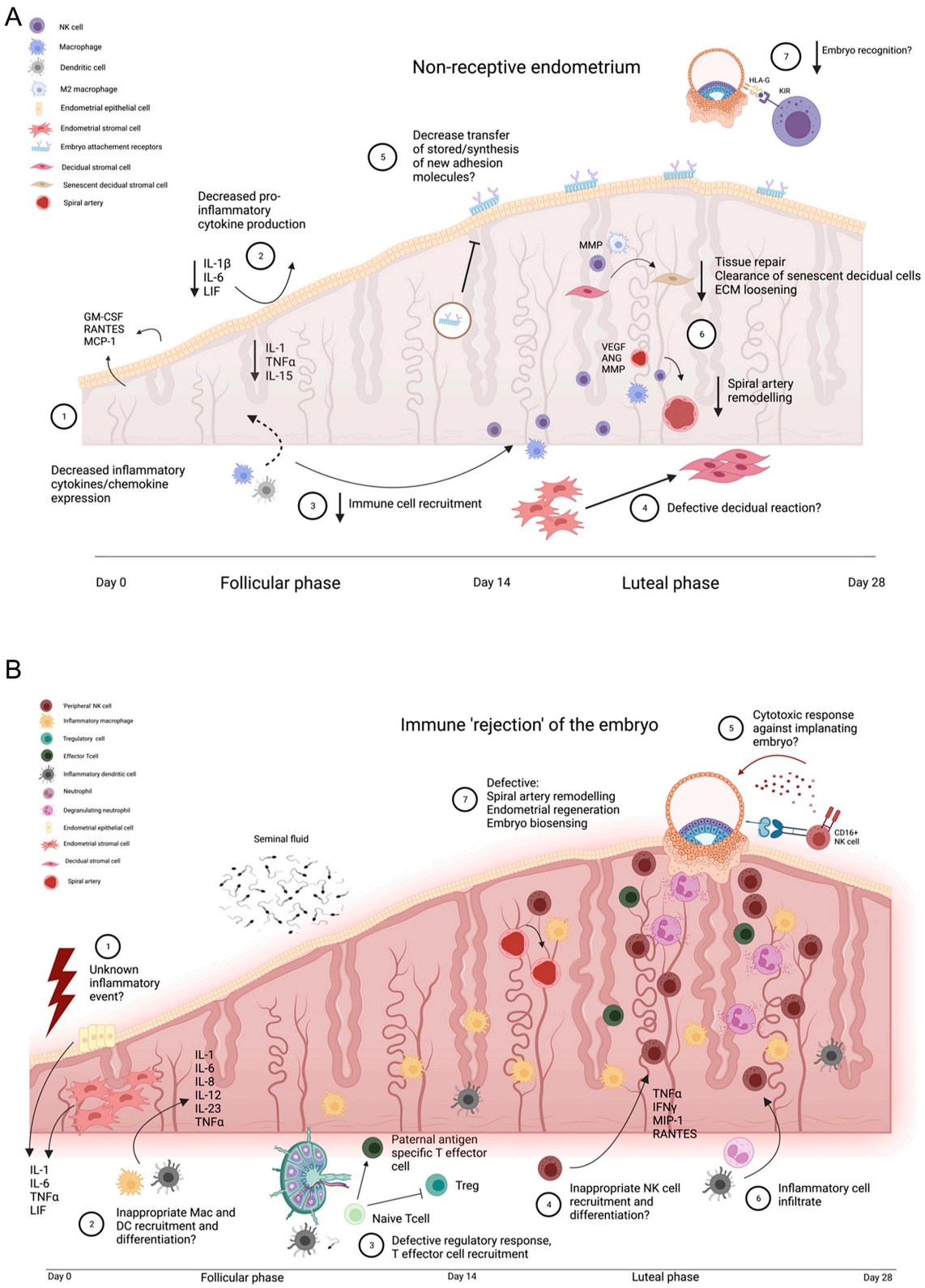

Figure 2 Insufficient endometrial inflammatory response could lead to implantation failure. Insufficient local production of pro-inflammatory cytokines decreases endometrial macrophage (Mac) and dendritic cell (DC) recruitment to the endometrium (Fig. 2a-1). The importance of DC and Mac recruitment is evidenced by murine models, where depletion of endometrial Mac (Chambers *et al.* 2020) or DC (Blois *et al.* 2004) causes implantation failure or embryo resorption. This leads to decreased endometrial stromal cell/infiltrating leukocyte production of

Figure 2 (Continued)

pro-inflammatory cytokines (IL-6, IL-8, IL-15, GM-CSF, MIP-1 and TNFα) (Fig. 2a-2) as well as insufficient stimulus for further NK cell and Mac recruitment and their phenotypic differentiation into implantation-specific effector cells (Fig. 2a-3) (Dekel *et al.* 2010). This may have a deleterious effect on the decidual reaction (Gnainsky *et al.* 2010) and expression of endometrial adhesion molecules, both leading to decreased endometrial receptivity (Gnainsky *et al.* 2015) (Fig. 2a-4 and -5). In addition, defective spiral artery remodeling (Zhang & Wei 2021), extracellular matrix loosening (Smith *et al.* 2009), endometrial repair (Zenclussen & Hammerling 2015) and regeneration (Brighton *et al.* 2017) could also contribute to decreased endometrial function during the WOI (Fig. 2a-6). Decreased NK function may also contribute to implantation failure cell through an inability to biosense the implanting embryo (Fig. 2a-7) (Kong *et al.* 2021). Overactive endometrial inflammatory response could lead to implantation failure. An overactive immune response is also hypothesized to lead to RIF. However, whether there is an instigating event (pathogen-driven endometritis (Liu *et al.* 2020a), sterile inflammation (Zhu *et al.* 2021), metabolic disorders (Koc *et al.* 2017), oxidative stress (Samimi *et al.* 2019)), defective ability of the endometrium to resolve inflammation (Drizi *et al.* 2020) or inability of the endometrium to respond appropriately to the implanting embryo (Wang *et al.* 2021b) in unknown (Fig. 2b-1). Regardless, endometrial inflammation is hypothesized to cause endometrial stromal and epithelial cells to produce pro-inflammatory cytokines, shifting DC and Mac phenotypic differentiation away from their proposed tolerogenic roles. This has several consequences including further pro-inflammatory cytokine secretion (Fig. 2b-2), inability to activate an appropriate early T_{reg} response (Aluvihare *et al.* 2004, Zenclussen *et al.* 2005, Li *et al.* 2017a) and favoring Th1, Th17 and CD8+ T cell chemotaxis to the endometrium (Guo *et al.* 2021) (Fig. 2b-3). A pro-inflammatory immune environment affects NK cell recruitment and differentiation (Diaz-Hernandez *et al.* 2021) (Fig. 2b-4). NK cells in patients with inflammatory RIF are postulated to exhibit enhanced cytotoxic potential (CD16 expression) (Comins-Boo *et al.* 2021, Huang *et al.* 2021), pro-inflammatory cytokine/chemokine secretion and attraction of an inflammatory cell infiltrate to the endometrium (Fig. 2b-5, 6) (Diaz-Hernandez *et al.* 2021, Comins-Boo *et al.* 2021, Tersoglio *et al.* 2021). These NK cells exhibit altered embryo biosensing, inability to contribute to vascular remodeling or endometrial regeneration necessary for embryo implantation (Wang *et al.* 2021b) (Fig. 2b-7). All these inflammatory changes are proposed to upset the normal immune remodeling of the endometrium and contribute to implantation failure through immune 'rejection' of the embryo.

