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REVIEW

Role of immune cells in the establishment of implantation and maintenance of pregnancy and immunomodulatory therapies for patients with repeated implantation failure and recurrent pregnancy loss

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Abstract

Background: Immune cells play an important role in the establishment of pregnancy, and abnormalities in the immune system can cause implantation failure and miscarriage.

Methods: Previous papers have been summarized and the role of immune cells in reproduction is reviewed.

Results: The immune environment in the uterus changes drastically from before implantation to after pregnancy to maintain pregnancy. In allogeneic pregnancies, immature dendritic cells (DCs) that induce immune tolerance from outside the uterus flow into the uterus, and mature DCs that remain in the uterus express programmed cell death ligand 2, which suppresses the immune response. Macrophages are classified into M1-macrophages, which induce inflammation, and M2-macrophages, which suppress inflammation; M1-macrophages are required for luteinization, and M2-macrophages induce the differentiation of endometrial epithelial cells to enable implantation. Regulatory T cells, which suppress rejection, are essential for the implantation and maintenance of allogeneic pregnancies. Implantation failure and fetal loss are associated with decreased numbers or qualitative abnormalities of DCs, macrophages, and regulatory T cells. The clinical usefulness of immunomodulatory therapies in patients with repeated implantation failure and recurrent pregnancy loss has been reported.

Conclusion: The provision of individualized medical care in cases of implantation failure or miscarriage may improve clinical outcomes.

KEYWORDS
dendritic cells, M2 macrophage, recurrent implantation failure, recurrent pregnancy loss, Treg

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1 | INTRODUCTION

Mammals are fertilized inside the female body, the fetus develops in the uterus, and finally, the mother delivers the baby. This mode of reproduction is more effective than that of fish, amphibians, reptiles, and birds in protecting fetuses from predation. However, it was necessary to develop a new system in which the mother's immune cells do not reject the fetus, which is a semi-allograft, but tolerate it.[1–5] The mother's immune system is greatly altered in the uterus where pregnancy is established, especially at the site of implantation. By inducing immune tolerance to fetal antigens, the fetus is not rejected by maternal immune cells, and pregnancy continues.[1–4] Maternal macrophages (Mφ) involved in luteinization[6,7] and Mφ and dendritic cells (DCs) play important roles in the differentiation of endometrial epithelium and stroma and in angiogenesis in the decidua.[8,9] Thus, abnormalities in the maternal immune cells can lead to implantation failure and miscarriage.[10–15] Understanding changes in the maternal immune system associated with pregnancy is important from the standpoint of reproductive medicine and can help in the development of new treatments for implantation failure and pregnancy loss. In addition to endocrine system, which is conventionally known to play an important role in reproduction, it is now known that immune system and endocrine systems complement each other and work in concert to establish pregnancy.[16–18] In this review, the role of immune cells in mammalian implantation and the establishment of pregnancy is explained, followed by a discussion of treatments for repeated implantation failure (RIF) and recurrent pregnancy loss (RPL) from an immunological perspective.

2 | UTERINE LYMPHOCYTES ARE ACTIVATED BY RECOGNITION OF THE FETAL ANTIGENS

When a blastocyst is attached to the endometrial epithelium, no aggregation of maternal lymphocytes is observed in the endometrial stromal tissue; however, when the embryo breaks through the endometrial epithelial basement membrane and invades the endometrial stroma, many lymphocytes gather around the embryo.[19] This is very similar to the phenomenon that occurs when cancer cells penetrate the epithelial basement membrane and invade the stroma, where many lymphocytes infiltrate around the cancer cells.[20]

Conventionally, lymphocytes in the uterus were believed to be inactive because they can tolerate the fetus. Importantly, lymphocytes in the decidua increased in size, similar to activated lymphocytes.[4] Therefore, we examined whether the lymphocytes in the decidua were truly activated using flow cytometry.[21,22] The results showed that the activated markers such as CD69 and human leukocyte antigen (HLA)-DR antigens were detected in approximately 60% and 70% of the $CD4^+$ T cells in the decidua (approximately 1% and 10% in the peripheral blood), approximately 70% and 80% of the $CD8^+$ T cells in the decidua (approximately 3% and 20% in the peripheral blood), and approximately 70% and 80% of the $CD16^-CD56^{bright}$ natural killer (NK) cells in the decidua (approximately 10% and 20% in the peripheral blood), respectively, indicating that the lymphocytes in the decidua are in an activated state. These activating lymphocytes produce vascular endothelial growth factor (VEGFα), angiopoietin, and interleukin (IL)-8, which are involved in angiogenesis; interferon-γ (IFN-γ), which acts in the remodeling of the spiral artery; and macrophage-colony stimulating factor (M-CSF), granulocyte macrophage-CSF (GM-CSF), and granulocyte-CSF (G-CSF), which are involved in trophoblast growth and differentiation and are actively involved in the establishment of pregnancy.[23–28]

The fetal and maternal immune cells are not in direct contact with each other. Maternal immune cells are in direct contact with placental epithelial cells (syncytiotrophoblasts) of fetal origin.

