Chapter

Recent Advances in Immunotherapeutic Approaches for Recurrent Reproductive Failure

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Abstract

Human reproduction is an insufficient process, disturbed by various factors, such as immunologic aberrations of mother. Immunologic abnormalities, including cellular and humoral immunity imbalance, cause dysregulated immune responses against embryo, fetus, and associated components and lack of maternal immunotolerance, which compromise the maintenance of pregnancy. Therefore, evaluation of immunologic parameters, including cellular and humoral immunity assessment (T and B lymphocyte, T helper subtypes, NK cells, cytokines, and autoantibodies), especially in women with a history of pregnancy loss or implantation failure, would help clinicians to manage the disorder and prevent next unfavorable pregnancy outcomes. Moreover, several immunomodulatory approaches have been introduced to modulate the abnormal immunologic responses in patients who experience reproduction failure, especially those diagnosed with immunologic basis. Anticoagulants, corticosteroids, intravenous immunoglobulin, immunosuppressive medications used in inhibition of graft rejection, such as calcineurin inhibitors, recombinant cytokines, and cell therapy approaches, are among these modalities. Here, we discuss the proposed mechanisms of immunologic abnormalities involved in the etiopathogenesis of reproduction disorders, besides the suggested immunologic tests and immunotherapeutic approaches which may be helpful in management of these disorders.

Keywords: reproductive immunology, immunotherapy, recurrent pregnancy loss, repeated implantation failure

1. Introduction

Human reproduction is an incompetent process, as about 70% of conceptions is lost before the first trimester [1]. Approximately, 85% of pregnancy losses are related to failure in implantation or losses prior to clinical diagnosis of pregnancy and only 15% of pregnancy losses are related to clinical miscarriages [2].

Recurrent pregnancy loss (RPL), also known as recurrent miscarriages (RM) and recurrent spontaneous abortion (RSA) or habitual abortion, alongside repeated or recurrent implantation failures (RIF), are among the reproductive disorders,

which are included in a broad term called recurrent reproductive failure (RRF) [3]. According to the updated guidelines, including American Society of Reproductive Medicine (ASRM, 2012) and European Society of Human Reproduction and Embryology (ESHRE, 2017) guidelines, RPL is determined as two or more pregnancy losses [4–6]. However, it is determined as three or more consecutive pregnancy losses before the 20th week of gestation, by world health organization (WHO) [7]. As most of the losses happen earlier than clinically recognized or the first missed period, it is difficult to estimate the accurate incidence of RPL. However, it is estimated that RPL accounts for 12–15% of all pregnancies [8]. RPL is divided into two categories, including primary RPL and secondary RPL. Series of pregnancy losses without a previous successful birth is called primary RPL, while a series of pregnancy losses followed by a previous live birth is known as secondary RPL [9].

RIF is also a distressing condition for young couples and an obstacle for human reproduction. Embryo implantation is a critical step in human reproduction. The "window of implantation" is a short and delicately regulated time in which the endometrium is ready for embryo penetration and attachment [10]. A failure in the embryo and the endometrium cross-talk may compromise the embryo attachment and cause implantation failure. In spite of increasing application of assisted reproductive technology (ART) and in vitro fertilization (IVF) still about 10% of couples experience unfavorable outcomes [11]. There are multiple definitions for RIF, based on number of transferred embryos [3–10], unsuccessful IVF cycles (2–6 cycles; the most common, 3 IVF cycles) [12] or both [13]. However, the preimplantation genetic diagnosis (PGD) consortium of ESHRE, defines RIF as >3 failed high quality embryo transfers (ETs) [11, 14].

The exact pathogenesis of RIF and RPL has yet to be understood. However, there are several heterogeneous risk factors, including chromosomal and anatomical abnormalities, infections, endocrine disorders, thrombophilia, and lifestyle. [15]. Nevertheless, the etiology of almost 50% of RRFs remains unclear and may be related to maternal immune system abnormalities [16]. Considering the embryo or fetus as a semi-allograft, pregnancy shares similar properties with allogeneic transplantation [17]. In order to survive in a hostile microenvironment, fetus antigens must be recognized and tolerized by maternal immune system. Any abnormalities in the regulatory mechanisms of immune system, which are responsible for establishment of maternal tolerance, may compromise the maintenance of the pregnancy [18]. Here we discuss the different aspects of immune system, which contribute to the pathogenesis of reproductive failure. Immuno-etiology of RRF is summarized in **Figure 1**.

1.1 Cellular immunity

There are solid evidence about the contribution of T cell subsets and their balance in the process of pregnancy, especially the balance between T helper type1 (Th1) and Th2 cells. Th17 and regulatory T (Treg) cells are the other critical population [19]. Embryo implantation requires an aseptic inflammation, created by a shift toward Th1-like cells and cytokines, in the first trimester [3]. Following the implantation, predomination of Th2 responses is required for protection of fetus and balancing the Th1 responses [19]. Th1 associated cytokines, such as interferon-ɣ (IFNɣ) and tumor necrosis factor-α (TNFα), adversely affects the pregnancy, inducing inflammation and thrombotic events in blood vessels of uterus, while Th2 associated cytokines, such as interleukin-4 (IL-4) and IL-10, are known to suppress Th1 immunity and cytokines [16].

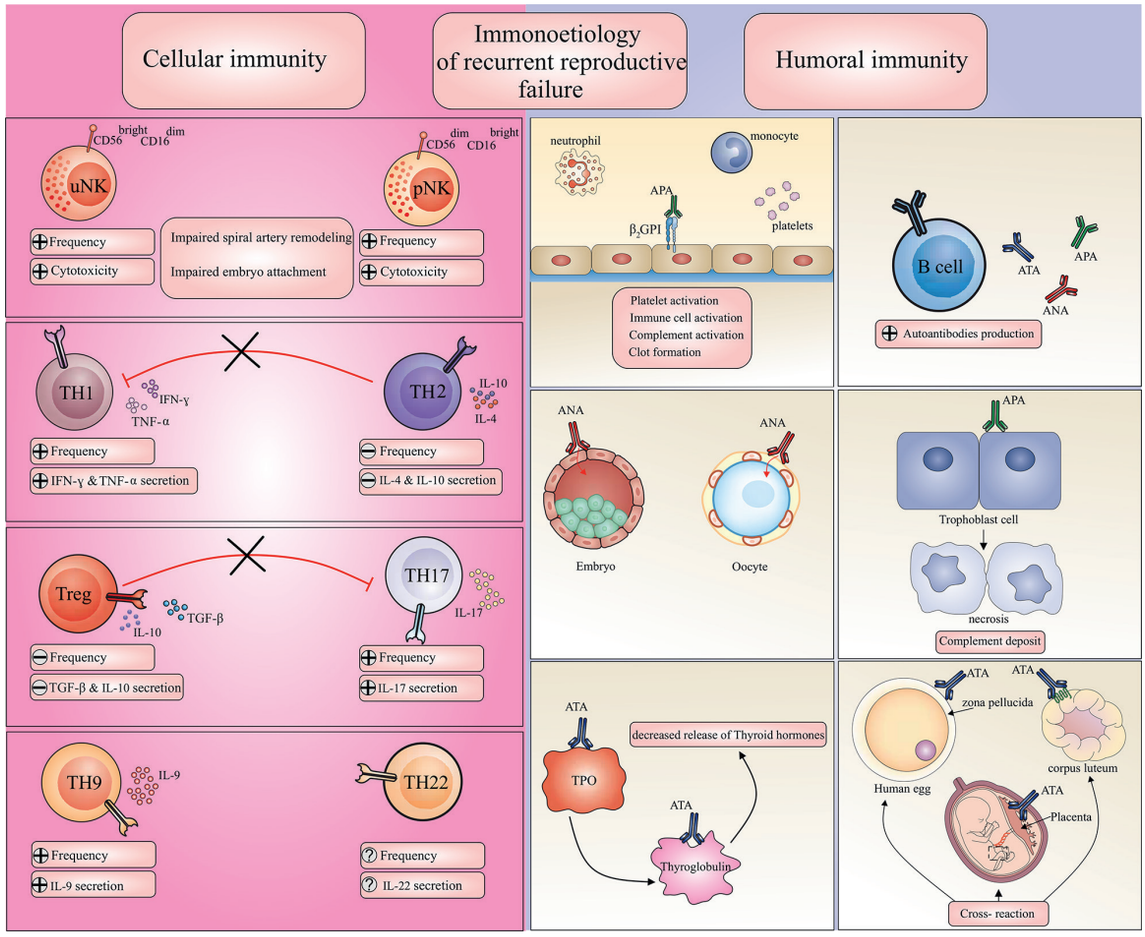

Figure 1.
Immuno-etiology of recurrent reproductive failure. Abnormalities of cellular and humoral immunity are both involved in the pathogenesis of RRF. Cellular immune system abnormalities which are involved in the pathogenesis of RIF and RPL include elevated frequency and cytotoxicity of both uNK and pNK cells, along with upregulation of Th1, Th17, and Th9 cells and associated cytokines. In contrast, immunoregulatory arm is weakened because of reduced frequency and cytokine secretion of Th2 and Treg cells. On the other side, over-production of autoantibodies such as APA, ANA, ACA, and ATA by B lymphocytes in dysregulated humoral immunity, is involved in the pathogenesis of RRF by various mechanisms including cross-reaction with oocyte, placenta, and other vital antigens for human reproduction, inducing thyroid dysfunction, clot formation and necrosis of trophoblast cells. Abbreviation: uNK: uterine natural killer; pNK: peripheral natural killer; TH: T helper; Treg: T regulatory; TNFα: tumor necrosis factor α; IFNγ: interferon γ; IL-4: interleukin-4; IL-10: interleukin-10; IL-17: interleukin-17; TGFβ: Transforming growth factor beta; TPO: thyroid peroxidase; ATA: anti-thyroid antibody; APA: anti-phospholipid antibody; ANA: antinuclear antibody; ACA: anti-cardiolipin antibody; β2GPI: Beta-2-Glycoprotein I.

On the other hand, $CD4^+CD25^+FoxP3^+$ Treg cells play a pivotal role in establishment of maternal immunotolerance toward fetus [20]. Treg cells suppression is mediated through secretion of immunosuppressive cytokines, such as IL-10 and transforming growth factor (TGF-β), or by cell–cell contacts [21]. The frequency of peripheral Treg cells is upregulated in implantation. Following the implantation, Treg cells frequency reaches the highest level in second trimester and decreases after delivery [22]. According to the literature, the frequency of peripheral and uterine Treg cells is downregulated in RPL and RIF women, compared with control group [23–25]. In contrast, Th17 population is upregulated in the decidua and peripheral blood pregnancy complication, besides elevated Th17/Treg ratio [26]. It has been also reported that activation of decidual NK (dNK) is induced by Th17 cells that contribute to vascular dysfunction and embryo resorption [27]. Infertile women exhibited an increased ratio of Th17/$CD4^+$ Treg cell, when compared to normal fertile controls [28]. Therefore,

Th17/Treg cells ratio has the potential to be a biomarker in women with a great risk of reproductive failure.