Endometrial scratch

Insufficient endometrial inflammation is hypothesized to inhibit implantation through deficient recruitment or inadequate leukocyte activation during the WOI, leading to decreased pro-inflammatory cytokine production, endometrial receptivity, tissue repair and angiogenesis (Gnainsky *et al.* 2010, Gnainsky *et al.* 2015, Yu *et al.* 2019) (Fig. 2a). Endometrial scratch therapy (EST) prior to embryo transfer (ET) is used as a method to augment endometrial immune cell recruitment and activation through a wound healing response, causing stromal cells to release inflammatory and chemoattractant cytokines required to promote implantation (Fig. 3a) (Gnainsky *et al.* 2015). However, *in vivo*/*in vitro* confirmation of this mechanism has not been possible thus far. While EST has not been shown to consistently improve LBRs in subfertile women undergoing natural conception or IUI (Bui *et al.* 2021), IVF (Lensen *et al.* 2019, Lensen *et al.* 2021) or in patients with ≥1 previously failed IVF attempt undergoing ET (Sar-Shalom Nahshon *et al.* 2019, Van Hoogenhuijze *et al.* 2021), it may be most useful in patients with RIF (Dekel *et al.* 2014). Unfortunately, a recent meta-analysis which included five RCTs (522 patients) (Karimzadeh *et al.* 2009, Baum *et al.* 2012, Shohayeb and El-Khayat 2012, Shahrokh-Tehraninejad *et al.* 2016, Matsumoto *et al.* 2017) did not find that EST performed in the luteal phase of the cycle preceding ET or in the follicular phase of the ET cycle improved LBR in patients with ≥2 failed ET attempts (relative risk (RR): 1.22 (95% CI: 0.52–2.82), $P=0.65$) (Sar-Shalom Nahshon *et al.* 2019). Similarly, a 2021 Cochrane review failed to demonstrate enhanced LBR in patients with ≥2 previously failed IVF-ET attempts undergoing EST (2 RCT, 533 patients; OR: 0.91 (95% CI: 0.62–1.31), I^2 48%), concluding that subgroup analysis was not feasible because of high heterogeneity between studies (Lensen *et al.* 2021). Looking more closely at these included studies, Olesen *et al.* randomized 117 women with ≥3 previous good-quality blastocyst transfer failures to EST in the luteal phase prior to ovarian stimulation vs no scratch (Olesen *et al.* 2019). While there was a trend toward more live births in the intervention group, this was not significant (LBR: 26/66 (39.4%) vs 12/51 (23.5%), RR: 1.67 (0.94–2.98), $P=0.069$). A larger open-label RCT study of 454 patients with ≥2 previous IVF-ET failures also failed to show improved LBR with EST performed between day 3 of the preceding cycle and day 3 of the ET cycle compared to women with <2 previous failed ET (estimated interaction OR: 0.63; 95% CI: 0.35–1.15; $P=0.14$) (Lensen *et al.* 2019). Last, in a meta-analysis subgroup analysis performed by Vitagliano *et al.* (7 studies, 702 patients), while patients with ≥2 previously failed ET had a higher LBR with EST (RR: 1.64, 95% CI: 1.21–2.21, $P=0.001$), this was likely driven by cofounding factors such as hysteroscopy, antibiotics or prednisolone administered to the patients randomized to EST (Vitagliano *et al.* 2018). As such, EST is not currently recommended in Canada for RIF (Shaulov *et al.* 2020); however, as with other immunomodulatory therapies, the intervention may be most useful in select patients whose characteristics are still being identified (Ledee *et al.* 2020b, Rahmati & Ledee 2020).

Peripheral blood mononuclear cells

Unexplained reproductive failure has been associated with the paucity or aberrant phenotypes of decidual immune cells (Kofod *et al.* 2018, Negishi *et al.* 2018), leading to the hypothesis that intrauterine administration of autologous PBMCs prior to implantation restores normal endometrial function. Autologous PBMCs are obtained from the patient, cultured in the presence of human chorionic gonadotropin (hCG) and infused into the uterus prior to implantation. hCG-activated PBMCs are thought to enhance endometrial receptivity and embryo attachment by several mechanisms. These include the secretion of inflammatory cytokines and chemokines required for trophoblast invasion, increased production of matrix metalloproteases and VEGF required for

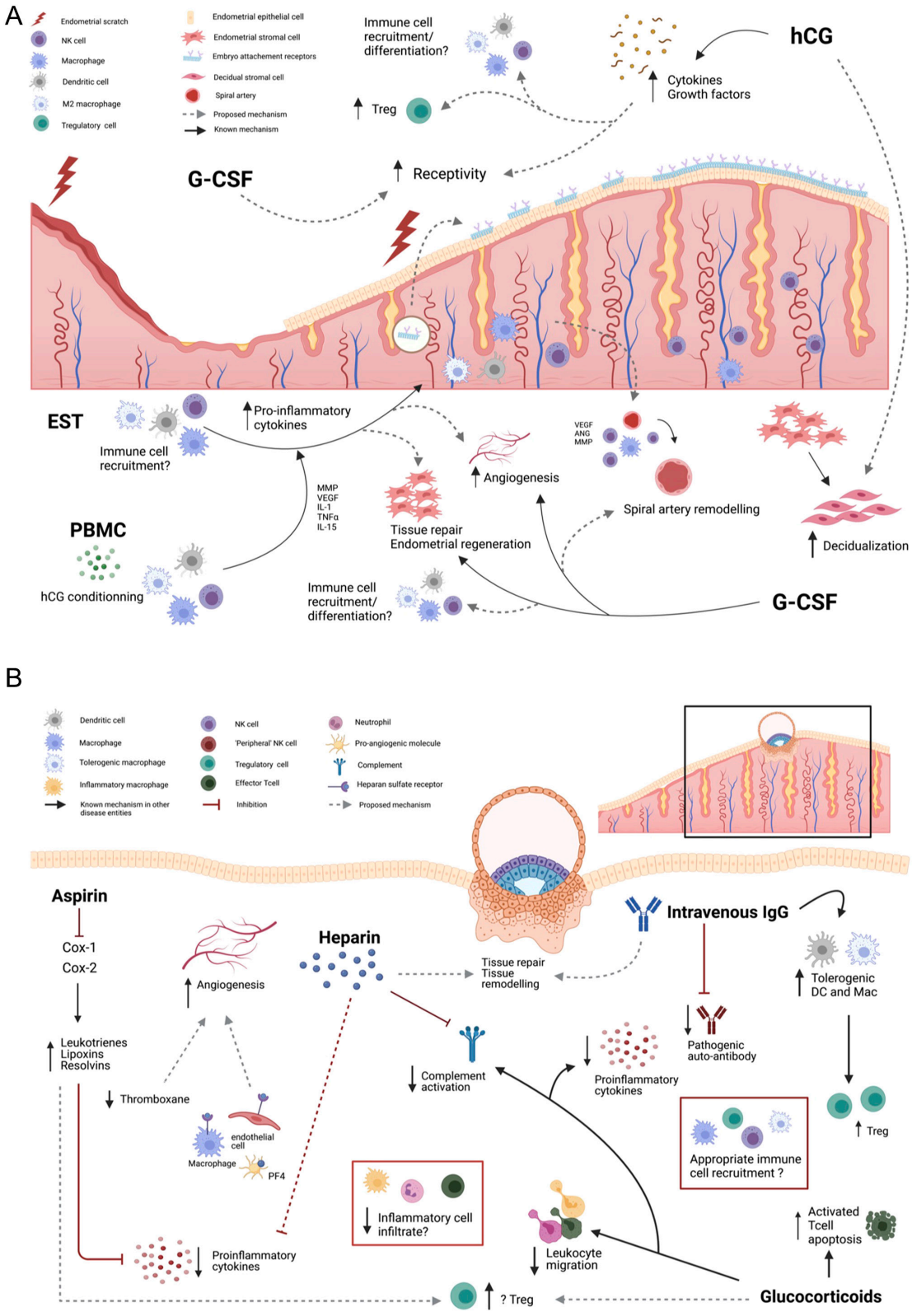

Figure 3 (A) Proposed mechanisms of select immunotherapies. Endometrial scratch therapy (EST) creates local trauma which enables the recruitment of immune cells to assist in the wound repair response. These cells secrete pro-inflammatory cytokines which promote endometrial receptivity, endometrial regeneration and angiogenesis. Intrauterine peripheral blood mononuclear cell (PBMC) infusion is thought to stimulate the secretion of cytokines and chemokines required for trophoblast invasion as well as enhance the production of other factors such as MMP