T-cell receptors recognize antigens bound to the major histocompatibility complex (MHC). Therefore, neither maternal $CD4^+$ nor $CD8^+$ T cells can recognize the antigens expressed on villous trophoblasts because neither MHC class I nor II molecules are expressed on syncytiotrophoblasts. In contrast, extravillous trophoblasts (EVT), which invade decidual stromal cells, express only HLA-C, an MHC class I molecule. Therefore, maternal $CD8^+$ T cells can react with HLA-C antigens of paternal origin expressed on EVT.[29–31]

Whether lymphocytes in the decidua are activated as a result of recognizing fetal antigens or paternally derived HLA-C antigens has not yet been fully proven. However, some data suggest that maternal T cells recognize fetal or paternal antigens. When maternal lymphocytes recognize fetal antigens, they are activated and proliferate at the feto-maternal interface, which should result in an increase in clonally proliferated T cells with the same T-cell receptor: (1) in peripheral blood, clonally proliferated $CD4^+$ T cells and $CD8^+$ T cells are rarely observed, (2) the decidua contains clonally expanded $CD4^+$ T cells and $CD8^+$ T cells, and (3) T cells with the same T-cell receptor sequence were observed in the decidua in the first and second pregnancies,[32–34] suggesting that clonally proliferating T cells recognize the same paternal HLA-C or fetal antigens expressed on EVT in the first and second pregnancies.

In summary, lymphocytes likely recognize and activate fetal/paternal antigens in utero. These lymphocytes secrete many cytokines that are involved in the establishment of pregnancy; however, the question has been raised as to why the fetus is not rejected.

3 | WHY IS THE FETUS NOT REJECTED DESPITE THE ACTIVATION OF INTRAUTERINE LYMPHOCYTES?

3.1 | Increased regulatory T (Treg) cells contribute to the maintenance of pregnancy

During pregnancy, Treg cells, which suppress immune activation and rejection, increase in the decidua[2,12–15,35] (Figure 1). Blood estrogen and progesterone levels increase during pregnancy and induce an