Another type of immunologic cells, which are involved in trophoblast invasion and vascular remodeling, are natural killer (NK) cells. A population of NK cells, known as uterine NK (uNK) cells ($CD56^{bright}CD16^{dim}$), are the predominant population of mucosa of uterine, which represent 70% of population of leukocyte in feto-maternal interface [29]. UNK cells differ from peripheral NK (pNK) cells ($CD56^{dim}CD16^{bright}$) and present a strong immunomodulatory activity and less cytotoxicity, in comparison with pNK cells [30]. In spite of confirmed involvement of uNK cells in the pathogenesis of reproduction failure, a majority of studies also highlight the contribution of pNK cells in these complications [30, 31]. In addition to the significant impact of increased frequency of uNK cells in pathophysiology of RPL [32], it has been confirmed that elevated frequency and cytotoxicity of pNK cells also contribute to implantation failure and miscarriage [33].

There are only a small number of studies evaluating the role of Th9 and Th22 cells in pathogenesis of reproductive complications. Th9 cells are a subpopulation of Th2 cells, with different functions and phenotype, which produce IL-9. Th9 cells are involved in anti-tumor immunity and pathogenesis of immune-mediated disorders [19]. According to animal experiments, production of IL-9 increases in the pregnancy and exhibits a regulatory role for inflammatory responses which compromise the maintenance of pregnancy. The decreased proportion of decidual Th9 and Treg cell has been confirmed to be related to parturition in mice [34]. IL-22, which is mainly produced by Th22 cells, is involved in promotion of trophoblast cells proliferation, as well as viability. Furthermore, protection of trophoblast cells from pathogens and infiltrated immune cells is mediated by IL-22 [35]. There are limited and conflicting data about the role of Th22 and IL-22 in pregnancy complications. It has been reported that RPL women have a decreased expression of IL-22 receptor, in comparison with control [36]. In the other hand, there are reports about the increased amount of IL-22 of sera in RPL women [37], in contrast, lower gene expression of IL-22 was detected in decidua of these patients [38]. Further studies are required to understand whether IL-22 expression is associated with RPL.

1.2 Humoral immunity

It has been confirmed that some auto-antibodies also contribute to the pathogenesis of RIF and RPL, such as anti-phospholipid antibodies (APAs), anti-nuclear antibodies (ANAs), and anti-thyroid antibodies (ATAs) [39]. Presence of these autoantibodies, regardless of presence of an autoimmune disorder, has been correlated with reproduction failures [40].

Antiphospholipid syndrome (APS), an autoimmune thrombophilia, is associated with the presence of anti-cardiolipin antibodies (ACAs), lupus anticoagulant (LA), and anti-β2-glycoprotein-1 (β2GPI) antibodies. ACAs recognize cardiolipin, a phospholipid of cell membranes, and are the most common antibodies of APS [41]. β2GPI is a cardiolipin-binding factor, which is recognized with anti-β2GPI antibodies, and LA includes various types of autoantibodies [42]. There are accumulated evidence of a direct interaction between serum positivity for APAs and pregnancy complications [42]. The risk of pregnancy wastage increases with higher antibody positivity, as triple positive women experience more pregnancy complications in comparison with double-positive women [41]. Indeed, after recognition of antigens, such as β2GPI, by associated antibody, an intra-placental coagulation-mediated thrombosis may take

place, leading in poor pregnancy outcomes [43]. It has been suggested that anti-β2GPI antibodies are capable of recognizing antigenic determinants on trophoblast, stromal decidual, and endometrial endothelial cells [44]. This antibody–antigen reaction would prevent the invasion of trophoblast, inducing apoptosis of trophoblast cells by complement-mediated reactions and recruitment of immune cells, such as neutrophils and monocytes; additionally, a pro-inflammatory microenvironment is created by production of inflammatory products, such as TNF-α, reactive oxygen species (ROS), and chemokines [44, 45]. Lately, there are conflicting data about the contribution of some non-conventional APAs, including antibodies that recognizes prothrombin, phosphatidylethanolamine, and annexin V, in the pathogenesis of obstetric complications [46, 47]; however further investigations are required to confirm the involvement of these APAs in pathogenesis of RIF and RPL.

ANAs, targeting the determinants of cytoplasm and nucleus, are detected in rheumatic and autoimmune diseases, such as systemic lupus erythematosus. In spite of conflicts, it has been reported that increased prevalence of ANA is associated with adverse pregnancy outcomes such as RPL [48] and RIF [49]. A recent meta-analysis confirmed the positive correlation between the presence of ANAs and higher risk for RPL and highlighted the importance of screening test for ANA in women with RPL risk [50]. The exact mechanism of ANAs is not fully understood; however, it is estimated that the presence of ANA adversely affects the quality and development of embryo by inducing the immune complex, which deposits in placental tissue and activates complement cascade [41, 51].

Anti-thyroglobulin (TGAb), anti-thyroid peroxidase (TPOAb), and anti-thyroid stimulating hormone (TSH) receptor (TRAb) antibodies are ATAs found in thyroid autoimmunity (TAI) and recognize antigenic determinants of thyroglobulin, thyroid peroxidase, and TSH receptors (TSHR), respectively [52]. Attachment of these antibodies to associated antigens may disturb the production, secretion, and function of thyroid hormones. Furthermore, ATAs are capable of passing the placental barrier, which enables them to impair the development of the fetus [53]. It has been confirmed that ATAs-positive women, especially positive for TGAbs and TPOAbs, are more prone to pregnancy adverse outcomes. Presence of ATAs increases the risk of miscarriage three times higher, according to the results of a meta-analysis [54]. On the other hand, prevalence of ATAs is also higher in RPL women [41]. Furthermore, ATAs-positive women experience impaired oocyte quality, lower grade A embryos, and implantation rate, in comparison with healthy controls [55]. Sometimes, in spite of overall euthyroidism, ATAs are capable of inducing a slight deficiency in thyroid hormones, which impairs embryo development after implantation [56]. The exact mechanism of ATAs' action in the pathogenesis of obstetric complications has yet to be elucidated. However, there are some suggested mechanisms including dysfunction of thyroid, cross-reaction of ATAs with extra-thymic antigens, such as placenta, zona pellucida, follicular fluid antigens, human chorionic gonadotropin receptors (hCGR), and formation of immune complexes [41, 57, 58]. In addition, the presence of ATAs is a sign of a generalized immune abnormality including abnormal frequency and function of T cell subsets, B lymphocytes, NK cells, and subsequent abnormal cytokines production [59, 60].

Celiac disease is the other disorder that increases the risk of RPL in untreated patients. Celiac is an autoimmune enteropathy of gluten-sensitive susceptible individuals, which involves the mucosa of small intestine. Prevalence of celiac is 1% in general population, while it increases to 2.7% in infertile women. Indeed, celiac women have higher risk for recurrent miscarriage, premature birth, or decreased fetal growth,

compared with healthy women [61, 62]. A meta-analysis also indicated a higher risk for celiac in RPL women, when compared to the general population [63]. Anti-transglutaminase and anti-endomysial antibodies are the serum markers of celiac patients, which are recommended to be screened in women with risk of reproductive failure [64]. According to a relevant study, in celiac women, anti-transglutaminase antibody is capable of recognizing antigenic determinants on syncytiotrophoblast, inhibiting the transglutaminase action in the placenta and disturbing the placental function [65]. However, there are studies that do not support the correlation between celiac disease and presence of anti-transglutaminase and anti-endomysial antibodies with adverse pregnancy outcomes, in which screening for these autoantibodies was not recommended in women with RPL history [66–68]. Further investigations are required in order to better understand the correlation between celiac disease and reproductive failures.

1.3 Human leukocyte antigen (HLA) sharing

As suggested by evidence, recurrent miscarriage is associated with elevated rate of HLA sharing. HLA molecules, encoded by a great number of genes on chromosome 6, are known by their broad polymorphism, so the chance of HLA similarity between two individuals is very low. HLA molecules are divided into HLA class I (HLA-A-G antigens) and HLA class II regions (HLA-DR, DQ, and DP antigens) [18]. As suggested by evidence, an increased rate of RPL is associated with higher frequencies of identical HLA-A and HLA-B alleles; however, some studies did not show any relation between HLA sharing and RPL incidence [18]. Increased HLA–sharing with father may suppress the production of blocking antibodies, such as anti-paternal cytotoxic antibodies (APCA), anti-idiotypic antibodies (Ab2), and mixed lymphocyte reaction blocking antibodies (MLR-Bf), which mask the paternal antigens and prohibit their recognition by maternal immune system. Lack of these antibodies compromises the maintenance of pregnancy. Lymphocyte therapy is one of the immunotherapeutic approaches for pregnancy complications, which is able to induce the production of these antibodies [69].

There are increasing evidence about the contribution of immunologic abnormalities in etiopathogenesis of RPL and RIF, including predomination of Th1 and Th17 cells and related cytokines and downregulation of Th2 and Treg cells alongside their cytokines [70], elevated Th1/Th2 and Th17/Treg ratios, increased frequency and function of uNK cells and pNK cells [30], presence of APAs [71] or other autoimmunities like autoimmune thyroiditis [72]. According to the literature, 30.5% of RPL women have increased frequency of NK cells, and 31.6% of them have increased cytotoxicity of NK cells, additionally, 20% of RPL patients and 30% of RIF patients have APAs [73].

Nowadays, there is a strong need for biomarkers and clinical assays for detection of immune abnormalities, besides the helpful immunotherapeutic approaches to improve the immunologic aberration in RIF and RPL patients. This review aims to discuss the immunological approaches in the diagnosis and treatment of pregnancy complications in women with immune-etiology reproductive failures.

2. Immunological tests for RIF and RPL

Analyses of immunologic parameters, addressing immune abnormalities in RIF and RPL women, were not routinely offered by guidelines. In fact, immunological diagnosis tests are generally suggested in the case of “idiopathic” or “unexplained” RIF or RPL, when the other risk factors, including genetic and anatomic

complications and infection are excluded. LA, ACA, and anti-β2GPI antibodies screening are more often suggested [74]. On the other hand, it seems that analysis of blood and endometrial immunologic biomarkers prior to immunotherapies would be helpful in selection of a proper candidate, proper therapeutic modality, investigation of altered parameters, and understanding the mechanism of action of therapeutic agent. Here, we summarized the proposed test for evaluation of immunologic imbalances of RIF and RPL women, including cellular and humoral tests [75]. Immunophenotyping and functional assays, besides evaluation of cytokines concentration and autoantibodies titer, are among the proposed immunologic tests.