Figure 3 (Continued)

and VEGF required for endometrial and vascular remodeling. Recombinant human G-CSF may aid in endometrial leukocyte recruitment and differentiation and promote angiogenesis and possibly endometrial repair, possibly enhancing endometrial receptivity. G-CSF is also thought to promote local immune tolerance through its effect on antigen-presenting cells and on T-regulatory cells. Human chorionic gonadotropin (hCG) is secreted by the human embryo. HCG may contribute to decidualization and enhance endometrial receptivity though its ability to induce endometrial cytokine and growth factor secretion. hCG may contribute to early maternal embryo tolerance by influencing the recruitment and phenotypic differentiation of endometrial leukocytes as well as by promoting T-regulatory cell responses. (B) LDA inhibits COX, inducing the production of anti-inflammatory and vasoactive molecules. These molecules may act on endometrial innate immune cells by decreasing their response to inflammation and on the adaptive immune system by enhancing T-regulatory cell responses. Heparins are thought to reduce inflammation by neutralizing pro-inflammatory molecules such as complement components, cytokines and chemokines. Heparins may also stimulate angiogenesis and enhance tissue repair, possibly improving endometrial receptivity by aiding the decidual response. Glucocorticoids (GCs) are broad immunosuppressants and anti-inflammatories; they act by modifying leukocyte gene expression. They can inhibit complement activation and reduce the ability of leukocytes to produce pro-inflammatory cytokines and migrate to areas of inflammation; they have also been shown to induce apoptosis of activated T cells. IVIg has many different effects on the immune system. It inhibits the secretion of pro-inflammatory cytokines and chemokines, autoantibodies and complement. IVIg may improve regulatory responses through the induction of tolerogenic macrophages and dendritic cells and activation of T-regulatory cells.

effective endometrial and vascular remodeling (Nakayama *et al.* 2002, Ideta *et al.* 2010, Yu *et al.* 2014) (Fig. 3a); the exact mechanistic effect of PBMCs has yet to be shown *in vivo*. The relative low cost and low potential for side effects makes this procedure an attractive treatment for patients with RIF. However, local PBMC treatment in humans has not been shown to consistently improve IVF-ET outcomes in RIF.

In a recent meta-analysis, Yakin *et al.* did not find that PBMC treatment prior to IVF-ET improved LBR. Authors pooled data from two RCTs (Madkour *et al.* 2016, Yu *et al.* 2016) and three controlled cohort studies (Yoshioka *et al.* 2006, Okitsu *et al.* 2011, Li *et al.* 2017b) encompassing 1173 patients; however, only two studies included patients with RIF (Yoshioka *et al.* 2006, Yu *et al.* 2016). Upon subgroup analysis, PBMCs improved CPR in women with RIF (OR: 2.69, 95% CI: 1.53–4.72; $P=0.001$; heterogeneity; I^2: 38.3%). While PBMC treatment did not significantly impact LBR overall (OR: 1.65, 95% CI: 0.84–3.25; $P=0.14$; heterogeneity; I^2: 73.1%), there was no LBR subgroup analysis performed for patients with RIF (Yakin *et al.* 2019).

Three meta-analyses performed subgroup analyses of LBR in patients with ≥ 3 IVF-ET failures. Maleki-Hijiagha *et al.* included data from one RCT (Yu *et al.* 2016) and three controlled cohort studies (Yoshioka *et al.* 2006, Okitsu *et al.* 2011, Li *et al.* 2017b), totaling 504 patients 267 treated with PBMC and 237 controls. The LBR was higher in the PBMC-treated group (RR: 1.93, 95% CI: 1.35–2.76; P <0.001) (Maleki-Hajiagha *et al.* 2019). Similar results were published by Yang *et al.* (2020) and by Pourmoghadam *et al.* (2020a) after analysis of the same data. While these results seem encouraging, several limitations must be acknowledged. Most studies based patient selection for PBMC treatment on personal preference, introducing a selection bias (Yoshioka *et al.* 2006, Okitsu *et al.* 2011, Li *et al.* 2017b); most were unblinded, thus eliminating the possibility of measuring the placebo effect (Yoshioka *et al.* 2006, Okitsu *et al.* 2011, Yu *et al.* 2016, Li *et al.* 2017b); and many did not comment on embryo/blastocyst quality (Okitsu *et al.* 2011, Yu *et al.* 2016), which is an important confounding factor when assessing LBR differences between interventions. For example, Li *et al.* (Li *et al.* 2017b) was the only prospective cohort study that discussed endometrial preparation as well as day and quality of embryos transferred in their prospective cohort. They found that patients with ≥4 IVF-ET failures treated with PBMCs had a higher LBR per embryo transfer cycle than control patients (33.3% (16/48) vs 9.58% (2/21) $P=0.038$); however, the percentage of patients receiving blastocyst transfer was also higher in the PBMC group (25% (12/48) vs 9.52% (2/21)) but not statistically significant (Li *et al.* 2017b). Furthermore, none of the above studies included a control group. Could the 'success' of the PBMC treatment have been due to local endometrial injury? Indeed, endometrial scratching may improve CPRs in well-selected RIF patients by creating a favorable inflammatory endometrial environment as discussed above (Ledee *et al.* 2017). This was addressed by Pourmoghadam *et al.* (2020b) who performed an RCT in which 100 women with ≥3 failed IVF-ET and low Th17/T-regulatory cells (Treg) were randomized to receive intrauterine PBMC ($n=50$) or phosphate-buffered saline ($n=50$) prior to embryo transfer. Despite randomization, maternal age and BMI were higher in the placebo group. Embryo quality, day 3 or day 5 transfer and endometrial preparation was similar in both groups. The LBR in the PBMC-treated group was higher than in the placebo group (38% (19/50) vs 20% (10/50), $P=0.047$) (Pourmoghadam *et al.* 2020b), suggesting that the success of PBMC treatment success was likely mediated by PBMC–embryo crosstalk and promotion of the trophoblast's invasive potential rather than by endometrial injury.

While PBMC treatment may offer some hope for patients with RIF, PBMC stimulation protocols, dose and timing of administration remain to be standardized. This procedure is relatively inexpensive (<$500 Canadian dollars) and is associated with almost no side effects, comparable to those observed with intrauterine insemination. It may be reasonable to propose this type of procedure to RIF patients provided they are appropriately counseled on the paucity of scientific evidence, and the procedure is performed in a laboratory experienced in this type of treatment, capable of respecting safety standards (Table 1) (Table 2).

Of note, autologous intrauterine infusion of PRP has also been proposed as a pro-inflammatory treatment to improve implantation rate in patients with RIF (Maleki-Hajiagha *et al.* 2020, Mouanness *et al.* 2021). Two RCTs report significantly better implantation and CPRs with PRP (Nazari *et al.* 2020, Zamaniyan *et al.* 2021) but none detail LBR. PRP will not be discussed further in this review.

Table 1 Summary of meta-analysis and RCT studies detailing the use of immunomodulation in patients with unexplained recurrent implantation failure (≥3 failed embryo transfers (IVF-ET)). Of note, low-dose aspirin and corticosteroids are not featured in this table for lack of RCTs.