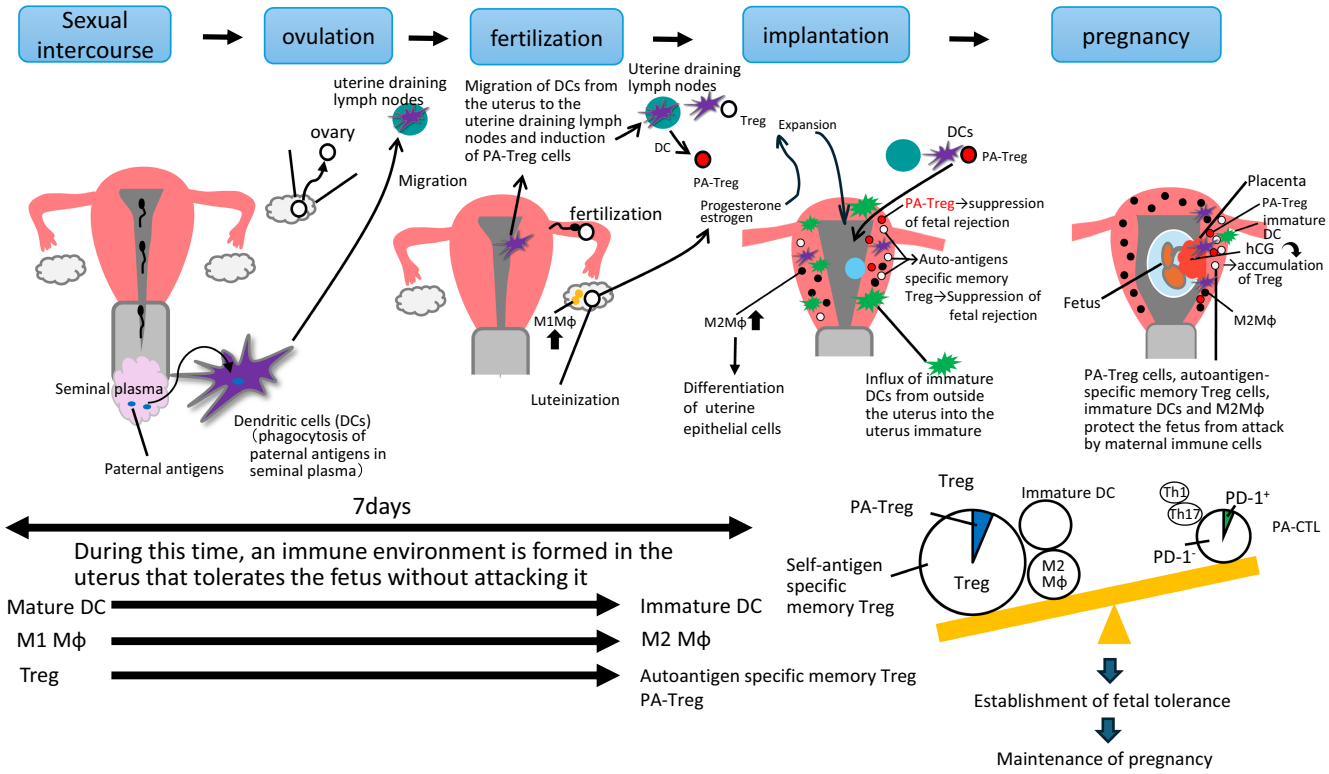

FIGURE 1 Changes in the intrauterine immune environment from sexual intercourse to the establishment of pregnancy. We show the changes in the immune environment in the uterus from sexual intercourse to pregnancy, focusing on DCs, Mφ, and Treg cells. These cells coordinate hormonal changes to help establish pregnancy. PA-Treg, Paternal antigen specific Treg.

increase in Treg cells.[36,37] Additionally, human chorionic gonadotropin (hCG) secreted from chorionic villi accumulates in Treg cells at the site of implantation by inducing chemokine (C-C motif) ligand 2.[38,39] Progesterone produced by the corpus luteum not only increases the number of Treg cells and their function at implantation to prevent fetal rejection but also contributes to fetal development by promoting remodeling of the uterine spiral artery.[40]

In mouse experiments, depletion of Treg cells at the time of implantation causes implantation failure in allogeneic pregnancies, but implantation is successful in syngeneic pregnancies.[10,11,13] In addition, depletion of Treg cells in early pregnancy causes fetal loss in allogeneic pregnancies but not in syngeneic pregnancies.[11,13,41,42] Therefore, Treg cells at the site of implantation prevent rejection of the fetus, which is a semi-allograft, and contribute to establishing implantation and continuing the pregnancy after implantation. Treg cells also contribute to fetal development by increasing maternal blood flow to the intervillous space by promoting the remodeling of the uterine spiral artery.[43] In other words, Treg cells protect the fetus and placenta from attacks by maternal immune cells. Once pregnancy is established, Treg cells are also involved in placentation. Treg cells are broadly classified into fetal/paternal antigen-specific Treg cells and memory Treg cells for self-antigens.[5,30,31,44,45] Treg cells are also classified as natural Treg cells, which develop in the thymus, and induced Treg cells, which develop in the periphery.[46] At the time of implantation and early pregnancy, natural and memory Treg cells increase in the decidua,[47–49] whereas induced Treg cells and fetal/paternal antigen-specific Treg cells increase in the third trimester of pregnancy.[47–49] In early pregnancy, as in cancer, antigen-nonspecific immunosuppressive memory Treg cells protect the fetus from rejection,[50,51] while in late pregnancy, fetal antigen-specific Treg cells are thought to protect the fetus from rejection[30,31,45,47] (Figure 1). Fetal/paternal antigen-specific Treg cells are present even after parturition and promptly increase in the next pregnancy to contribute to placentation and pregnancy maintenance.[45] In summary, antigen-nonspecific memory Treg cells and fetal antigen-specific Treg cells cooperate to maintain pregnancy.