2.1 Cellular tests

- T, B, and NK immunophenotyping
- Ratio of T CD4/T CD8
- Ratio of Th1/Th2
- TNF-a and IL-10 positive T $CD4^+$
- Regulatory T cells frequency
- NK cells cytotoxicity
- NK cells activator and inhibitory receptors
- Th17/Treg cells
- HLA-typing

2.2 Humoral tests

- ANAs (anti-DNA, anti-histone, anti-Smith (Sm) antibody, anti-ribonucleoprotein, anti-Jo, autoantibodies against topoisomerase (anti-Scl), anti-SSA/Ro, anti-SSB/La antibodies)
- APAs on ≥2 situations with at least 12 weeks intervals and < 5 years prior to clinical manifestations
- Conventional APA (ACA, LA, anti-β2GPI antibodies)
- Non-conventional APA (anti-annexin V antibodies, anti-phosphatidylethalonamine antibodies)
- Anti-transglutaminase and anti-thyroid (TPO and thyroglobulin) antibodies
- Anti-sperm antibodies
- T helper cells cytokines
- APCA

- Anti-HY and anti-HLA antibodies
- Anti-transglutaminase and anti-endomysial antibodies

3. Immunotherapy of RRF

As suggested by evidence, immunologic aberrations play a critical role in pathogenesis of reproductive disorders including RIF and RPL. Obviously, several immunotherapeutic approaches have already been introduced for the management of these complications, such as immunosuppressive and immunomodulatory agents. According to literature, anticoagulants, corticosteroids, and immunosuppressive medications used in inhibition of graft rejection, such as calcineurin inhibitors, recombinant cytokines, and cell therapy approaches are among the immunotherapeutic agents which have been used in animal experiments and clinical trials, in order to modulate the abnormal immune responses and improve the pregnancy consequences. However, the ambiguous evidence provided by this literature need further clarification, as most of these approaches have yet to achieve routine clinical applications, due to concerns about their efficiency and safety. Therefore, further investigations are required to determine the efficacy and safety of novel immunotherapeutic strategies for pregnancy complications. Here, we examine the present immunotherapies, their mechanisms, and related studies, which have been conducted for the management of RIF and RPL patients, especially those with an immunological background. We first

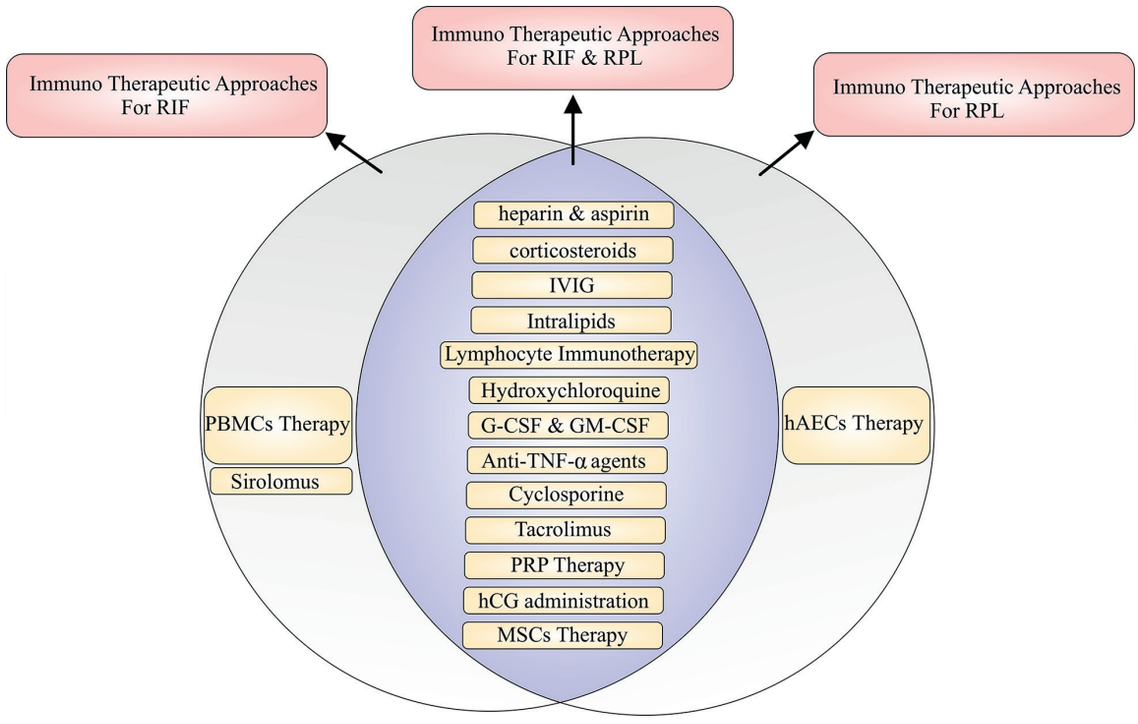

Figure 2.
The classification of immunotherapeutic approaches used for RRF, based on their application for RIF and/or RPL patients. Abbreviation: RPL: recurrent pregnancy failure; RIF: recurrent implantation failure; IVIG: Intravenous immunoglobulin; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; Anti-TNF-α: anti-tumor necrosis factor α; PRP: platelet-rich plasma; hCG: Human chorionic gonadotropin; MSC: mesenchymal stem cells; hAECs: Human amniotic epithelial cells; PBMCs: Peripheral blood mononuclear cells.

describe the proposed mechanisms of action of the immunotherapeutic modalities; afterward, the studies that utilized these agents and related systematic reviews and meta-analyses, for RIF and RPL patients, are described respectively. The classification of these immunotherapeutic approaches, based on their application for RIF and/or RPL patients, is presented in **Figure 2**.

3.1 Immunotherapeutic approaches for RIF and RPL patients

3.1.1 Heparin and aspirin

Heparin is a structural analog of heparan, which is present in reproductive tract and plays a pivotal role in reproduction. Heparin and heparan are capable of binding to growth factors and their receptors, antithrombin, and molecules of extracellular matrix [76]. In addition to anti-thrombotic effect of heparin during implantation, it is able to improve placentation, especially in women with thrombophilia [77]. On the other hand, the proteins involved in the blastocyte invasion and adhesion to endometrium and trophoblastic differentiation are modulated by heparin and it is due to the action of heparin on metalloproteinases, cadherin-E, heparin-binding epidermal growth factor, and free insulin-like growth factor [78]. The anti-inflammatory effect of heparin is also reported, as heparin interferes with the activation of complement [77]. Anti-inflammatory effect of aspirin, a non-steroidal anti-inflammatory drug (NSAIDs), is attributed to the inhibition of prostaglandins synthesis; besides, aspirin is able to induce acetylation of cyclooxygenase-2, which leads to the production of aspirin-triggered lipoxins (ATLs) from arachidonic acid. Aspirin also prohibits platelet generation and exhibits anti-thrombotic effects [79].

According to the ESHRE, ASRM, German/Austrian/Swiss Society of Obstetrics and Gynecology (DGGG/OEGGG/SGGG), and the Royal College of Obstetricians and Gynecologists (RCOG) guidelines, low-dose aspirin and heparin are recommended for treatment of APS [18]. Different studies also reported the positive effect of combination of aspirin and heparin in pregnancy complications, accompanied by APS [80]. Evaluation of effect of heparin therapy around the time of implantation, at/or after egg collection, or at the time of embryo transfer, in subfertile women during assisted reproduction, demonstrated that heparin was able to increase the rate of live birth in comparison with control group [76]. According to the results of Potdar et al. systematic review and meta-analysis, adjunct low molecular weight heparin (LMWH) in women with a history of ≥3 RIF significantly increased the live birth rate in comparison with control group. However, the implantation rate did not show any significant difference [81].

Study by Badawy et al. included 340 women, divided into two groups, one group received LMWH enoxaparin, and the other group received folic acid tablets. The results demonstrated that heparin decreased the incidence of recurrent miscarriages and increased the mean birth weight [82]. A systematic review, investigating the efficacy and safety of aspirin and heparin therapy, in women with at least two unexplained miscarriages, with or without inherited thrombophilia showed no beneficial effect of aspirin and heparin; so, the anticoagulants in women with unexplained RM were not confirmed confirmed in this systematic review [83]. While the cochrane systematic review of Hamulyak et al. confirmed that combination of heparin plus aspirin during pregnancy is capable of improving the live birth rate in RPL women with APS and the efficiency of combination of heparin and aspirin was more than efficiency of aspirin alone [84].

Heparin may be a helpful choice in reproduction complications in the case of known APS or thrombophilia; however, evaluation of the efficacy of heparin in women with reproduction failure has shown almost no improvement of clinical pregnancy or live birth rate in women without known case of thrombophilia. More research is needed about the efficiency of heparin therapy alone or in combination with aspirin in both known and unknown cases of thrombophilia, in order to further evaluate potential benefits of this treatment strategy, and to gain consensus on the ideal treatment.

3.2 Corticosteroids

Along the line of corticosteroids, prednisolone has been widely used in immune-mediated reproductive disorders due to its anti-inflammatory and immunomodulatory effects. The suggested action mechanisms for prednisolone in pregnancy complications include decreasing the Th1/Th2 ratio, secretion of Th1-related cytokine, and downregulation of frequency and cytotoxicity of NK cells [85].

According to the literature, prednisolone may be a helpful choice in improvement of implantation rate of women undergoing IVF procedure, especially the women who are positive for APAs and ANAs [86, 87]. There are evidence about the positive effect of prednisolone, alone or in combination with heparin, on RIF patients through modulation of elevated frequency and function of NK cells [88]. Indeed, expression of glucocorticoid receptors by uNK cells makes these cell to be highly affected by prednisolone [89]. Evaluation of endometrial biopsy of RIF patients indicated that over-activation of immune system in RIF women was modulated after prednisolone administration. The mRNA expression of IL-18/tumor necrosis factor-like weak inducer of apoptosis (TWEAK), which is a reflector of Th1/Th2 ratio, was significantly decreased post-treatment [90]. In addition, a recent study also demonstrated that the dysregulated Th17/Treg axis in RIF patients was modulated by creating a shift toward Treg cell responses after prednisolone administration [91, 92]. On the other hand, there are studies in which prednisolone showed no benefit in the improvement of the outcomes in RIF patients [93]. The results of a recent systematic review about the effect of prednisolone administration in women undergoing IVF or intracytoplasmic sperm injection (ICSI) indicated almost no significant difference in live birth and clinical pregnancy rate of corticoid versus no corticoid or placebo group [94].

Corticosteroid administration for RPL women who have elevated frequency of uNK cells, in cycle days 1–21, was capable of decreasing the frequency of uNK cells [95]. Combination of prednisolone with aspirin and heparin also seemed to be more helpful in pregnancy complications in unexplained recurrent miscarriage, as was confirmed in the study of Gomaa et al. [96]. Evaluation of endometrial samples of RM women showed that the increased percentage of uNK ($CD56^{+}CD16^{-}CD3^{-}$) in RM patients was decreased posttreatment with prednisolone [95]. According to in-vitro experiments, elevation of HLA-G expression post-glucocorticoid therapy may decrease the incidence of RM [97]. Meta-analysis of Don et al. also confirmed that prednisolone may improve pregnancy outcomes in women with idiopathic RM, but its effect was not significant in women undergoing ICSI [98].