Study	Study details	Intervention	Outcome	Comments
Endometrial scratch				
Vitagliano *et al.* 2018 (37)	Meta-analysis RIF ≥2 failed IVF-ET treatment (*n* = 348) Control(*n* = 354)	Treatment: multiple different scratch timing Control: no treatment	LBR for scratch: RR 1.64, 95% CI (1.21–2.21); Multiple cofounders	No evidence that endometrial scratch improves LBRs
Sar-Shalom Nahshon *et al.* 2019 (28)	Meta-analysis RIF ≥2 failed IVF-ET treatment (*n* = 66) Control(*n* = 51)	Treatment: luteal phase endometrial scratch in cycle preceding ET Control: no treatment	LBR for scratch: RR 1.22, 95% CI: 0.52–2.82, *P* = 0.65	
Lensen *et al.* 2021 (26)	Meta-analysis RIF ≥2 failed IVF-ET Treatment (*n* = 232) Control(*n* = 222)	Treatment: luteal phase endometrial scratch in cycle preceding ET Control: no treatment	LBR for scratch: OR 0.9, 95% CI 0.63–1.27, *P* = 0.6	
Peripheral blood mononuclear cell (PBMC)				
Maleki-Hajiagha *et al.* 2019 (56), Yang *et al.* 2020 (52)	Meta-analysis RIF ≥3 failed IVF-ET Treatment (*n* = 267) Control (*n* = 237)	Treatment: intrauterine infusion of PBMC prior to ET Control: no treatment	LBR for PBMC: RR 1.93, 95% CI: 1.35–2.76; *P* < 0.001	Possibly effective for RIF
Pourmoghadam *et al.* 2020a (55)	RCT RIF ≥ 3 failed IVF-ET Low Th17/Treg ratio Treatment (*n* = 50) Control (*n* = 50)	Treatment: intrauterine infusion of PBMCs prior to ET Control: PBS equivalent	LBR for PBMC: 38% LBR for control group: 20% *P* = 0.047	
Granulocyte colony-stimulating factor (G-CSF)				
Aaleyasin *et al.* 2015 (69)	Multicenter RCT RIF≥3 failed IVF-ET Treatment (*n* = 56) Control (*n* = 56)	Treatment: 300 µg G-CSF s/c 1h prior to ET Control: no G-CSF	CPR: OR = 2.94, 95% CI = 1.23–8.33	No evidence that s.c. or intrauterine G-CSF prior to IVF-ET improves LBR
Davari-Tanha *et al.* 2016 (71)	RCT RIF≥3 failed IVF-ET Treatment (*n* = 40) Placebo (*n* = 40) Control (*n* = 20)	Treatment: G-CSF 300 µg (1mL) intrauterine at the time of OR Placebo: saline equivalent Control: catheter passage without injection	CPR similar across groups	
Arefi *et al.* 2018 (70)	RCT RIF ≥3 failed IVF-ET Treatment (*n* = 34) Control (*n* = 34)	Treatment: 300 µg of G-CSF subcutaneously 30 min before ET Control: no treatment	LBR G-CSF (17/32) 53.1% Control (7/20) 35% *P* = 0.09	
Kalem *et al.* 2020 (72)	RCT RIF ≥3 failed IVF-ET (≥4 failed good-quality embryos), age <40 years Treatment (*n* = 82) Control (*n* = 75)	Treatment: G-CSF 30 mIU (1mL) intrauterine on the day of hCG trigger Placebo: saline equivalent	LBR G-CSF: (12/82) 14.6%, placebo: (13/75) 17.3% *P* = 0.668	
Human chorionic gonadotropin (hCG)				
Xie *et al.* 2019 (96)	Meta-analysis RIF≥3 failed IVF-ET Treatment: 410 Control: 460	Treatment: Intrauterine hCG (500 IU) 10 min–3 days prior to ET Control: no hCG	LBR for hCG: RR 1.52, 95 % CI 1.18–1.96, *P* = 0.001	Possibly effective for RIF
Huang *et al.* 2017 (97)	RCT ≥2 failed IVF-ET Treatment (*n* = 62) Saline (*n* = 49) Control: *n* = 50	Treatment: Intrauterine hCG 3 days prior to ET (1000 IU)Placebo: saline equivalent Control: no intervention	Ongoing pregnancy hCG 32/62 (51.6%) Placebo 22/49 (44.9%) *NS Control 13/50 (26%) *P*<0.05	
Low molecular weight heparin (LMWH)				
Potdar *et al.* 2013 (124), Yang *et al.* 2018 (125)	Meta-analysis RIF ≥3 failed IVF-ET Treatment (*n* = 85) Control (*n* = 77)	Treatment: enoxaparin 1 mg/kg/day or 40 mg subcutaneous starting on the day of OR until 12 weeksControl: no treatment	LBR for heparin: RR 1.36, 95% CI: 0.82–2.26, *P* = 0.24	No evidence that preconceptual LMWH improves LBRs

(*Continued*)

Table 1 Continued.

Study	Study details	Intervention	Outcome	Comments
Intralipids				
Al-Zebeidi *et al.* 2020 (165)	RCT RIF ≥3 failed IVF-ET cycles Treatment ($n=71$) Control ($n=71$)	Treatment: intralipid 20% (100 mL) on the day of ET and on the day of pregnancy test Control: no intralipids	LBR for intralipids: OR = 1.37, 95% CI 0.55–3.36, $P=0.49$	No evidence that intralipids improve LBR in patients with RIF
IVIG				
Stephenson & Fluker 2000 (180)	RCT ≥2 failed IVF-ET Treatment ($n=26$) Control ($n=25$)	Treatment: IVIG 0.5 g/kg within 72 h prior to transfer,second dose 4 weeks later if viable pregnancyPlacebo: saline equivalent	LBR IVIG: 4/26 (15%) Placebo: 3/25 (12%)	No evidence that IVIG improves LBR in patients with unexplained RIF

CPR, clinical pregnancy rate; IVF, *in vitro* fertilization; IVIG, intravenous immunoglobulin; LBR, live birth rate; LMWH, low-molecular-weight heparin;, OR, oocyte retrieval; RCT, randomized controlled trial.

Granulocyte colony-stimulating factor

During the menstrual cycle, ovarian hormones regulate the stepwise and concerted expression of a network of cytokines and their receptors in the endometrium. Their timely expression is crucial for ensuring endometrial growth and receptivity for pregnancy as well as endometrial immune cell function. Of these cytokines, colony-stimulating factors are prominently featured. Granulocyte macrophage colony-stimulating factor has been implicated in endometrial regeneration after menses, and recombinant human G-CSF has been shown to directly enhance the endometrial expression of genes involved in embryo adhesion, cell migration and local angiogenesis. G-CSF possibly aids in local immunomodulation by providing a stimulus for endometrial leukocyte recruitment and phenotypic differentiation, promoting T_{reg} responses and by downregulating antigen presentation; however, this has not yet clearly been shown *in vivo* (Mahnke & Enk 2005, Rahmati *et al.* 2014, Würfel 2015, Zhao *et al.* 2015, Liu *et al.* 2020b) (Fig. 3a).

G-CSF's safety profile and pleiotropic roles during implantation makes it an attractive IVF adjunct. In patients with RIF, Wurfel *et al.* first reported improved pregnancy rates after a single *s.c.* dose of G-CSF on the day of ET (Würfel 2000). Since this publication, other groups have evaluated adjuvant G-CSF for RIF patients with conflicting results. While three meta-analyses have reported that G-CSF improves CPRs in patients with ≥2 failed IVF-ET (Zhang *et al.* 2019, Jiang *et al.* 2020, Kamath *et al.* 2020), positive results were mainly driven by unpublished conference abstract data and unblinded studies. High study heterogeneity made comparative analysis difficult to perform, and G-CSF's impact on LBR was unfortunately not recorded, limiting the clinical utility of these

Table 2 Summary of recommendations.

Intervention	Recommendations	Treatment considerations
Scratch therapy	No evidence	
Peripheral blood mononuclear cells (PBMCs)	Consider in patients with ≥3 IVF-ET failures	PMBC infusion must be performed in a laboratory equipped to respect cell culture safety standards
Granulocyte colony-stimulating factor	No evidence	
Human chorionic gonadotropin	Consider for patients undergoing cleavage stage embryo transfer or in patients with ≥ 2 IVF-ET failures	Intrauterine administration ≥500 IU within 3 days prior to ET
Low-dose aspirin (LDA)	Consider starting LDA prior to conception for patients with ≥2 IVF-ET failures	LDA should not be given to patients that are at increased risk of bleeding; LDA should be started after oocyte retrieval in patients undergoing fresh ET
Low-molecular-weight heparin (LMWH)	Consider in patients with ≥3 IVF-ET failures with age ≥36 or with inherited thrombophilia	Start LMWH prior to conception but after oocyte collection at prophylactic doses. Screen for thrombocytopenia regularly during treatment -Stop LMWH at 12 weeks' gestation
Glucocorticoids	RCT currently underway Can be considered for patients with ≥3 IVF-ET failures	Use lowest effective dose (≤20 mg prednisone/day or equivalent) for the shortest amount of time (≤14 days) Avoid in patients with metabolic risk factors (BMI >30, diabetes, hypertension)
Intralipids	No evidence	
Intravenous immunoglobulin (IVIG)	Can be considered patients with ≥3 RIF who have failed other immunomodulatory treatment or with immune anomalies	Viral serologies (hepatitis B and Rubella) must be obtained prior to IVIG treatment. Administer as a slow infusion to minimize side effects. IVIG should be a last resort option because of possible shortages and high costs

IVF, *in vivo* fertilization; RCT, randomized controlled trial; RIF, recurrent implantation failure.

studies. Unfortunately, these meta-analyses did not distinguish between s.c. (systemic) or intrauterine administration of G-CSF.