4 | IMMUNE CELLS OTHER THAN TREG CELLS THAT CONTRIBUTE TO ALLOGENEIC PREGNANCY MAINTENANCE

In peripheral blood, 70%–75% of lymphocytes are T cells, 5%–20% are NK cells, and 10% are B cells. In the decidua, $CD16^{-}CD56^{bright}$ NK cells (uterine NK: uNK cells), which are present in only 0.5% of peripheral blood, comprise 80%, $CD16^{+}$ NK cells, which are predominantly peripheral blood NK cells, are present only in 2%–3%.[20,21,27] In the decidua, T cells are present in only 10%–15% of the population, and the B cell population is only 1%. The cytotoxic activity of uNK cells is low compared with that of $CD16^{+}$ NK cells, and they do not directly attack trophoblasts. However, when stimulated by

IL-2 or IFN-γ, their cytotoxic activity increases, leading to miscarriage.[28,52–54] uNK cells produce VEGF, G-CSF, M-CSF, IL-8, IFN-γ, etc., and are involved in angiogenesis.[23–28,55] These cytokines are useful for angiogenesis and trophoblast proliferation and differentiation. In addition, uNK cells in the endometrium of women who have given birth highly express NKG2C and LILRB1, recognize HLA-E and HLA-G expressed on EVT, produce VEGFα for angiogenesis and IFN-γ for spiral artery remodeling, and play a role in placenta formation.[56] Thus, uNK cells, such as Treg cells, have a memory function to remember previous pregnancies. This explains the increased risk of preeclampsia in the first pregnancy and the lower incidence of preeclampsia in parous women.[57]

T-cell activation requires the recognition of antigen peptides by T-cell receptors and co-stimulatory signals via the binding of CD80/86 complexes or B7 molecules on antigen-presenting cells, DCs, and CD28 on T cells. CD80/86 complexes on DCs in the uterus are downregulated.[8,58–60] Activation of T cells without co-stimulation leads to the unresponsiveness of T cells, resulting in immune tolerance of the fetus, suggesting that downregulation of CD80/86 complexes on DCs prevents fetal rejection. Additionally, indolamine 2,3-dioxygenase (IDO), an enzyme that degrades tryptophan, reduces the cytotoxic activity of $CD8^+$ T cells by decreasing tryptophan levels. IDO is expressed in trophoblast cells and in DCs and Mφ in the decidua, preventing maternal $CD8^+$ T cells from attacking fetal and placental cells.[61–63] IDO expression is downregulated in miscarriages, suggesting that IDO plays an important role in pregnancy maintenance.[61,63,64]

Programmed cell death protein 1 (PD-1) is a receptor expressed on the surface of activated T cells. Programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2) are ligands that bind to PD-1 and are expressed in cancer cells.[65,66] When PD-1 expressed on activating T cells binds to PD-L1 or PD-L2 expressed on cancer cells, these activated T cells cannot attack the cancer cells. Trophoblasts express PD-1; therefore, PD-1 expressing -$CD8^+$ T cells and -$CD4^+$ T cells are unable to attack the placenta, and the fetus is protected from maternal T-cell attack.[67] Recently, immune checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 antibodies have been used for cancer therapy. Complications such as preterm delivery, HELLP syndrome, low birth weight, and placental insufficiency have been reported in a few cases due to the use of immune checkpoint inhibitors as cancer therapy during pregnancy.[68] In human recurrent miscarriage cases, decidual macrophage-derived nitric oxide downregulates PD-L1 expression in trophoblasts, leading to a decrease in Treg cells.[69] These clinical events suggest that PD-L1 expression in trophoblasts is involved in the induction of mother-to-child tolerance.

Trophoblasts also produce IL-10 and transforming growth factor-β, which nonspecifically suppress immune responses and prevent fetal rejection by the maternal immune system.[70,71]

Thus, through Treg cells and various immunosuppressive mechanisms, the fetus, a semi-allograft, is protected from attacks by maternal immune cells.

5 | IMPLANTATION AND IMMUNITY

5.1 | Treg cells and implantation

In the mouse system, a decrease in Treg cells during the implantation period causes implantation failure in allogenic pregnancies, whereas implantation is successful in syngeneic pregnancies.[10,13] Since embryonic cells express major histocompatibility antigens, it is likely that Treg cells suppress the attack on the embryo by $CD8^+$ T cells that recognize fetal/paternal antigens. Paternal antigen-specific Treg cells increase in the uterine regional lymph nodes 1 day before implantation in mice[42,44,72] (Figure 1). The removal of the seminal vesicle, which produces seminal plasma, results in no increase in paternal antigen-specific Treg cells or a decrease in the number of implantations, suggesting that seminal plasma plays an important role in the differentiation of paternal antigen-specific Treg cells[42,44,72] (Figure 1). Seminal plasma contains transforming growth factor-β and prostaglandin E2, which induce Treg cells,[72] as well as soluble paternally derived HLA-class I antigen.[73] It has been suggested that maternal DCs phagocyte these antigens, present paternal MHC-class I antigens to Treg cells, and induce paternal antigen-specific Treg cells in uterine regional lymph nodes.[74] These paternal antigen-specific Tregs quickly migrate to the uterus after implantation[44] (Figure 1).