There is still a requirement for studies to investigate the efficiency of corticotherapy in RIF and RPL women with immunologic abnormalities; studies which investigate the effect of corticosteroid on improvement of both the immunologic aberrations and pregnancy outcomes. In addition, given the contradictory results about the efficiency of prednisolone and considering the reported adverse effects, such as risk

of hypertension and diabetes, further powerful and well-designed placebo-controlled randomized trials with lower doses of prednisolone are required to identify the efficiency of treatment and specific risk factors.

3.3 Intravenous immunoglobulin G (IVIG)

IVIG is an immunomodulatory agent, consisting of natural antibodies and autoantibodies, Fab fragments of IgG, antibodies against antigenic determinants of bacteria, and different cytokines [85, 99]. IVIG is purified from the plasma of 1000 to up to 100,000 healthy donors, and is used for treatment of thrombocytopenia, kawasaki disease, graft versus host disease (GVHD), immune-mediated and pregnancy disorders [100]. There are numerous suggested mechanisms of action, by which IVIG improves the pregnancy outcome, including reducing the number and cytotoxicity of NK cells [101, 102], enhancing the frequency and function of Treg cells [103], inhibiting the production of autoantibodies by B lymphocytes, neutralizing the maternal autoantibodies by its anti-idiotypic antibodies [85, 104], inhibiting the deposition of complement fragment and membrane attack complex (MAC) [105], and upregulation of inhibitory receptors on antigen-presenting cells (APCs) [106]. A reduction in the number of Th1 cells and cytokines secretion and elevation in Th2 responses was also observed after IVIG administration in related studies [107], followed by a reduction in Th1/Th2 ratio [108]. Furthermore, the results of the study of Ahmadi et al. also demonstrated that IVIG is capable of increasing the frequency of Treg cells and mRNA expression of Treg transcription factor, FoxP3, and cytokines such as IL-10 and TGF- β. In addition, the mRNA expression of Th17-associated transcription factor, RORγt was reduced post-treatment [109]. Therefore, the other mechanism of action of IVIG may be attributed to modulation of Th17/Treg axis.

The pregnancy and live birth rates were significantly elevated in RIF patients with an increased level of circulating NK and/or NKT-like cells after IVIG therapy when compared to those not receiving IVIG patients [110]. Additionally, a systematic review and meta-analysis demonstrated that IVIG administration is associated with increased rate of implantation and pregnancy in women undergoing IVF/ICSI cycles, in comparison with placebo group. The results also indicated that IVIG receiving group had a lower rate of miscarriage, therefore the usefulness of IVIG administration was strongly supported in women who had a history of recurrent IVF failure, by this systematic review [111]. A systematic review of our group also confirmed the positive effect of IVIG on RIF patients, especially those with immunologic abnormalities. The results of this systematic review, which included two cohorts, two cross-sectional and one quasi-experimental study, revealed that there is a significant increase in the live birth and pregnancy rate of IVIG group in comparison with control group. However, the miscarriage rate was not significantly affected by IVIG [112].

Numerous studies have assessed the efficiency of IVIG in RPL women with or without known etiologies, including immunologic abnormalities. The group of Yousefi and colleagues evaluated the beneficial effects of IVIG in pregnancy complications, considering the various immunologic abnormalities involved in etiology. For instance, IVIG treatment in RM women with elevated frequency and function of peripheral NK cells resulted in significant decrease in the percentage and cytotoxicity of NK cells and expression of activating receptors. In contrast, expression of inhibitory receptors was significantly elevated, post-treatment. Pregnancy outcome was also improved as a result of IVIG therapy [113]. The other investigation by this group evaluated the effect of IVIG on alteration of Th1 and Th2 responses in RPL women with pre-treatment elevation of

NK cell frequency and cytotoxicity. After IVIG administration, the frequency, mRNA expression level of transcription factor, and secretion of Th1-related cytokine were significantly decreased. In contrast, these parameters for Th2 cells were increased, in comparison with control group. Furthermore, Th1/Th2 ratio was decreased post-treatment. 87.5% of IVIG treated group and 41.6% of untreated groups had live birth [114]. As a new risk factor for recurrent miscarriage, Th17 and Treg cell balance was evaluated in RM patients after IVIG administration. Before and after intravenous administration of 400 mg/kg of IVIG, every 4 weeks through 32 weeks of gestation, the immunologic parameters were evaluated. The results indicated that IVIG therapy is capable of down-regulating Th17 frequency, while Treg frequency is upregulated, in comparison with untreated group. Rate of pregnancy was 86.3% in IVIG treated group and 42% in untreated group [115]. This study confirmed the results of the study of Kim et al. [116]. Exhausted T cells, exhausted Tregs and Treg cell alteration were also evaluated post-IVIG therapy in RM patients. Blood samples were collected twice, prior to treatment at the time of positive pregnancy test and after the latest IVIG administration. The results indicated a significant elevation in frequency of Treg cells and a significant reduction in frequency of exhausted Tregs, in comparison with untreated group; however, the frequency of exhausted T cells was not affected by IVIG. The pregnancy outcome was also significantly higher in IVIG-treated RM patients [117].

There are reports about the lack of beneficial effects of IVIG treatment on the improvement of pregnancy outcomes in obstetric complications, for instance, the study of Christiansen et al. [118] and Stephenson et al. [119], In accordance with these studies, positive effect of IVIG on the improvement of live birth was not confirmed in systematic review of Wang et al. [120]. However, a recent systematic review and meta-analysis by Parhizkar et al. assessed the results of IVIG therapy on RPL women with immunologic abnormalities in five studies (two cohorts and three quasi experimental studies). The results revealed that IVIG therapy significantly increased the live birth rate, when compared with untreated group and emphasized the efficiency of IVIG for RPL patients, especially those with immunologic aberrations [121].

As reported by several studies, IVIG may be used in combination with other therapeutic approaches, such as prednisolone and TNF-α inhibitor, in order to improve the pregnancy consequences. The combination of TNF-α inhibitor and IVIG showed promising results in improvement of implantation, clinical pregnancy, and live birth rate in women with elevated Th1/Th2 cytokine ratio, who undergo IVF cycles [122]. Furthermore, combination of IVIG with prednisolone also was helpful as indicated by the study of Nyborg et al. [123].

Keeping in view of all the above studies, there are controversies about the efficiency of IVIG in the improvement of pregnancy outcomes alongside immunologic abnormalities in RM and RIF women. Small sample size, lack of randomization, using the less potent IVIG like Gamimune, which was not able to effectively suppress elevated NK cells [75], and lack of evaluation of immune abnormalities prior to treatment, are among the reasons for the heterogeneities of studies. It is inferred that final conclusion about the efficiency of IVIG in improvement of pregnancy outcomes requires more well-designed and powered prospective and randomized controlled trials with appropriate sample size and protocols.

3.4 Intralipids

Intralipids are 20% parenteral sterile fat emulsion, with main components of polyunsaturated fatty acids (PUFAs), especially linoleic acid, in addition to soybean oil,

egg phospholipids, glycerin, and water [124]. According to the literature, intravenous administration of intralipids is capable of suppressing the proliferation of immune cells, by altering the composition of cell membrane phospholipids, which subsequently modulates the fluidity and receptors of membrane [124]. Reducing the cytotoxicity of NK cells and inhibition of Th1 responses are attributed to fatty acids and soybean oil of intralipids, respectively [125]. Intralipids also diminish the signals, which are required for T and B lymphocyte activation, by inhibition of IL-2 production and downregulating pro-inflammatory mediators such as IL-1β and TNF-α [15, 126]. However, intralipids are often known to influence the NK cell expansion and function [127].

There are studies that reported the effect of intralipids on NK cell function in women with reproductive disorders [128, 129], and its ability to improve clinical pregnancy and live birth rate in RIF patients [126]. Results of a similar study demonstrated that abnormal NK cell function in patients who received intralipids was modulated to the normal range, after the first or second infusions [129]. According to a systematic review, a significant elevation in clinical pregnancy and live birth rate was observed after intravenous intralipid in RIF women [130]. There are several studies that indicated no beneficial effect of intralipid administration for RIF patients including the study of Shreeve et al. [124] and Check et al. [131].

Intralipids were also capable of elevating the live birth rate in RM patients, according to the meta-analysis of Placais et al., in which live birth was observed in 70% of pregnancies of women with elevated pNK cells, who received intralipids, when compared to untreated group [132]. The other recent systematic review and meta-analysis showed the efficiency of intralipids administration on live birth rate in unexplained infertility and RM patients with known immunological risk factors, but still not as a routine intervention for reproductive disorders [133]. On the contrary, there are studies that do not confirm the beneficial effect of intralipid supplementation in RSA women, even with elevated NK cells such as the study of Dakhly et al. [134].

There is heterogeneity across the studies, which evaluate the efficiency of intralipids in reproductive failures. Moreover, there are limited data about the exact mechanism of action of intralipids for decreasing the elevated number and cytotoxicity of NK and about the safety of intralipids administration during pregnancy. Large-scaled and well-designed research are required for safe conclusions on the efficiency of intralipid therapy in reproductive disorders.

3.5 Lymphocyte immunotherapy (LIT)

LIT or peripheral blood mononuclear cell (PBMCs) therapy includes paternal lymphocyte immunization (PLI), third-party lymphocyte immunization, or insemination of patients' own lymphocytes, in which the lymphocytes are gathered and administrated to the prospective mother [69].

The proposed mechanisms by which LIT improves pregnancy outcomes include stimulation of the maternal immune system in order to produce antibodies, such as APCA, and Ab2, including anti-T cells receptor (TCR) idiotypic antibodies, MLR-Bf, and progesterone-induced blocking factor (PIBF), which avoid recognition of paternal HLA antigens by maternal immune system, especially T and NK cells, by blocking these antigens [135, 136]. Furthermore, it is reported that LIT is capable of reducing the activity of NK cells, downregulating the expression of maternal IL-2 receptors [137], creating a shift toward Th1 responses, improving the Th1/Th2 equilibrium and increasing Treg responses over Th17 responses [138, 139]. Considering the amount and dose of lymphocytes (100–500 × 10^6 cells) [140], route (Intradermal, intravenous

and fewer subcutaneous, intracutaneous and intramuscular routes) [141] and time (before pregnancy, during pregnancy, before and during pregnancy: the most helpful) of administration [69], there are multiple protocols for LIT [85].