Looking more closely at the published RCTs evaluating G-CSF use in patients with RIF, Aleyasin led a multicenter RCT in which 112 well-matched patients with ≥3 IVF-ET failures received either 300 µg G-CSF subcutaneously 1 h prior to ET or no additional treatment. Clinical pregnancy with positive fetal heartbeat was higher in the G-CSF group (OR 2.94 95% CI 1.23–8.3, $P=0.05$) (Aleyasin *et al.* 2016), but LBR was not assessed. In their RCT, Arefi *et al.* did not find that G-CSF 300 µg administered subcutaneously 30 min before blastocyst transfer improved LBR compared to no treatment in 52 women with ≥3 IVF-ET failures (Arefi *et al.* 2018). Davari-Tanha *et al.* randomized 100 women with ≥3 failed IVF-ET to receive 300 µg of intrauterine G-CSF ($n=40$), intrauterine saline injection (placebo $n=40$) or intrauterine catheter passage alone (control $n=20$) on the day of oocyte retrieval. Patients in the treatment group had higher implantation rates than in the placebo and control groups (12.3% vs 6.1% (saline) and 4.7% (control), $P=0.04$). However, CPRs were similar (Davari-Tanha *et al.* 2016). In their recent RCT, Kalem *et al.* did not find that intrauterine G-CSF administered on the day of hCG trigger improved LBR over placebo in 157 patients <40 years with failure of ≥4 IVF-ET (Kalem *et al.* 2020). There was a trend toward more miscarriages and more early preterm births (≤28 weeks) in the G-CSF group, although neither was statistically significant (Kalem *et al.* 2020).

This finding raises important safety questions regarding G-CSF use prior to oocyte collection. Indeed, in murine studies, high doses of colony-stimulating factor have an adverse effect on blastocyst development, raising the proportion of mosaic/aneuploid embryos (Elaimi *et al.* 2012). While s.c. or intrauterine G-SCF administered peri-conceptually is well tolerated and does not seem to affect fetal or perinatal outcomes (Aleyasin *et al.* 2016, Davari-Tanha *et al.* 2016, Eftekhar *et al.* 2016, Arefi *et al.* 2018, Cruz *et al.* 2019), G-CSF administration prior to oocyte collection has not been appropriately studied. In a conference abstract publication, Boxer *et al*. found that the miscarriage rate decreased in patients receiving G-CSF for severe chronic neutropenia compared to those who declined treatment during the conception and early pregnancy phase (Boxer *et al.* 2010). However, as neutrophils may be important for implantation and early pregnancy (Schumacher & Zenclussen 2019), severe neutropenia itself may be an independent risk factor for miscarriage. Preconceptual G-CSF administration would likely improve pregnancy outcomes in neutropenic patients, and these results cannot be extrapolated to a population with normal baseline neutrophil counts (Sauss *et al.* 2018).

In summary, while peri-conceptual s.c. or intrauterine G-CSF administration is likely safe and well tolerated, it does not seem to be effective in improving LBRs in RIF patients. Routine use of G-CSF for RIF is not currently justified (Table 2).

Human chorionic gonadotropin

hCG is produced by the human embryo prior to implantation. The luteal phase endometrium expresses hCG receptors, and hCG modulates the endometrial expression of numerous cytokines and growth factors which directly impact endometrial decidualization and receptivity (Licht *et al.* 2003, Berndt *et al.* 2013, Bourdiec *et al.* 2013, Srivastava *et al.* 2013) as well as initiate early maternal–embryo crosstalk (Cameo *et al.* 2006). Many authors have also described its immunomodulatory role; hCG contributes to maternal tolerance of the embryo by enhancing recruitment, phenotypic differentiation and function of endometrial NK cells, dendritic cells (DCs) and T_{reg} (Fig. 3a) (Poloski *et al.* 2016, Zhang *et al.* 2016, Diao *et al.* 2017, Gong *et al.* 2017, Sauss *et al.* 2018). However, it is unclear if hCG exerts an immunomodulatory effect during implantation.

Recently, supplemental intrauterine application of hCG (IU-hCG) has been proposed to confer local endometrial benefits effects in infertile patients undergoing IVF-ET. Indeed, a recently published Cochrane meta-analysis summarized data from five RCTs reporting on LBRs (Mansour *et al.* 2011, Singh & Singh 2014, Aaleyasin *et al.* 2015, Wirleitner *et al.* 2015*a*,*b*), stratifying for embryo development stage. Infertile patients undergoing cleavage stage embryo transfer and receiving ≥500 U of IU-hCG prior to ET experienced a higher LBR in three RCTs (RR 1.57, 95% CI 1.32–1.87; N = 914; $I^2=0\%$; moderate-quality evidence). This was not observed in patients undergoing blastocyst transfer ($n=1666$) (Craciunas *et al.* 2018).

While the use of IU-hCG is still debated in infertile patients undergoing IVF-ET, it remains scantly studied in patients with RIF. In their systematic review and meta-analysis, Xie *et al.* extracted data from three RCTs and three cohort studies, each evaluating the use of IU-hCG in improving IVF-ET outcomes in patients with ≥2 IVF-ET failures. The LBR was reported in three studies (870 women) (Singh & Singh 2014, Volovsky *et al.* 2018, Liu *et al.* 2019) and was higher in the IU-hCG-treated group than in the non-hCG group (27.8 % vs 18.0 %; RR 1.52, 95 % CI 1.18–1.96, $P=0.001$). All studies administered hCG ≥ 500 units within 3 days prior to ET (Xie *et al.* 2019). Interestingly, Huang *et al.* published a prospective randomized study where 162 patients with ≥2 failed IVF-ET received 1000 units of IU-hCG ($n=62$) or IU-saline placebo ($n=49$) 3 days prior to their planned ET. Outcomes were compared to a separate cohort which did not receive any treatment ($n=50$). Ongoing pregnancy rates were similarly higher in both hCG and saline placebo groups compared to the control group. The authors hypothesized that local endometrial injury rather than hCG treatment explained the higher success rates in the treatment groups (Huang *et al.* 2017).

hCG plays an important role in improving endometrial function and IVF outcomes in fresh autologous cycles. Therefore, it is mechanistically attractive to infer that local hCG administration may provide similar benefits for patients with RIF, but the effectiveness of this approach remains to be demonstrated in a randomized controlled setting using appropriate controls. Like Huang *et al.*, such RCTs should incorporate a separate group receiving IU-placebo to determine if hCG itself or endometrial injury explains improved IVF-ET outcomes. Pending such studies, IU-hCG (1000 U) 10–30 min prior to embryo transfer may represent a low-risk and relatively inexpensive approach for improving IVF-ET outcomes in RIF patients or in patients undergoing cleavage stage embryo transfer (Table 2).

Anti-inflammatory strategies

In this section, we discuss strategies hypothesized to inhibit endometrial inflammation (Fig. 3b).

Aspirin

Aspirin is a ubiquitous anti-inflammatory drug with many clinical applications. It acts by inhibiting cyclooxygenase (COX) and consequently the production of pro-inflammatory cytokines, prostanoids and thromboxane. Aspirin has been shown to decrease local recruitment of neutrophils, monocytes and T cells, inhibit antigen presentation to DCs and potentially enhance T_{reg} (Hussain *et al.* 2012). LDA has recently been proposed to improve endometrial receptivity by enhancing endometrial and uterine blood flow, presumably improving endothelial function through its vasoactive and anti-platelet properties (Zhang *et al.* 2022) (Fig. 3b). However, it is unclear if LDA exerts immunomodulatory functions in the endometrium. Despite being frequently used as an IVF adjunct (Kumar & Mahajan 2013), the routine incorporation of pre- or peri-conception LDA into IVF protocols has not been shown to improve CPR) or LBRs compared to placebo in two meta-analyses (Dentali *et al.* 2012, Siristatidis *et al.* 2016). Of note, some cohort studies have recommended against the empiric use of LDA, arguing that suppressing prostanoid synthesis may hinder implantation (Check *et al.* 1998, Akhtar *et al.* 2013a); however, this is not supported by recent RCT data (Siristatidis *et al.* 2016, Madani *et al.* 2019).

The authors are unaware of any RCT examining the effectiveness of LDA in patients with RIF. In a 2021 publication by Zhang *et al.*, 190 patients with ≥2 IVF-ET failures demonstrated lower endometrial and uterine perfusion indices compared to 105 fertile controls. While LDA administration improved blood flow velocity in the infertile patients, this was not clinically correlated with IVF outcomes (Zhang *et al.* 2022). In addition to improving endometrial perfusion, LDA may also exert effects on the endometrial immune system. LDA inhibits COX-1 and modifies COX-2, inducing the production of anti-inflammatory lipoxins, resolvins and prostacyclins. These have vasodilator, anti-inflammatory and anti-platelet functions and may act on the innate and adaptive immune systems by decreasing leukocyte response to inflammation and by inducing T_{reg}. LDA is also thought to improve endometrial perfusion and improve progesterone resistance and possibly endometrial receptivity in patients undergoing IVF (Poorani *et al.* 2016, Pahan & Pahan 2019) (Fig. 3b).