In humans, forkhead box P3 (Foxp3) mRNA expression, a gene essential for Treg cell differentiation, is decreased in the endometrium in cases of RIF,[75,76] and a decrease in the number of Treg cells in the endometrium and an increase in exhausted Treg cells have been reported in RIF cases.[77] These data indicate that Tregs play an important role in human implantation. The clinical pregnancy rate is higher when seminal plasma is administered vaginally during in vitro fertilization (IVF) or when spontaneous intercourse is performed.[78,79] These findings suggest that seminal plasma may induce paternal antigen-specific Treg cells and promote their implantation in humans.

5.2 | Dendritic cells and implantation

DCs are antigen-presenting cells. DCs present antigen peptides and activate T cells in an antigen-specific manner. In addition to antigen presentation, DCs play an important role in implantation[8,58,80,81] (Figure 1). In mice, DCs increase in the uterus during implantation and play an important role in decidua formation.[8] Artificial depletion of DCs at the time of implantation results in implantation failure in both allogeneic and syngeneic pregnancies.[8] This differs from the phenomenon in which implantation failure occurs only in allogeneic pregnancies in which Treg cells are depleted. Intrauterine DCs contribute to implantation by inducing angiogenesis in the decidua and the proliferation and differentiation of decidual stromal cells.[8,81] These reports indicate that DCs are involved in the differentiation of the decidua rather

than in suppressing fetal rejection. We investigated whether these increases were caused by the proliferation of DCs in the uterus or by influx from outside the uterus and found that most DCs were influxed from outside the uterus[58] (Figure 1).

DCs are broadly classified into mature DCs that activate immunity and immature DCs that induce immune tolerance. In mice, immature DCs increase only during allogeneic pregnancies, and mature DCs decrease immediately before implantation.[58] Most immature DCs are derived from extrauterine tissues and may suppress maternal immune responses in allogeneic pregnancies. After sexual intercourse, seminal plasma induces intrauterine DCs to differentiate into mature DCs, but most DCs migrate to the uterine regional lymph nodes[58] (Figure 1). Uterus-derived DCs are thought to take paternal antigens contained in seminal plasma, migrate to the uterine regional lymph nodes, and induce paternal antigen-specific Treg cells by presenting paternal antigens.[74] Paternal antigen-specific Treg cells in the uterine lymph nodes migrate immediately to the uterus after implantation to prevent rejection of the embryo[44] (Figure 1). In contrast, a small number of mature DCs that remain in the uterus express high levels of PD-L2, thus preventing PD-1-expressing maternal $CD8^+$ T cells from attacking the fetus.[58]

In summary, intrauterine DCs not only induce angiogenesis in the decidua and proliferation and differentiation of decidual stromal cells to ensure successful implantation but also induce immune tolerance by immature DCs and PD-L2-expressing mature DCs to prevent rejection of the embryo as a semi-allograft.

5.3 | Macrophages and implantation

Macrophages (Mφ) are broadly classified into M1Mφ, which induce inflammation and activate immunity, and M2Mφ, which suppress inflammation.[82] When CD11b-diphtheria toxin receptor mice were treated with a small amount of diphtheria toxin, CD11b positive cells (M1Mφ and M2Mφ) were removed during implantation, resulting in implantation failure.[6] A detailed examination revealed that implantation failure resulted from low blood progesterone levels due to inadequate luteinization of the ovary.[6] However, when M2Mφ was removed in CD206-diphtheria toxin receptor mice with diphtheria toxin, implantation failure was observed despite normal luteal formation and elevated blood progesterone concentrations.[7] This suggests, indirectly, that M1Mφ is required for luteinization (Figure 1). Embryos from M2Mφ-depleted female mice were harvested and transferred to pseudopregnant mice, where they implanted normally, indicating that implantation failure was not due to poor embryo quality in M2Mφ-depleted mice.[9] Endometrial epithelial cells from M2Mφ-eliminated mice expressed the cell proliferation marker Ki67. Furthermore, Wnt4, Wnt7B, and β-catenin expression were also upregulated, suggesting that the Wnt-β-catenin pathway contributes to the proliferation of uterine epithelial cells.[9] In addition, the removal of M2Mφ also resulted in the dominance of M1Mφ in the uterus and increased tumor necrosis factor-α mRNA expression, which induces inflammation.[9] In other words, intrauterine M2Mφ prevents excessive inflammation caused by M1Mφ, regulates the proliferation of endometrial epithelial cells, and promotes the differentiation of endometrial epithelial cells, which may lead to successful implantation (Figure 1).