3.5.1 Intradermal LIT

There are evidence that confirms the positive effect of LIT in improvement of pregnancy outcome in women with immunologic abnormalities by inducing maternal tolerance toward fetus and decreasing the risk of pregnancy wastage; however, most of these studies emphasize the beneficial effect of LIT, especially intradermal LIT, on RPL patients more than RIF women [142, 143]. The recent systematic review of Cavalcante and colleagues indicated that the use of LIT would be a beneficial treatment in RM patients; however, it was not recommended for RIF patients [144]. Gao et al. investigated the immunologic parameters of pre- and post-intradermal paternal lymphocyte immunization in women with unexplained RSA. Before LIT, RSA patients showed an increased rate of lymphocyte counts, CD4/CD8 cell ratios, and frequency of NK cells, in comparison with control group. LIT was capable of reducing all the mentioned parameters in RSA women, except T cell frequency, which was increased post-treatment. Considering the abnormal activation of immune system in RSA patients, lymphocyte immunotherapy was helpful in modulation of these abnormalities [145]. In addition, it has been confirmed that intradermal paternal or third-party lymphocyte immunization increased the $CD4^{+}CD25^{bright}$ T cells frequency in RSA women while decreasing the percentage of $CD4^{+}CD25^{dim}$ cells. This study suggested that $CD4^{+}CD25^{+}$ regulatory T cells serve as a biomarker for monitoring the efficiency of LIT in RSA patients [146]. LIT was also capable of elevating the pregnancy outcome in RSA patients. Abortion rate was significantly decreased after LIT in RSA women, in comparison with patients who received routine treatment. Furthermore, LIT significantly increased the pregnancy success rate [147]. On the contrary, there are studies that reported no beneficial effect of LIT for the improvement of pregnancy outcomes in RM patients [134]. However, the efficiency and safety of LIT for RM patients were confirmed in the systematic review and meta-analysis of Cavalcante et al. [139].

3.5.2 Intrauterine LIT

Intrauterine administration of each patient's own PBMCs, mostly used for RIF women, is suggested to improve the immunologic balance of endometrium which is required for successful implantation and pregnancy, in addition to enhancing the endometrial receptivity [148]. Indeed, PBMCs improve the invasion of trophoblast and implantation by increasing the expression level of matrix metalloproteinase-2 (MMP-2) and MMP-9 and decreasing the expression of tissue inhibitors of metalloproteinase [149]. PBMCs also create a shift toward Th2 prominent responses by induction of progesterone from luteal cells [150]. Peripheral blood of each patient is collected 3–5 days before the embryo transfer, subsequently, PBMCs are isolated and infused into the uterine cavity via an intrauterine insemination catheter [151]. It has been observed that co-culture of PBMCs with human chorionic gonadotropin (HCG), corticotropin-releasing hormone (CRH), and human menopausal gonadotropin (HMG), prior to infusion, may be a useful approach in improving the rate of implantation, as the secretion of essential cytokines and mediators for implantation would be increased [152, 153].

There are solid evidence of the positive effect of intrauterine implementation of PBMCs, leading to favorable outcomes in RIF patients [154], including the study of Yoshioka and colleagues [155]. The results of our previous study also confirmed the efficiency of intrauterine administration of autologous hCG-activated PBMCs in improving the live birth rate and decreasing the miscarriage rate of RIF patients with a history of at least three IVF/ET failures [156]. The systematic review of Wu et al., demonstrated that PBMC therapy improved clinical pregnancy implantation and live birth rate of RIF patients, in comparison with placebo or no treatment group [149]. The next systematic review and meta-analysis indicated that clinical pregnancy and live birth, irrespective of embryo stage and cycle type, were increased after PBMC therapy [150]. The most recent systematic review belongs to our group, in which we investigated the effect of intrauterine PBMC-therapy before IVF in women with at least three IVF/ET failures. The results demonstrated that PBMC therapy in RIF women is associated with significantly higher implantation, pregnancy, and live birth rate and reduced miscarriage rate, in comparison with non-treated group [157].

Further RCTs are still required with a larger population and high-quality study design and less heterogeneous study populations, for recommending PBMC administration as a helpful immunologic approach in treatment protocol of RIF patients.

3.6 Hydroxychloroquine

Hydroxychloroquine, known as an anti-malaria drug, has been considered an immunomodulatory drug in inflammatory and autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis [158]. The proposed mechanisms of action of hydroxychloroquine from the immunologic point of view, include downregulation of prostaglandins and inflammatory cytokines such as TNF-α and IFN-γ, reducing the antigen presentation and chemotaxis of immune cells, blocking the receptor signaling of B and T lymphocytes [159], restoring the Th1/Th2 balance and creating a shift toward Th2 responses [160], promotion of Treg cells, inhibition of phospholipase activity and lysosomal acidification and prevention of platelet aggregation and matrix metalloproteinases action [161]. In recent years, hydroxychloroquine has gained attention for its immunomodulatory properties in improvement of reproductive disorders. The efficiency of hydroxychloroquine in reducing the titer of autoantibodies in APS has been reported, therefore it serves an anti-thrombotic effect [158]. In addition, binding of anti-β2GPI antibodies to phospholipid bilayers in trophoblasts is decreased by hydroxychloroquine [160]. In other words, hydroxychloroquine saves the fusion and differentiation of trophoblast, which were compromised because of APAs [162].

In the study of Ghasemnejad-berenji et al., the effect of 400 mg/per day of oral hydroxychloroquine was investigated on immunologic parameters of RIF women with increased TNF-α/IL-10 ratio. Post-treatment with hydroxychloroquine, the serum level of TNF-α was significantly downregulated, while the serum level of IL-10 was increased. Moreover, the expression of Th1 cells transcription factor, T-bet, and Th2 transcription factor, GATA-3, were significantly decreased and increased, respectively, in comparison with pre-treatment [163]. A clinical trial that evaluated the effect of hydroxychloroquine on Th17/Treg axis in RIF women, reported that hydroxychloroquine was able to downregulate the function and cytokines of Th17 cells, while Treg cells function and cytokines were significantly upregulated post-treatment. The expression level of Th17 and Treg cells associated

transcription factors was significantly decreased and increased, respectively. However, no significant difference in pregnancy outcomes was observed post-treatment with hydroxychloroquine [161].

The effectiveness of hydroxychloroquine was also evaluated in RPL patients, but the studies are limited. Hydroxychloroquine administration for autoimmune-related RPL women who did not gain benefit of the low-dose aspirin and LMWH in previous pregnancies, indicated that hydroxychloroquine was able to significantly increase the live birth rate, gestational age at delivery and the mean birth weight, in comparison to placebo group [164]. There are some ongoing clinical trials assessing the impact of hydroxychloroquine on RPL or RM women [165–167]. Nevertheless, the results of a systematic review by Yang et al. suggested that combination of hydroxychloroquine with current treatment regimens used in the prevention of RM in APS patients, including low-dose aspirin and heparin, has been shown to have beneficial effects. However, this study also suggested further large-scale and well-designed RCTs to confirm these findings [168].

3.7 Granulocyte colony-stimulating factor (G-CSF)

G-CSF is a cytokine, produced by various types of cells, such as monocytes and macrophages, endothelial, decidual, and bone marrow cells, which act as a stimulator of neutrophils differentiation, proliferation, and function [169]. According to experimental studies, G-CSF positively affects trophoblast growth and placenta metabolism and supports the embryo [170]. It is also reported that G-CSF promotes Th2-type responses, and cytokines decreases the cytotoxicity of NK cells and enhances the function of Treg cells [15]. Moreover, G-CSF is involved in endometrial vascular remodeling which is essential for implantation [171].

According to the literature, G-CSF is capable of enhancing the thickness of endometrium and improves the quality of embryo [172]. As shown in an endometrial ex vivo model, the endometrial genes which are involved in fetus adhesion, cell migration, and remodeling of endometrial vascular are regulated by recombinant human G-CSF [173]. Subcutaneous injection of G-CSF prior to embryo transfer in RIF women, resulted in increased clinical pregnancy and implantation rates when compared to control group [174]. Study by Xu et al. showed positive effect of intrauterine administration of G-CSF for thickening of thin endometrium and significantly increasing the embryo implantation and clinical pregnancy rates [175]. Systematic review and meta-analysis of Jiang et al. indicated that G-CSF administration was capable of enhancing the implantation and clinical pregnancy rate by both the intrauterine and subcutaneous routes, however, subcutaneous injection was more efficient [176]. Almost the same beneficial effect was reported in Li. et al. meta-analysis, about the effect of transvaginal perfusion of G-CSF [177].

G-CSF treatment was also capable of elevating the Foxp3 expressing cells, indicating Treg cells, in the decidua of RPL women. Expression of G-CSF and vascular endothelial growth factor (VEGF) in trophoblast was also upregulated as a result of G-CSF treatment. These findings showed the efficiency of G-CSF therapy for RPL women, probably by modulating the immune responses by induction and recruitment of Treg cell in decidua of RPL women [178]. Subcutaneous G-CSF administration for primary RM patients showed that 82.8% of women who received G-CSF, had live birth, while the rate of live birth was 48.5% in placebo group [179]. However, a RCT by Eapen et al. indicated no improvement in pregnancy outcome after administration of recombinant human G-CSF in the first trimester of pregnancy for RPL patients

[180]. Lack of beneficial effect after intrauterine G-CSF injection was also confirmed in another RCT including RM women [181].

Considering the heterogeneities in studies, besides inexpensive cost of G-CSF and no report of the newborn's abnormalities or malformations and minor maternal side effect [75], G-CSF has the potential to be a promising approach in management of reproductive disorders. Nevertheless, administration of G-CSF for improvement of pregnancy outcome and immunologic aberrations in RIF and RPL women, requires further high-quality researches.

3.8 Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF is a cytokine, produced by T lymphocytes, macrophages, endothelial cells, and fibroblasts, which stimulates the differentiation, survival, and activation of granulocytes and macrophages [182]. GM-CSF is also produced by epithelial cells of uterine glands or lumen during the pregnancy, furthermore, placental trophoblasts express GM-CSF receptor [75]. GM-CSF production increases significantly during embryo implantation and pregnancy, especially in the first trimester; however, the elevated production of GM-CSF in normal pregnancy, is not observed in reproductive disorders [15]. It is estimated that GM-CSF is essential for normal development of blastocyst, through inhibition of apoptosis and stimulation of glucose uptake by blastocyst [183]. The addition of GM-CSF to the embryo culture medium, enhanced the survival of transferred embryo, implantation rate in addition to live birth rate [184]. The same improvement in the implantation and progressive clinical pregnancy rate was obtained in the study of Tevkin et al. [185]. Study by Akgul et al. indicated that GM-CSF activity was decreased in decidua of RPL patients, while moderate and severe GM-CSF activity was observed in fertile women. In addition, GM-CSF rate and distribution were different in various compartments of decidua [186].

Considering the positive effects of GM-CSF on human reproduction, it may be effective in women with reproductive disorders; however, there are limited studies evaluating the effect of GM-CSF in RIF and RM patients, which highlights the importance of further large-scale studies.

3.9 Anti-tumor necrosis factor-α (anti-TNF-α) for RIF patients

Anti-TNF-α medications target TNF-α cytokine and are utilized for the treatment of autoimmune disorders like rheumatoid arthritis [187]. These medications, including adalimumab (humira-fully human recombinant immunoglobulin G1 monoclonal antibody) and etanercept (dimeric Fc fusion protein), reduce inflammation, thus they are suggested to be useful in improving the pregnancy outcome in reproductive disorders [188]. Elevated level of TNF-α is responsible for higher Th1 type responses, increasing the rate of prostaglandin E2, uterine muscle contraction, and activation of coagulation cascade, which leads to thrombosis of placental vascular and adverse pregnancy outcome [189]. In fact, TNF-α is involved in thrombosis-mediated fetal loss by increasing the expression of fibrinogen-like protein 2 (FGL2), a fibrinogen-related prothrombinase, which induces the synthesis of thrombin, deposition of fibrin and activation of C5 component of complement and neutrophils [75].