LDA has not been associated with increased maternal or fetal side effects, even at doses up to 160 mg daily (Schisterman *et al.* 2014, Rolnik *et al.* 2017, Blomqvist *et al.* 2018, Levine *et al.* 2019). Empiric preconceptual LDA represents a low-risk and low-cost intervention for patients with RIF, provided LDA is started after oocyte collection to mitigate the risk of procedural bleeding (Table 2). Interestingly, recent evidence suggests that patients undergoing frozen embryo transfers (FET) have an increased risk of hypertensive disorders of pregnancy, possibly associated with the lack of corpus luteum (Singh *et al.* 2020, Wang *et al.* 2020). In the absence of contraindications, we believe that LDA should be considered for patients with RIF, especially if undergoing FET. However, further studies are needed to evaluate LDA's true clinical value for RIF.

Heparins

In addition to their anti-thrombotic role, unfractionated heparin and LMWH are thought to exhibit anti-inflammatory properties by preventing complement activation and neutralizing pro-inflammatory cytokines and chemokines, thus preventing the migration of leukocytes to areas of inflammation (Mulloy 2019, Bikdeli *et al.* 2020). Heparin fragments can bind to macrophages, endothelial cells as well as pro-angiogenic molecules VEGF and fibroblast growth factor 2) and are thought to improve implantation by stimulating angiogenesis (Li & Vlodavsky 2009), promoting tissue repair, enhancing endometrial receptivity and improving local blood flow (Tersigni *et al.* 2012) (Fig. 3a). However, it is unclear if heparins exert immunomodulatory properties in the endometrium. The benefit of LMWH would theoretically be most apparent when started prior to conception; this is feasible in an RIF population as time to conception is predictable. Timing LMWH administration with IVF-ET limits the length of LMWH usage and lowers the risk of bleeding, heparin-induced thrombocytopenia and thrombocytopenia (Akhtar *et al.* 2013b) while possibly enhancing its immune modulatory potential (Clark 2013).

In a 2013 meta-analysis, Potdar *et al.* analyzed three RCTs (Qublan *et al.* 2008, Urman *et al.* 2009, Berker *et al.* 2011) comparing outcomes in women with ≥3 failed IVF-ET treated with enoxaparin ($n=127$) to those receiving either placebo or no treatment ($n=118$). Enoxaparin started after oocyte collection or on the day of IVF-ET showed improved LBR (RR=1.79, 95% CI: 1.10–2.90, $P=0.02$) and a reduced miscarriage rate (RR= 0.22, 95% CI: 0.06–0.78, $P=0.02$) compared to control groups. However, one study (Qublan *et al.* 2008) included patients with RIF and thrombophilia. When analyzing data from women RIF without thrombophilia, the observed treatment benefit was no longer statistically significant (LBR: RR 1.36, 95% CI 0.82–2.26, $P=0.24$) (Potdar *et al.* 2013). These results were confirmed in a later meta-analysis by Yang *et al.* (Yang *et al.* 2018).

Of note, a 2011 retrospective review of patients with ≥2 IVF-ET failures suggested a higher LBR per cycle in patients treated with LMWH compared to placebo (29.53% (17/57) vs 17.19% (88/512); $P=0.006$), the effect being more notable in women over 36 years of age (35.71% (10/28) vs 15.53% (50/322); $P=0.007$) (Lodigiani *et al.* 2011). However, a later subgroup analysis of an RCT led by the same authors found no benefit of Parnaparin 4250 IU or 6400 IU started prior to conception vs placebo in 44 patients with RIF, including those over 36 years of age ($n=31$) (Lodigiani *et al.* 2017), but this study was underpowered as target patient enrollment was not reached. Unfortunately, several other studies evaluating the use of adjuvant heparin or LMWH in patients with ≥2 IVF-ET failures could not be included in this analysis because they failed to evaluate LMWH exclusively (Siristatidis *et al.* 2018, Sung *et al.* 2021), failed to report appropriate outcomes (Hamdi *et al.* 2015, Tormene *et al.* 2015) or did not include a control group (Grandone *et al.* 2014).

Systematic preconception use of LMWH in patients with RIF is mechanistically attractive and prophylactic doses of LMWH have a favorable side effect profile. Unfortunately, studies have been disappointing thus far. While it is possible that certain RIF patient subgroups may benefit from LMWH, such as those with thrombophilia (Qublan *et al.* 2008) or advanced maternal age (Lodigiani *et al.* 2011), available data are scant. Indeed, thrombophilia workup in patients with RIF remains controversial (Clark 2013), and the effect of maternal age on the function of the endometrium is largely unknown. Since both thrombophilia and advanced maternal age are associated with an increased risk of thrombosis during pregnancy (Croles *et al.* 2017, Sheen *et al.* 2018), it is conceivable that an increased tendency to produce micro-thrombosis, defective spiral artery remodeling or vasospasm at the site of implantation could lead to RIF. Therefore, the clinical utility of LMWH in these patient populations merits further investigation (Table 2). Awaiting these studies, a prophylactic dose of LMWH started on the day of ET and continued until 12 weeks of gestation with monthly assessment of platelet levels can be considered in select RIF patients, such as those with advanced maternal age and thrombophilia.

Last, it has been hypothesized that the combination of LDA and heparin may be more effective to improve outcomes in RIF patients by better preventing thrombosis and inflammation of decidual vessels, reducing oxidative stress and enhancing the production of anti-apoptotic proteins than either intervention alone (Johnson *et al.* 1997, Lea *et al.* 1997). While there is no evidence that combination therapy enhances live birth in subfertile patients having failed ≥1 IVF-ET (Akhtar *et al.* 2013a) or in patients with RIF (≥10 failed IVF-ET) and serologic positivity for antinuclear and/or anti-phospholipid antibody (Stern *et al.* 2003), the authors are unaware of appropriately powered and controlled trials in uRIF patients.

Corticosteroids

Glucocorticoids act as broad immune suppressors and have potent anti-inflammatory effects; as such, they have been used to treat patients with suspected immune mediated reproductive failure for over 25 years. They act by modifying leukocyte gene expression and decrease leukocyte production of pro-inflammatory cytokines as well as their ability to migrate to areas of inflammation. GCs also have been shown to induce apoptosis of activated CD4 T cells (Krigstein & Sacks 2012) (Fig. 3b). However, normal implantation and decidualization are inflammatory events, mediated by innate leucocytes which are recruited to the endometrium during the menstrual cycle. These leukocytes differentiate locally to aid with endometrial receptivity, angiogenesis and embryo recognition (Negishi *et al.* 2018). Thus, it is unsurprising that GC use for unselected patients with RIF has been relatively ineffective. Furthermore, GCs have a myriad of side effects and must be used with caution during pregnancy. Patients need to be monitored for the development of gestational diabetes (Leung *et al.* 2015) and hypertension (Ponticelli & Moroni 2015); prolonged GC use during pregnancy has been associated with an increased incidence of pre-term birth (Laskin *et al.* 1997) and case reports of fetal adrenal suppression (Kurtoglu *et al.* 2011).

While routine pre-conception GC use is not supported for women undergoing IVF (Boomsma *et al.* 2012), including low dose (Moffitt *et al.* 1995, Ubaldi *et al.* 2002, Duvan *et al.* 2006, Revelli *et al.* 2008) or high dose GC (Polak de Fried *et al.* 1993, Lee *et al.* 1994, Moffitt *et al.* 1995), small case series have suggested that GC may improve pregnancy rates in patients with autoantibodies undergoing IVF (Ando *et al.* 1996, Hasegawa *et al.* 1998, Zhu *et al.* 2013) or in patients with a history of RIF (Lee *et al.* 1994, Geva *et al.* 2000).