We examined the expression levels of CD206 mRNA, a marker of M2Mφ, and CD68 mRNA, a marker of M1Mφ, in endometrial biopsy specimens from patients with infertility in the mid-secretory phase. In the next IVF, the ratios of CD206/CD68 mRNA were significantly higher in successful pregnancies than in unsuccessful pregnancies,[9] suggesting that the M2Mφ dominant environment is useful for the establishment of pregnancy. G-CSF, which promotes differentiation into M2Mφ from M1Mφ,[83] significantly increases the clinical pregnancy rate in IVF in RIF cases.[84,85] These clinical outcomes may explain how G-CSF is linked to the treatment of implantation failure through its mechanism of action.

5.4 | Efficacy of immunomodulatory therapy for patients with RIF

RIF is defined as three or more unsuccessful intrauterine transfers of good-quality embryos in women aged <40 years. In addition to uterine malformations, endometrial polyps, endometrial adhesions, endometritis, and genetic abnormalities in the parents, intrauterine immune abnormalities are also risk factors.[86] Therefore, several immunotherapies have been attempted for RIF cases.

Subcutaneous injection of G-CSF significantly increased the clinical pregnancy rate (risk ratio 2.29, 95% CI [1.58–3.31]) in patients with RIF using meta-analysis[84] (Table 1). Intrauterine administration of G-CSF also significantly increased the rate of clinical pregnancy (risk ratio 1.53, 95% CI [1.00–2.33]) in patients with RIF.[84] A recent network meta-analysis revealed that subcutaneous injection of G-CSF was more effective than the intrauterine administration of G-CSF.[85]

Intrauterine infusion of autologous peripheral blood mononuclear cells stimulated with hCG significantly increased the clinical pregnancy rate (risk ratio 2.18, 95% CI [1.58–3.00])[17,18,84] (Table 1). As hCG activates Treg cells and causes them to migrate,[37,38] these effects may improve the implantation rate. It is also unclear which peripheral blood mononuclear cells stimulated with hCG are responsible for the improvement of implantation failure. However, the optimal number of mononuclear cells to be infused and the optimal dose of hCG stimulation have not yet been determined (Table 1). The lymphocytes must be cultured in their own facilities. Future multicenter studies should be conducted under specific conditions to verify the efficacy of intrauterine mononuclear cell therapy in patients with RIF.

Two observational studies of intrauterine hCG infusion have demonstrated a significant increase in the clinical pregnancy rate (odds ratio 1.81, 95% CI [1.23–2.65]).[84,85] These results and the intrauterine infusion of peripheral mononuclear cells stimulated with hCG may improve the implantation rate by improving Treg cell

TABLE 1 Immunomodulatory therapies for patients with repeated implantation failure (RIF) and recurrent pregnancy loss (RPL).

Treatment	Immunological mechanisms	Problems
RIF		
• Intrauterine administration of auto logous peripheral blood mononuclear cells (PBMCs) activated by hCG	• Treg function↑ • Variety of cytokines produced by PBMCs improve the receptivity of the endometrium	• Not clear which cells improve RIF • Few studies • No uniform protocols • Cell culture needs to be carried out at own institution
• Subcutaneous G-CSF administration	• Treg induction • Tolerogenic DC induction • Inhibition of Th1-type immunity	• More higher quality studies are needed • Side effects of G-CSF such as fever and malaise
• Intravenous immunoglobulin (IVIG)	• Treg expansion • NK cell cytotoxicity↓ • Neutralization of autoantibodies	• Few studies • High cost
• Seminal plasma	• Induction of paternal antigen-specific Treg	• The study subjects are IVF cases, not RIF
RPL		
• Intravaginal progesterone therapy for RPL patients with 3 or more miscarriages and genital bleeding in the current pregnancy	• Induction of cytokine secretion with immunomodulatory effects • Differentiation of uNK cells • Induction of M2Mφ • Suppression of neutrophil activation	• Few studies • Requires conditioning of progesterone dosage, timing of initiation of administration, etc.
• IVIG for unknown etiology of RPL patients with 4 or more in the very early stage of pregnancy	• Treg expansion • Attenuation of NK cell cytotoxicity • Neutralization of auto-antibodies	• Few studies • Requires conditioning of IVIG dosage, timing of initiation of administration, etc.
• Low molecular weight of heparin for RPL patients with 3 or more miscarriage	• Regulation of complement activation • Regulation of activation of granulocytes	• Few studies • Efficacy of unfractionated heparin has not been reported