The study of Santiago et al., which used etanercept for endometrial preparation at the time of embryo transfer in women suffering from RIF, indicated 75.9% of embryo implantation and 62.7% of ongoing pregnancy/live birth rate, post-treatment [190]. The effect of Adalimumab on pregnancy complications is often investigated in

combination with other therapeutic approaches such as IVIG. For instance, the study of Winger et al. investigated the efficiency of adalimumab alone or in combination with IVIG in RIF patients, who had an increased Th1/Th2 ratio. The implantation rate for adalimumab receiving group was 31% (4/13), while it was 59% (50/85) in combination with adalimumab and IVIG. The clinical pregnancy and live birth rates were also higher in combination treatment group [122]. Another investigation by this author, evaluated the effect of preconception Adalimumab and IVIG in group I with severe TNF-α/IL-10 cytokine elevation, before the conception and treatment (>39.0) and group II with a moderate TNF-α/IL-10 ratio (>30.6 and ≤ 39.0). The implantation, clinical pregnancy, delivery, and live birth rate was higher in group II when compared to group I; however, the difference was not significant. The TNF-α/IL-10 ratio was also significantly decreased post-treatment. This study supported the beneficial effect of modulating the elevated inflammatory cytokines in improving the success rate of IVF cycles with immunomodulatory approaches, such as anti-TNF-α and IVIG [191].

It has been proved that the frequency of TNF-α producing Th1 cells, and TNF-α/IL-10 ratio is significantly higher in RPL patients [192]. Increased serum concentration of TNF-α in immune-dependent RM patients was decreased after treatment with etanercept [193]. In the study of Fu et al. etanercept was able to downregulate the levels of TNF-α and NK cell activity and increased the rate of live birth, in refractory RSA patients with immunologic abnormalities [194]. Moreover, etanercept was able to significantly downregulate the activity of NK cells, however, no significant difference was observed in Treg cells level. Therefore, beneficial effect of etanercept in RM patients was attributed to the immunomodulatory effect of etanercept [195]. Combination of TNF inhibitors and IVIG for the treatment of RSA women was investigated in study of Winger et al. Study population was divided into three groups, receiving anticoagulant (group I), anticoagulant and IVIG (group II) and anticoagulant, IVIG and etanercept or adalimumab (group III). The live birth rate was 19%, 54%, and 71% for groups I, II, and III, respectively. Moreover, a significant increase was observed in the pregnancy outcome of group III, in comparison with group I [196].

3.10 Cyclosporine

Cyclosporine, an immunosuppressive agent, is widely utilized in order to prevent graft rejection post-transplantation and in treatment of autoimmune disorders [197]. Cyclosporine impairs both humoral and cellular immunity and prevents IL-2, TNF-α, and IFN-γ expression and T cell proliferation, by inhibiting calcium-dependent signaling pathways [198]. According to the literature, cyclosporine upregulates IL-4 secretion and creates a shift in favor of Th2-type responses in addition to suppression of Th1 lymphocytes and associated cytokines [199]. Furthermore, NK cells, macrophages, and dendritic cells' function is also impaired by cyclosporine [85]. Cyclosporine is capable of improving the trophoblast invasion by regulation of MMP9 and MMP2, in first trimester [200]. Indeed, animal investigations proved the positive effect of cyclosporine on trophoblast cells and its ability in inducing maternal immunotolerance by downregulation of co-stimulatory molecules, upregulation of inhibitory mediators, and modulation of Th1/Th2 and Th17/Treg equilibrium [201, 202].

There are limited studies that explore the efficiency of cyclosporine in improvement of outcomes of RIF patients. In a recent retrospective cohort study by Cheng et al., the beneficial effect of cyclosporine after embryo transfer on pregnancy

outcome was investigated among RIF patients. Implantation, clinical pregnancy, and live birth rate of subjects were significantly improved post-cyclosporine application, while there was no elevation in the risk of obstetric and pediatric complications after this treatment protocol [17]. It is inferred that the beneficial effect of cyclosporine in improvement of pregnancy outcomes is associated with immunomodulatory effect of this agent, which hampers maternal immune system's attack on the embryo. Cyclosporin effect was also explored on women with a history of unexplained transfer failure in frozen-thawed embryo transfer (FET) cycles in the study of Qu et al. However, the results of this study showed no significant differences between cyclosporine-treated group and control group in implantation, clinical pregnancy, and take-home baby rate [203].

Refractory immune RSA patients who were positive for APS were treated with cyclosporine after unsuccessful treatments with aspirin, prednisone, heparin, LIT, and IVIG. Cyclosporine was capable of reducing the titer of autoantibodies besides 76.92% successful pregnancy was achieved [204]. In the study of Ling et al. the effect, safety, and mechanism of low-dose cyclosporine in RSA patients were assessed. At the time of positive pregnancy test, 100 mg/day oral cyclosporine was started for treatment group for 30 days, control group received progesterone. Immunologic parameters were evaluated pre- and post-treatment. CD3 level of maternal blood was upregulated while CD8 level was downregulated after treatment. Moreover, the live birth rate was significantly higher in cyclosporine group. No side effects and adverse pregnancy outcomes were reported [205]. In the study of Azizi et al., 76 RPL women were recruited (38 in cyclosporine group, 38 in control group) and alteration of immunologic parameters besides pregnancy outcome were assessed pre- and post-treatment. According to the results, the frequency of Th1 cells, Th1/Th2 ratio, expression of T-bet, Th1-related transcription factor, and secretion of IFN-ɣ and TNF-α were significantly downregulated after cyclosporine administration, when compared to pre-treatment. Control group exhibited no significant differences. Moreover, cyclosporine significantly upregulated the frequency of Th2 cells, expression of GATA-3, and secretion of IL-10. A significant elevation in the rate of successful childbirth was observed in cyclosporine group [206]. Another study evaluated the effect of cyclosporine on Th17/Treg axis in peripheral blood of RSA patients. The study group included 30 women with normal early pregnancy, 25 RSA women, 27 pregnant women with RSA history receiving progesterone, and 24 pregnant women with RSA history receiving cyclosporine. Cyclosporine significantly increased the frequency of Treg cells, production of IL-10 and TGF-β, and decreased Th17 cells, by upregulation of co-inhibitory molecules expression [207]. A recent RCT by Zhao et al. Investigated the effectiveness of intrauterine perfusion of cyclosporine in RSA women with endometrial alloimmune dysfunction. Live birth rate of cyclosporine group was significantly higher than control group, while the frequency of $CD56^+$ cell and $CD57^+$ cell at the luteal phase of the second menstrual cycle was lower [208]. A recent meta-analysis, in which effects of oral immunosuppressants were assessed on pregnancy outcome of RM patients, indicated that cyclosporine or prednisolone was able to significantly enhance the rate of live birth (OR = 3.6, 95% CI: 2.1–6.15, $p < 0.00001$) and ongoing pregnancy (OR = 8.82, 95% CI: 2.91–26.75, $p = 0.0001$) in idiopathic RM patients. Rate of miscarriage was decreased post-treatment. However, the study reported significant heterogeneity and a moderate-to-severe risk of bias [209].

There is still a lack of high-quality evidence about cyclosporine efficiency for RSA and RIF patients. Due to limited evidence, cyclosporine is not recommended for these patients and cyclosporine application must be limited to clinical trials.

3.11 Sirolimus

Sirolimus, also known as rapamycin, is an immunomodulator agent approved by FDA for prevention of solid organ transplant rejection, furthermore, anti-tumor effect of sirolimus has been also documented. The immunosuppressive effect of sirolimus is mediated by its inhibitory action on mammalian target of rapamycin (mTOR) kinase pathway, blocking the downstream of co-stimulatory signals [210]. The proposed mechanisms of action of sirolimus for modulation of immune system include expansion of Treg cells and prevention of the differentiation of Th17 cells, inhibition of B and T lymphocytes proliferation by prevention of IL-2 and IL-4 production, and attenuation of inflammatory responses [211, 212].

According to a report from the national transplantation pregnancy registry (NTPR), more than 14,000 female transplant recipients worldwide, had a history of successful pregnancies, therefore, it is concluded that sirolimus is not a contraindication for pregnancy [213]. In addition, animal studies also confirmed the positive effect of sirolimus on gestation. An animal study on murine model of RIF demonstrated that Sirolimus was able to promote the expansion of Treg cells in the depletion of regulatory T cell (DEREG) mice and improved the implantation rate [214]. A phase II randomized clinical trial by Ahmadi et al. evaluated the immunomodulatory effect of Sirolimus on immunologic abnormalities in RIF women with a history of at least 3 implantation failures. Patients with increased Th17/Treg ratio, who received Sirolimus showed an expansion of Treg cells, besides a reduction in frequency of Th17 cells and Th17/Treg ratio. Subsequently, an elevated rate of clinical pregnancy and live birth was observed in treated group, compared with non-treated control group [215].

There is no study assessing the efficiency of sirolimus in improvement of pregnancy outcomes in RPL women and by today, sirolimus has been used in animal model of RIF and for improvement of pregnancy consequences of RIF women in Ahamdi et al. study. Nevertheless, there are limited evidence about the efficiency of sirolimus in reproductive failure. Considering the immunomodulatory effect of sirolimus, it has the potential to provide a promising option to ameliorate reproductive disorders on immunologic basis.

3.12 Tacrolimus

Tacrolimus (FK506), is an immunosuppressive agent, approved for inhibition of allograft transplant rejection, by diminishing the recipient's immune systems' alloreactivity toward graft [216]. It has been also documented that Tacrolimus is also efficient in management of GVHD and autoimmune disorders, such as rheumatoid arthritis and degenerative inflammatory brain diseases [15]. Binding of Tacrolimus to FK506 binding protein (immunophilin FKBP12), and subsequent creation of a complex with calcineurin, prevents the production of IFN-γ, IL-2, TNFα, IL-1β, and IL-6 and activation and proliferation of T lymphocyte [85].

It is postulated that tacrolimus may be a plausible choice in management of reproductive disorders, such as RIF and RPL, especially in patients with an elevated level of Th1 cells, as it was investigated in the study of Nakagawa et al. In this prospective cohort study, RIF patients with elevated Th1 ($CD4^+$/IFN-γ^+)/Th2 ($CD4^+$/IL-4^+) ratio, received tacrolimus 2 days before embryo transfer, continued until a positive pregnancy test. The results indicated that RIF women who received tacrolimus had a significantly higher rate of clinical pregnancy and live birth rate, compared to control group, while the miscarriage rate was significantly decreased post-treatment [217].