In patients with RIF, several cohort and controlled cohort studies have assessed the clinical utility of preconceptual GC. Forges *et al.* studied 211 patients with ≥2 IVF-ET failures who had positive anti-ovarian antibodies. Patients were treated with prednisolone 0.5 mg/kg preconceptually, and outcomes following GC therapy were compared to each patient's previous failed cycle. GC therapy resulted in a significant increase in clinical pregnancies (38% vs 14%, $P=0.0001$) and live births (26% vs 0%, $P=<0.0001$) vs no treatment (Forges *et al.* 2006); but one must question the validity of a study with an LBR of 0% in one arm. Mottaram *et al.* performed a subgroup analysis in a large matched-case study and did not find that adjuvant treatment with doxycycline, aspirin and prednisolone 25 mg improved the LBR over standard treatment in patients with ≥3 IVF-ET failures (57/175 (32.6%) vs 54/155 (34.8%)) (Motteram *et al.* 2015). Similarly, in a retrospective cohort study, Siristadis *et al.* did not find that treatment with LMWH and prednisolone (dose not specified) improved the LBR over standard treatment in patients with ≥2 previous IVF-ET failures (15/57 (26.3%) vs 9/58 (15.5%), $P= 0.152$) (Siristatidis *et al.* 2018). Currently, a large RCT is underway to evaluate the clinical utility of preconceptual prednisone use in patients with RIF (Lu *et al.* 2020).

Preconceptual GC administration may be useful for a subset of patients with RIF. However, appropriate patient selection remains challenging, and there are no agreed-upon characteristics or biomarkers to identify whom may benefit most (Ledee *et al.* 2018). The administration of GCs during pregnancy must also be weighed against the plethora of maternal and fetal side effects associated with GC use. Administering a low-to-moderate dose (equivalent ≤20 mg of prednisone/day) prior to IVF-ET and for <14 days in women with RIF and without metabolic risk factors may mitigate some of the risks associated with GC therapy (Table 2). However, there is insufficient evidence thus far that this approach improves pregnancy outcomes.

Intralipid

Intralipid is a fat emulsion used as a lipid and calorie source for patients requiring total parenteral nutrition (TPN). While intralipid infusions are well tolerated, it's safety has not been established in an obstetric population. Intralipids can bind to peroxisome proliferator- activated receptors (PPARs) on macrophages and DCdendritic cells, preventing pro-inflammatory cytokine secretion and antigen presentation to CD-1- restricted T cells respectively; intralipids are also thought to suppress natural killer (NK) cell cytotoxicity (Ota et al. 1985, Mayer et al. 2003, Khan & Vanden Heuvel 2003, Roussev et al. 2007, Coulam 2021). However, the immunomodulatory

properties of intralipid are still debated, and the clinical impact and mechanism remains to be defined (Shreeve & Sadek 2012) and areis not further detailed in Fig. 3b. Interest in Intralipid treatment for RIF is ex,trapolated from the recurrent pregnancy loss (RPL) literature, where it is used as a controversial adjunct in patients with suspected immune- mediated miscarriages (Genest et al. 2022). As in RPL, RIF studies are mixed in terms of intralipid efficacy (Coulam 2021).

Al-Zebeidi *et al*. published results of a matched RCT comparing iIntralipid to placebo in 142 women with ≥3 failed IVF-–ICSIhelp_outline cycles. The LBR was similar in both groups (13/71 (18.3%) vs 10/71 (14.1%); OR: 1.37, 95% CI: 0.55-3.55–3.36, $P=0.49$). Adverse events were not recorded (Al-Zebeidi *et al*. 2020). Similarly, Singh et al. randomized 105 women with secondary infertility and ≥ 1 IVF-ET failure to receive iIntralipids ($n=52$) or placebo ($n=50$). The LBR was higher in the iIntralipid group (18/52 (34.6%) vs 7/50 (14%), RR: 2.5; 95% CI: 1.13-5.13–5.4, $P=0.023$); however, these patients did not meet RIF criteria, and more patients in the intralipid group had day 5 blastocyst transfers then in the placebo group (OR: 3.66, 95% CI: 1.04-12.04–12.84). Of note, one congenital anomaly (congenital diaphragmatic hernia), 2 two cases of gestational hypertension, 2 two cases of gestational diabetes and one patient with severe preeclampsia were recorded in the Intralipid group. One patient in the control group delivered twins prematurely, both of which later died of sepsis (Singh et al. 2019).

In a recent meta-analysis of four studies including two unpublished conference abstracts (El-Khayat & El Sadek 2015, Gamaleldin *et al.* 2018, Singh *et al.* 2019, Al-Zebeidi *et al.* 2020), Zhou (Zhou *et al.* 2020) *et al.* concluded that only the Singh *et al*. study (Singh *et al.* 2019) appeared to be of 'adequate quality and with low risk of bias'; however, this study did not include women with only RIF and may not be comparable to the three others in terms of populations studied. In the pooled analysis of all studies, there was a significant increased LBR in the women receiving intralipids (RR: 1.98, 95% CI: 1.39–2.80); however, excluding both unpublished abstracts, the LBR was not statistically significant (RR: 1.66, 95% CI: (0.9–3.08), high heterogeneity) (Zhou *et al.* 2020). Of concern, in an abstract publication, Gamaleldin *et al.* randomized 97 patients with RIF to receive intralipids or placebo, and while the LBR was similar in both groups, three congenital malformations occurred in the intralipid group while none were recorded in the control group (Gamaleldin *et al.* 2018). Most recently, Erlich *et al.* retrospectively described the outcomes of 93 women with RIF treated with intralipids compared to an age-matched control group of 651 women undergoing IVF. While the LBR in both groups was similar, it is unclear if the age-matched controls were also matched for clinical characteristics (Ehrlich *et al.* 2019).

Intralipid is a popular adjunct to IVF and is offered by many clinics (Coulam 2021), but there is no convincing evidence that it improves outcomes in patients with RIF. While intralipid success may be contingent on patient selection (for example, patients with high peripheral NK cell activity or with endometrial immune activation) (Lédée *et al.* 2018, Coulam 2021), this remains to be validated in a well-powered RCT using appropriate controls. Furthermore, intralipid is safe in patients with a legitimate medical indication for it, but its safety during pregnancy has not been ascertained. Indeed, one RCT (Singh *et al.* 2019) reported more adverse pregnancy outcomes and congenital anomalies in the intralipid group. These undesirable outcomes may be due to chance alone, but before offering intralipids on a wide scale for patients with RIF, larger studies are required to assess the safety of intralipid treatment prior to conception and during pregnancy.

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG is a plasma-derived product which contains polyclonal immunoglobulin G. IVIG acts by multiple mechanisms to dampen immune responses, many of which could improve pregnancy outcomes in patients with immune-mediated RIF. Indeed, IVIG exerts direct immunomodulatory effects through the neutralization of pro-inflammatory cytokines, chemokines, pathogenic autoantibodies and complement. IVIG indirectly increases the activation threshold of macrophages, possibly inducing polarization to M2 macrophages, and induces tolerogenic DCs which enhance T_{reg} cell responses (Gelfand 2012) (Fig. 1). However, the exact mechanism(s) by which IVIG improves reproductive outcomes is RIF patients is unknown. IVIg is safe and well tolerated during pregnancy, and maternal side effects occur at the same frequency as in the general population (Caress *et al.* 2010, Brinker & Silk 2012, Feldman & Whitington 2013, Katz-Agranov *et al.* 2015, Pacheco *et al.* 2016).

Unlike RPL, there are few studies evaluating the efficacy of IVIG for RIF patients selected based on clinical criteria alone; most RCTs have recruited patients with peripheral blood immune anomalies. De Placido *et al.* randomized 39 patients with ≥2 early miscarriages or ≥3 failed IVF-ET to receive either IVIG (18 patients) or placebo (21 patients) prior to embryo transfer. Patient characteristics were not detailed otherwise. CPRs and miscarriage rates were similar between groups, but LBR or pregnancy outcomes were not assessed (De Placido *et al.* 1994). Similarly, Coulam *et al.* administered IVIG to 29 women with RIF, defined as no implantation after transfer of ≥12 embryos. Two subgroups were compared: Group I were effective embryo producers (≥50% fertilization rate and/or production of ≥3 embryos) while Group II were not. In Group I, 9/16 (56%) women conceived and 7/16 (44%) had a live birth, while in Group II, 1/13 (8%) of women conceived ($P=0.02$) and none had a live birth ($P=0.0002$). Despite there not being an adequate control group, this study suggests that IVIG may be effective in women with RIF and adequate ovarian reserve with otherwise poor IVF prognosis (Coulam *et al.* 1994).