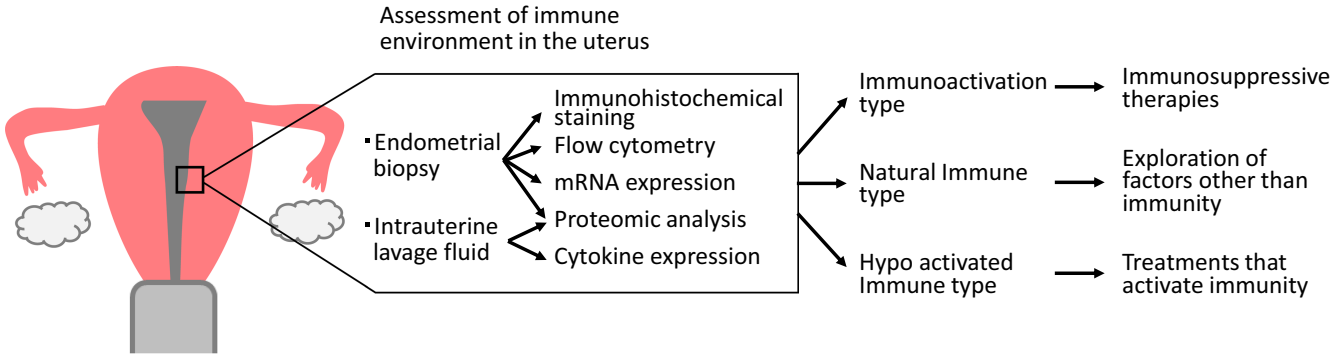

FIGURE 2 Treatment strategy for RIF and RPL cases based on a diagnosis of the immune environment in the uterus. The pathogeneses of RIF and RPL differ among patients. Therefore, it is important to investigate immune function abnormalities in the uterus and provide the most appropriate treatment for each condition.

function and increasing the number of Treg cells through the migration of Treg cells into the uterus, which may lead to an improved prognosis. The immune environment of the uterus after intrauterine administration of hCG requires further investigation.

High-dose intravenous immunoglobulin (IVIG) therapy increases the number of Treg cells, decreases NK cell activity, and suppresses inflammation.[87-90] A significant increase in the clinical pregnancy rate (odds ratio 2.08, 95% CI [1.28–3.36]) has been reported in patients with RIF treated with IVIG by observational studies[84] (Table 1). As discussed below, IVIG therapy at 4–5 weeks of gestation significantly increases the rate of live births in patients with four or more previous miscarriages and unknown risk factors. IVIG therapy also increases the number of effector Treg cells with high immunosuppressive activity in peripheral blood.[87]

Other methods, such as the endometrial injury method, which induces inflammation of the endometrium, and intralipid administration,

which suppresses the cytotoxic activity of NK cells, slightly, but not significantly, improve the prognosis of RIF cases in a meta-analysis.[84,85]

At present, there is no high-evidence-level treatment for RIF; however, the optimal treatment should be selected after first checking the immune environment in the uterus. However, it is difficult to determine the immune environment in the endometrium using peripheral blood. Therefore, it is necessary to determine the immune environment in the uterus using biopsies of the endometrial tissue or intrauterine lavage fluid, although this is invasive for patients (Figure 2).