The other prospective cohort study by this group included larger population of RIF patients, who had elevated Th1/Th2 ($CD4^+IFN\text{-}\gamma^+/CD4^+IL\text{-}4^+$) cells ratios (≥10.3) and were treated with tacrolimus. Dose of tacrolimus was adjusted based on the initial Th1/Th2 ratio. Th1 cells level were divided as low, medium, and high. Clinical pregnancy rates of low, middle, and high Th1 level groups were not statistically different. Successful ongoing pregnancy rate was statistically elevated in the low Th1 group when compared with the high Th1 group. However, the rate of live births was not significantly different between groups [218]. In Bahrami-Asl and colleagues' study, 10 RIF women with increased Th1/Th2 ratio were evaluated after tacrolimus treatment for expression of p53, leukemia inhibitory factor (LIF), IL-4, IL-10, IL-17, and IFN-γ in the endometrium. LIF, IL-10, and IL-17 expression were upregulated and IL-4, IFN-γ expression, and IFN-γ/IL-10 ratio were downregulated post-treatment. Moreover, rate of implantation, clinical pregnancy, and live birth were 40, 50, and 35% respectively, in RIF women without a history of previous successful pregnancy [219]. Considering the results of these studies, Th1/Th2 ratio may be a biomarker for predicting ART outcomes in RIF patients and selection of suitable candidates for tacrolimus administration. Of note, due to presence of a congenital heart abnormality in one of the babies in study of Nakagawa, careful considerations must be taken in administration of tacrolimus.

There are limited studies evaluating the effectiveness of tacrolimus for RPL and RSA patients. A case report study utilized tacrolimus for an RM patient with a history of 11 consecutive miscarriages in spite of receiving different treatments including low-dose aspirin, LMWH, prednisolone, and IVIG. After 12th conception, the patient showed an elevated rate of Th1/Th2 ratio, so she received tacrolimus (1 mg/d). In spite of this treatment, the patient miscarried. However, 13th conception of this patient was successful by receiving 2 mg/d of tacrolimus. The authors suggested the efficiency of immunosuppressive treatment with tacrolimus for RM patients with increased Th1/Th2 ratio [220].

There are studies that investigated the effectiveness of tacrolimus on both RIF and RPL patients. A study including 58 subjects, who were divided into two groups: (I) 31 subjects in RIF-alone group; and (II) 27 subjects in RIF-plus-RPL group was done by Hisano et al., in order to investigate the effect of tacrolimus on these patients. Frequency of Th1 was decreased after treatment in both groups, however, the reduction in Th1 frequency was delayed in Group II [221]. One hundred nine RIF or RPL women with increased peripheral Th1/Th2 ($CD4^+IFN\text{-}\gamma^+/CD4^+IL\text{-}4^+$) cell ratio received tacrolimus. One hundred thirteen babies, including 4 twins, were born. Obstetric complications including hypertension and one congenital abnormality were reported [222].

Further, well-designed and ideally randomized double-blind controlled studies are required to confirm the efficiency of tacrolimus in pregnancy complications with immunologic aberrations.

3.13 Platelet-rich plasma (PRP)

Recently, there is accumulating interest in the efficiency of intrauterine infusion of PRP in improving the pregnancy outcome in RIF patients. PRP is an autologous blood product, containing concentrated platelets in a small volume of plasma [223]. PRP may be able to promote endometrial receptivity through the various growth factors, cell adhesion molecules, and cytokines, stored in platelets' granules, including fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet-derived growth

factor (PDGF), VEGF, transforming growth factor (TGF), insulin-like growth factor I, II (IGF-I, II), connective tissue growth factor (CTGF) and IL-8 [151, 224]. Intrauterine administration of PRP induces the activation of platelets and release of above mentioned mediators in the uterus space, leading to cellular proliferation and differentiation, endometrial cell migration, and neu-angiogenesis alongside alteration of local immunologic responses [151]. In fact, platelets are capable of regulating immune responses. The TGFβ content of platelets mediates the immunosuppressive effect on T cells, furthermore, platelets can suppress the $CD8^+$ T cell's function [225].

There are limited studies investigating PRP therapy for reproductive disorders. Study by Nazeri et al. indicated that intrauterine infusion of 0.5 ml of PRP, 48 hours before ET, was effective in improvement of pregnancy outcomes in RIF patients [226]. Another study revealed that endometrial thickness, implantation rate, and per-cycle clinical pregnancy rate were higher in PRP administration [227]. The same positive effect of increasing the thickness of endometrium by PRP in RIF subjects was reported in the study of Mouanness et al. [228] and Coksuer et al. [229]. To our knowledge, there is only one study investigating the effectiveness of autologous PRP for improvement of pregnancy outcomes in RPL patients. Sixty-three RPL patients with a history of at least two previous pregnancy losses were divided into PRP receiving group and control group. The rate of clinical pregnancy and live birth was higher in patients who received intrauterine PRP. This study confirmed the efficiency of PRP administration in RPL women for the first time [230].

However, there is a lack of studies that investigate the effect of PRP on alteration of immunologic parameters in RIF and RPL women. Additionally, larger RCTs are still required to prove the efficiency of intrauterine PRP administration for reproductive failures.

3.14 Human chorionic gonadotropin (hCG)

HCG is an embryo-derived glycoprotein, which is involved in the process of implantation and regulation of endometrium receptivity. Prior to implantation, production of hCG is started by the blastocyst; after the implantation, the syncytiotrophoblast is responsible for hCG synthesis; furthermore, hCG is secreted by endometrium in the secretory phase [231]. Endometrial decidualization, receptivity, and immune system are regulated by hCG, through expressed hCG receptors on endothelium, in a paracrine manner. Additionally, the cytotrophoblast synthesizes a hyperglycosylated form of hCG, which plays a role in embryo implantation and trophoblast invasion [232].

The results of intrauterine infusion of hCG at the time of embryo transfer in RIF women, in an RCT, demonstrated increased implantation, pregnancy, clinical pregnancy, ongoing pregnancy, and live delivery rate in treated women [233]. Significantly higher implantation, clinical pregnancy, and ongoing pregnancy rate were observed in intrauterine administration of recombinant hCG (rhCG), prior to embryo transfer [234]. However, the positive effects of hCG were not confirmed in the study of Kathleen et al., in which no improvement in pregnancy outcome was achieved post-infusion of hCG [235]. Giuliani et al. investigated the effect of a single intrauterine hCG infusion at the time of embryo transfer on the distribution of NK cells in the uterine, in fertile oocyte donors. Stromal $CD56^+CD16^+$NK cells were evaluated in endometrial biopsies. Intrauterine hCG infusion was capable of enhancing the percentage of stromal $CD16^+$ cells; however, no statistical differences were observed in $CD56^+$ staining in hCG receiving group, compared with control group [232]. The

systematic review and meta-analysis of Gao et al. confirmed the efficiency of intrauterine injection of hCG in improvement of live birth, clinical pregnancy and ongoing pregnancy and implantation rate after IVF cycles. This study also emphasized that different effects of hCG on IVT-ET outcomes are related to different timing and dosages of hCG injection [236]. Further studies, including multicenter, randomized controlled trials, are suggested in order to confirm the conclusion of these meta-analysis, because of the great heterogeneity among the studies.

Swart et al. found that hCG supplementation during the mid-secretory phase for RPL women was able to significantly reduce the miscarriage rate [237]. The immunomodulatory effect of hCG was assessed in a cohort study, in which intrauterine infusion of hCG was administrated for infertile women with a decreased rate of $FoxP3^+$ Treg cells in mid-luteal phase. As a result, frequency of Treg cells and the clinical pregnancy rate were significantly elevated post-treatment [238]. The results of a cochrane database systematic review, assessing the efficacy of hCG in preventing further miscarriage in RM women indicated that hCG significantly decreased the miscarriage rate, but in the case of excluding two studies with lower methodological quality, no significant difference was observed. This study suggested well-designed RCTs of sufficient power and methodological quality to determine efficiency of hCG in RM patients [239].

It is concluded that there is a requirement for further studies including multicenter, randomized controlled trials, and preferential studies that consider immunologic aberration, in order to assess the efficiency of hCG administration for reproductive failures.

3.15 Mesenchymal stem cells (MSCs)

MSCs are stromal cells, derived from adipose tissue, umbilical cord blood, Wharton's jellies, endometrium, and amniotic fluid, which exhibit the ability of self-renewal, multilineage differentiation, secretion of multiple factors, and regulation of immune responses [240]. Considering these potentials, MSCs may be a promising approach for immunotherapy. According to the literature, the efficiency of MSCs, derived from different sources, has been evaluated in pregnancy-associated disorders, especially RPL, in animal models. According to animal studies, the action mechanisms of MSCs in improvement of pregnancy outcome in abortion-prone mice includes downregulation of Th1 cytokines, upregulation of Th2 cytokines, induction of switch of M1 macrophages to anti-inflammatory M2 type, reducing of lymphocytes proliferation, increasing the secretion of anti-inflammatory cytokines, such as IL-10 and TGF-β, which are mediated by mediators produced by MSCs or by cell–cell interaction [241].

Most of the animal studies were conducted on abortion-prone mice and RSA mouse models, however, only Tersoglio et al. study investigated the effect of MSCs on thin endometrium with repeated implantation failure. Endometrial changes were evaluated before and after administration of endometrial mesenchymal stem cells in 29 RIF patients with thin endometrium, hypo-responsive/unresponsive to estrogens. Endometrial thickness was increased significantly and immunologic parameters including T and B lymphocytes and NK cells were normalized post-treatment, resulting in an improvement in pregnancy outcomes [242].

Wharton jelly of human umbilical cord was utilized for isolation of MSCs for treatment of spontaneous-abortion rat model. Intravenous injection of bromocriptine was used for induction of abortion model, inducing degeneration of decidual cells. Transplantation of MSCs prevented the damage caused by injection and restored the

changes in expression and secretion of IL-10, IFN-γ, and IL-17, with IL-10 increasing and IFN-γ, IL-17 decreasing [243]. A recent study by Zhang et al. investigated the effect of umbilical MSCs on the expansion of Treg cells. Co-culture of MSCs with decidual Treg cells showed that MSCs are capable of promoting the expansion and suppressive function of decidual Treg cells besides elevating the IL-10 and TGF-β production, in vitro. Additionally, in vivo experiments, including transfer of bone marrow-derived MSCs to LPS-induced abortion model and spontaneous abortion model promoted the decidual Treg cell, meanwhile, the rate of absorption was decreased in both models [244]. The same immunoregulatory effect was confirmed for adipose-derived MSCs in abortion-prone mice, including reduction of IL-2 and IFN-γ and up-regulation of IL-4 and IL-10 production, reduction of IL-12, IL-2, and IFN-γ and upregulation of IL-4, IL-6, IL-10, and GM-CSF gene expression besides significant decrease in abortion rate [245]. Modulation of uNK cells and promotion of secretion of tolerogenic cytokines rather than inflammatory cytokines [246], switching off the decidual macrophages to an M2 phenotype and prevention of CD4+ T cells proliferation [247], decreasing the rate of Th1 cells while upregulating the Th2 responses and downregulation of lymphocytes proliferation against paternal antigens [246, 248] are among the immunoregulatory mechanism of MSCs, on animal models of abortion or RPL.