The largest prospective RCT to date was conducted by Stephenson *et al.* In this study, 51 women with ≥2 failed IVF-ET were randomized to receive IVIG 0.5g/kg within 72 h prior to embryo transfer or normal saline. Both groups were well matched in terms of maternal characteristics, but despite randomization, the saline group had more unexplained infertility (48%, 12/25) than the IVIG group (11.5%, 3/26, $P=0.004$). LBRs were similar in both groups (4/26 (15%) in the IVIG group vs 3/25 (12%) in the saline group, $P=0.52$). Importantly, this study was powered to detect a large effect of IVIG over placebo (45% live birth with IVIG vs 15%

with placebo), and the recruitment target was not reached (Stephenson & Fluker 2000).

Thereafter, multiple studies were published evaluating the use of IVIG in patients with measurable peripheral immune anomalies ranging from positive autoantibody to high NK cell levels or abnormal Th1/Th2 ratios. Li *et al.* performed a meta-analysis of 10 studies including 8207 participants. The studies included were very heterogenous; seven were placebo controlled, three compared IVIG to no treatment, and two started IVIG after pregnancy diagnosis. IVIG protocols varied widely, so did the use of other adjunctive therapies such as tumor necrosis factor inhibitors, LMWH and LDA. Patient selection was also very heterogenous; only four studies recruited patients with RIF and immune anomalies (Coulam & Goodman 2000, Heilmann *et al.* 2010, Moraru *et al.* 2012, Virro *et al.* 2012), the four others recruited patients with immune anomalies undergoing IVF without a history of RIF (Sher *et al.* 1998, Winger & Reed 2008, Winger *et al.* 2011) and two studies included patients with RIF without immunological anomalies (De Placido *et al.* 1994, Stephenson & Fluker 2000). Using the random effects model, Li *et al.* concluded that IVIG was effective in patients with RIF. LBRs were 406/816 (49.8%) with IVIg compared to 506/1599 (31.6%) without IVIg (pooled RR: 1.616 (1.243–2.101), I^2 58.2, $P=0.014$). However, these results must be interpreted with caution as study populations were heterogenous, and IVIG dose, timing of administration and administration with other immunomodulatory drugs differed between studies (Li *et al.* 2013).

Finally, Abdolmohammadi performed a systematic review to evaluate the effectiveness of IVIg in improving LBR in patients with ≥3 failed IVF-ET and high NK cells/cytotoxicity or high Th1/Th2 ratios. Of the four studies cited, two were retrospective cohort studies (Moraru *et al.* 2012, Chernyshov *et al.* 2016) and two were cross-sectional studies (Heilmann *et al.* 2010, Ramos-Medina *et al.* 2014); all studies included a matched control group that did not receive IVIG and all administered IVIG prior to embryo transfer at a dose of 0.2–0.5g/kg. When pooling data from both cohorts, the LBR was significantly higher in the IVIG-treated patients (OR = 2.17, 95% CI = 1.30–3.61, $P = 0.003$); however, studies were heterogenous ($I^2 = 90\%$) (Abdolmohammadi-Vahid *et al.* 2019).

It should be noted that IVIG is an expensive produce that is dependent on a ready supply of plasma. Especially in the era of COVID, where blood product shortages are a reality and resource allocation is an important consideration, IVIg may not be widely available for patients with RIF. With this is mind, IVIg therapy should be used as a last resort in well-selected RIF patients or used within a controlled research protocol. We recommend a single dose of IVIG 0.4–0.6g/kg 3–5 days before embryo transfer in patients <40 years old with unexplained good-quality () blastocyst transfer failures despite adequate endometrial preparation. Pre-IVIg serologies (hepatitis B and C, CMV, parvovirus and toxoplasmosis) should be obtained prior to infusion as first-trimester serologies are unreliable up to 3 months post IVIg infusion.

Concluding remarks

uRIF is a frustrating condition to treat for clinicians, especially when faced with a psychologically vulnerable population with high expectations. Immune dysregulation at implantation likely explains a subset of cases of uRIF, and immunomodulatory therapy is probably effective for some patients but remains controversial because of study heterogeneity and patient selection. An important limitation in the field of reproductive immunology is the inability to confirm that immunomodulatory therapy reestablishes normal endometrial physiology or corrects a presumed immune anomaly when it is successful. Until this is feasible, it will be difficult to determine which (if any) treatment is truly clinically effective.

Rapid development is occurring in the field of reproductive immunology, and we are progressively overcoming traditional barriers for the study of immunomodulatory treatments for RIF. Indeed, advances in our understanding of molecular mechanisms underlying immune-mediated RIF (IM-RIF) (Franasiak *et al.* 2021) are leading to novel candidate diagnostic biomarkers (Diaz-Hernandez *et al.* 2021, Piekarska *et al.* 2021) and therapeutic targets (Sadeghpour *et al.* 2020, Comins-Boo *et al.* 2021), as well as new treatments such as stem cell therapy (Saha *et al.* 2021). While efforts are being made to harmonize the definition of RIF (Cakiroglu & Tiras 2020), peripheral blood and endometrial immune tests are being developed and validated. Indeed, studies in which patients are selected on the basis of true RIF (Wang *et al.* 2021a) or abnormal peripheral blood or endometrial immune parameters (Kolanska *et al.* 2021) show overall better results with immunomodulation. Last, the immune adaptation required for successful implantation is complex and IM-RIF is likely multifactorial; one intervention alone may not improve reproductive outcomes. Interestingly, some studies propose that combination treatments, tailored to patient's laboratory work (Sung *et al.* 2021) or endometrial immune profile (Ledee *et al.* 2020a), successfully improve LBR in RIF patients. This potential shift toward precision personalized medicine offers hope for better diagnostic test development and better patient selection for future studies. Ultimately, it is conceivable that immune testing will be offered preemptively to all patients as part of their standard infertility workup to better tailor future treatments.

Despite the large strides made in the field in the last two decades, further research is required before the widespread application of individualized immunotherapy is feasible. As a scientific community, we must focus our energy on multicenter collaborative studies implicating both physicians and basic scientists to understand immune mechanisms involved in implantation and identify interventions best suited to a specific disease entity. Clinically, patient selection, definition of RIF and metrics used to describe patients must be harmonized to improve study homogeneity for future comparative analysis; this is paramount in our field since most RCT are small and often do

not reach statistical significance. Ideally, all patients receiving immunomodulatory therapy should be included in specific registries which would permit periodic practice audits, rapid identification of effective/ineffective therapies as well as therapy side effects or adverse maternal/fetal outcomes. From a basic science perspective, characterizing the endometrium during the WOI in both healthy and infertile patients with newly available technologies such as mass cytometry and single-cell RNA sequencing may shed new light onto normal physiology or pathological decidual immune responses. Furthermore, peripheral blood immune markers are routinely being used to diagnose and treat patients. These assays need to be validated and reproducible, and correlated with reproductive outcomes in fertile controls and in patients with 'explained' RIF. Further studies are also needed to determine if peripheral blood markers reflect endometrial immune physiology and whether these tests are clinically useful. As clinicians, we must remain aware of the fact that most therapies are used off-label with very little current scientific evidence of benefit. Patients must understand this and be adequately counseled on, and followed for, potential risks associated with each intervention to ensure free and informed consent as well as patient safety during treatment.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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GG: Conceived the review, coordinated co-author's contributions, contributed to the writing and editing of each section of the paper; SB Contributed to literature review and writing of most sections on immunotherapy, conception of the figures; WA: Contributed to the literature review and conception of the figures; CB: Contributed to writing the introduction and editing the document; JB: Contributed to unifying the definition of recurrent implantation failure and editing of the document; WB: Aided in the conception of the review and in the selection of papers to include; FD: Contributed to the sections on PBMCs and IVIg; PG: Contributed to the section of IVIg and editing of the document; MG: Contributed to the sections on aspirin and heparin; WJ: Contributed to the section on intralipids; IK: Contributed to the section on glucocorticoids; EK: Reviewed referenced articles to ensure statistical methods were sound for inclusion; LL: Contributed to the section on G-CSF; PM: Contributed to the section on PBMC; TS: Conceived and contributed the summary tables; CS: Contributed to the section on G-CSF and hCG; TT: Contributed to the section on hCG and review and editing of the paper; BDM: Contributed to the section on IVIg; CAL: Reviewed and edited the paper; NM: Reviewed and edited the paper.

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