It seems that MSCs derived from adipose, bone marrow, Wharton jelly, and other resources are helpful in improvement of pregnancy consequences and modulation of abnormal immune systems in animal models. It is estimated that MSCs could be efficiently used in the immunotherapy of patients with reproductive failures, however further well-designed RCTs are required to confirm these findings.

3.16 Human amniotic epithelial cells (hAECs)

hAECs are derived from the closest layer of the term placenta to the fetus and have a high potential for proliferation and multilineage differentiation capacity. hAECs have gained considerable attention in recent years because of their stem cell characteristics, which differentiate into various cell lineages [249]. hAECs are also able to produce prostaglandin E2 and TGF-β and possess immunomodulatory effects on the proliferation and function of immune cells. Activity of NK cells, switch of M1 type macrophages to M2 type cells, apoptosis induction in T and B lymphocytes, and prevention of NK cells and macrophage migration, are mediated by the cytokines secreted from hAECs [250]. Immunomodulatory potential and low immunogenicity due to reduced expression of major histocompatibility complex (MHC) type I, make hAECs a promising approach for clinical applications [250].

To our knowledge, there is only one study that investigates the effects of hAEC on the immune cells of unexplained RSA patients, in vitro. Co-culture of naive $CD4^+$ T cells of URSA patients with hAECs indicated that proliferation of naive $CD4^+$ T cells and secretion of Th1 and Th17 cytokines were inhibited by hAECs, while secretion of Th2 cytokines and differentiation of Tregs alongside production of Treg associated cytokines were increased. Considering the immunosuppressive ability of hAECs, the authors suggested that these cells may be a promising choice in treatment of RSA patients [251].

4. Future prospective

In recent years, considerable progress has been made in the management of reproductive failures, including immunotherapeutic approaches. However, there is

a paucity of clinical data and well-designed qualified studies in this field, especially studies in which candidates for immunotherapy are selected based on evaluation of immunologic aberration. In addition, there is an urgent need for experiments that evaluate the pre- and post-treatment alteration of immunologic parameters, in addition to pregnancy outcome, to further determine the mechanism of action of the immunologic treatment in obstetric complications. Moreover, a "benefit to risk" evaluation of these therapeutic agents is required in order to determine the probable risk of pregnancy adverse outcome or fetus malformation. Further research is also required to update the knowledge about the newly introduced immunotherapeutic agents such as calcineurin inhibitors. There is also a need for studies which determine the immunological status of uterine, rather than peripheral blood, as immune status of peripheral blood does not always reflect the uterine immune status.

Among immunomodulator and immunosuppressive therapies which have already been introduced to clinical practice for the management of reproductive complications, including RIF and RPL, some still need further investigations. Immunosuppressive medications, such as cyclosporine, sirolimus, and tacrolimus seem to be beneficial, however, future studies are required to determine the appropriate candidates besides side effects.

Nowadays, therapeutic modalities with minimal side effects and ethical issues, besides high efficiency have gained a great deal of attention. In recent years, there has been increasing attention on the role of endometrial microbiota in reproductive disorders. Administration of probiotic formulation that includes species of lactobacillus or bifidobacterium, as most commonly used probiotic strains, or other probiotic strains, by different routes has been investigated in literatures. Probiotics consist of bacteria or non-pathogenic yeast, which colonize the gastrointestinal tract and exhibit health benefits when applied to the body [252]. It has been suggested that probiotics are capable of modulating the abnormalities of immune system in animal models, including upregulation of suppressive immune cells and mediators and downregulation of pro-inflammatory microenvironment [253]. The data on probiotics efficiency and mechanisms in pregnancy complications and its immunomodulatory effect, are limited and conflicting due to the heterogeneity of the studies [254]. Therefore, well-designed high-quality randomized controlled trials are required to comprehend the effectiveness of probiotics in reproductive complications, considering the probable immunomodulatory effect of probiotics.

Stem cells are the other promising approaches, which have gained attention in recent years based on their wide sources, easy sampling, low immunogenicity, and minimum ethical issues, which make stem cells an attractive therapeutic modality. There is increasing evidence indicating the exciting results of MSC therapy in autoimmune diseases, cancers, GVHD and etc. Nevertheless, a small number of studies have been conducted investigating the effectiveness of MSC-based therapy in RIF and RPL women [255]. Considering the immunomodulatory action of MSCs, it can be a promising approach in reproductive disorders with immunologic basis. In addition, extracellular vesicles derived from MSCs have also provided a novel insight into cell-free therapies for treatment of various diseases, as a safer and more suitable treatment for clinical applications. Therefore, MSC-derived extracellular vesicles may serve as an attractive modality, with similar therapeutic and immunomodulatory effects with MSCs, in the field of reproduction disorders.

The proposed action mechanism of immunotherapeutic approaches in improvement of pregnancy consequences in RRF patients is summarized in **Figure 3**.

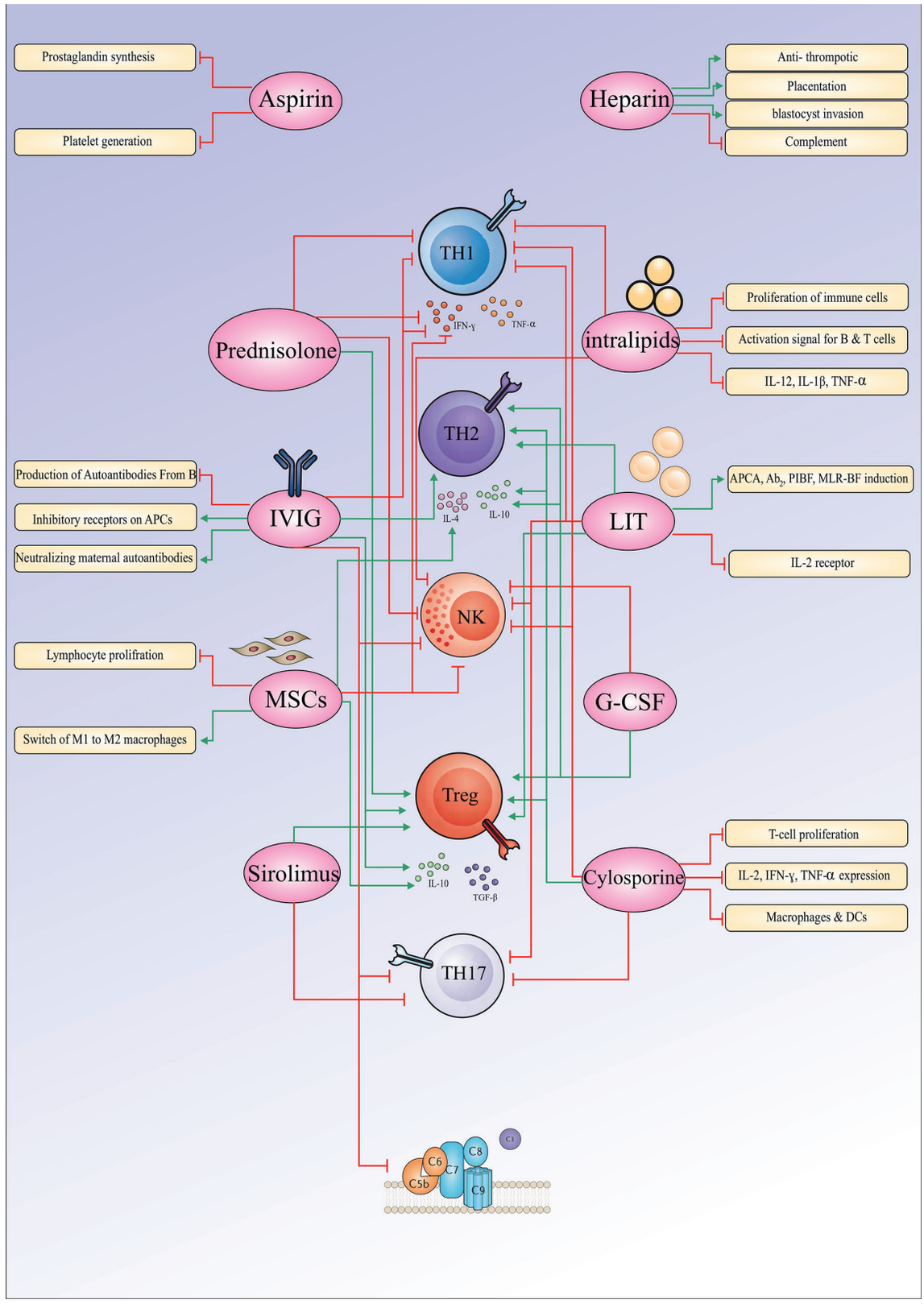

Figure 3.
The proposed action mechanism of immunotherapeutic approaches in improvement of pregnancy consequences in RRF patients. The common immune cells which are affected by immunotherapeutic modalities include Th1 and Th17 cells, which are downregulated by these approaches, in contrast, NK cell and Th2 and Treg cells frequency and/or cytokine secretion are upregulated by these immunologic methods. Abbreviation: NK: natural killer; Th1: T helper 1; Th2: T helper 2; Th17: T helper 17; Treg: T regulatory; TNFα: tumor necrosis factor α; IFNγ: interferon γ; IL-4: interleukin-4; IL-10: interleukin-10; IL-17: interleukin-17; TGFβ: Transforming growth factor beta; IVIG: Intravenous immunoglobulin; G-CSF: Granulocyte colony stimulating factor; MSC: mesenchymal stem cells; LIT: lymphocyte immunotherapy.

5. Conclusion

RRF is a frustrating condition for both couples and clinicians in the field of reproductive treatment and a significant concern for women who have already undergone ART treatments without favorable outcomes. Here, we discussed the immunologic aspects of reproductive failure, proposed mechanisms, and immunologic tests, besides the immunotherapeutic modalities. Given the limited number and quality of available research and heterogeneity of studies (sample size, patients' selection, dose, route, duration, etc.) which investigate the mechanism of immunologic imbalances in pathogenesis of RRF, further investigations are required to update the current knowledge about the immunoetiology of RRF. Additionally, considering the novelty of immunotherapy in the field of reproductive disorders, more experiments are required to determine the effectiveness of mentioned approaches in improvement of pregnancy consequences, besides their mechanisms and side effects. Nevertheless, the involvement of immunologic aberrations in pathogenesis of RRF (excluding other etiologies) and beneficial effects of immunotherapeutic approaches in the treatment of patients who are selected based on their immunologic basis, are indisputable. Therefore, selection of immunotherapeutic approach, based on the immunologic origin of complication, or personalized medicine in other word, is maybe the best solution for the dilemma.

Further advancement of the immunologic diagnostic test, is also helpful to assess the underlying immunoetiology of subject, prior to treatment. Nevertheless, considering the novel promising approaches, such as calcineurin inhibitors, MSCs therapy and related extracellular vesicles, and probiotics, already available therapeutic modalities, such as PBMC therapy and TNF-α inhibitors, immunologic-based RRF diagnosis and treatment have the potential to be the next great forthcoming development in the field of human reproduction.

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