6 | RPL AND IMMUNITY

RPL is defined as a history of two or more clinical miscarriages or stillbirths, with a frequency of 1%–4%.[91] The European Society of Human Reproduction and Embryology (ESHRE) counts biochemical pregnancy as pregnancy loss,[92] whereas it is not included in the U.S. and Japan.[91] The established risk factors for RPL include abnormal uterine morphology, hypothyroidism, chromosomal abnormalities in couples, and positive antiphospholipid antibodies.[91–93] RPL of unknown etiology is found in 50%–60% of cases. As discussed below, various immune abnormalities have been reported in RPL cases, and it is possible that the fetus is attacked by maternal immune cells and miscarries owing to maternal immune abnormalities. The following is a description of the immune system changes in RPL cases and the treatment of cases of unknown etiology.

6.1 | RPL from the perspective of Th1/Th2/Th17/Treg cell balance

$CD4^+$ T cells are broadly classified into Th1 cells, which induce cellular immunity and rejection; Th2 cells, which contribute to antibody production; Th17 cells, which induce inflammation and rejection; and Treg cells, which suppress immune activation and inflammation.[94] In the field of reproductive immunology, around 1990–2000, the Th1/Th2 ratio was examined in cases between RPL,[95] and it became clear that Th1 immunity was predominant in RPL. Later, with the identification of Th17 and Treg cells, the Th1/Th2/Th17/Treg cell balance was examined in RPL.[94,96] Although changes in immunological balance cannot be unified in RPL because risk factors differ from case to case, in many cases, the Th1/Th2/Th17/Treg cell balance showed Th1 immunity and Th17 immunity predominance and decreased Treg cells.[94–96] These changes in the immune system in RPL indicate that the environment is more likely to reject the fetus as a semi-allograft. Miscarriages can be caused by fetal chromosomal abnormalities, even in the absence of abnormal maternal immunocompetence. Since chromosomal abnormalities are found in approximately 50% of miscarriages, the remaining 50% may be caused by antiphospholipid antibodies, hypothyroidism, uterine malformations, and unknown risk factors such as maternal immune abnormalities. Currently, the etiology of maternal immune abnormalities is unknown. Preimplantation genetic testing for aneuploidy is now being performed to prevent miscarriages due to fetal chromosomal abnormalities, and the miscarriage rate has decreased.[97] However, there is a need to reduce the number of fetal losses with normal fetal chromosomes in cases of RPL of unknown etiology.

To understand the changes in the immune environment in cases of miscarriage, it is necessary to examine the changes in the immune system in miscarriage cases with normal fetal chromosomes. Since Th1 and Th17 cells are increased in patients with miscarriages with normal fetal chromosomes,[94–96,98] it is possible that Th1 and Th17 cells may reject the fetus, albeit indirectly. In mice, fetal loss occurs during allogeneic pregnancies when Treg cells are reduced.[3,11,13] Because Foxp3 is the master gene for Treg cells, immunostaining for Foxp3 can be used to examine the localization of Treg cells. The number of Treg cells in the decidua basalis of miscarriages with normal fetal chromosomes was significantly decreased compared with that in normal pregnancies, while the number of Treg cells did not decrease in miscarriages with abnormal fetal chromosomes.[99] In the decidua parietalis, which is far from the implantation site, there was no decrease in the number of Treg cells in either normal or abnormal fetal chromosome miscarriages.[99] The increase in Th1 and Th17 cells and the decrease in Treg cells in the decidua basalis, the site of the mother-fetus interface in miscarriages with normal fetal chromosomes, suggest that fetal rejection also occurs in human miscarriages. $CD8^+$T cells that recognize fetal/paternal antigens show no cytotoxicity against fetal cells expressing PD-1. PD-1^-CD8^+ T cells can attack EVTs expressing paternal antigens. In miscarriages with normal fetal chromosomes, decidual PD-1^-CD8^+ T cells specific for the paternal antigen were found to be increased,[34] suggesting that maternal $CD8^+$T cells attack the fetus.

M1Mφ is predominant in miscarriage cases,[60,100] and IDO expression in macrophages and DCs is decreased,[61] suggesting an immune environment in which the fetus is susceptible to rejection by the maternal immune system. Taken together, these findings strongly suggest that in human cases of miscarriage of unknown etiology with normal fetal chromosomes, miscarriage is caused by immune abnormalities. In these cases, the improvement of the abnormal immune environment may also be a treatment for RPL.

7 | TREATMENT OF RPL

In cases of RPL with known risk factors, treatment of each risk factor can provide a good prognosis. For example, low-dose aspirin and heparin therapy should be administered in antiphospholipid antibody-positive cases.[91–93] Treatment of hypothyroidism with levothyroxine prevents miscarriages. Genetic counseling should be provided to couples with chromosomal structural abnormalities. Preimplantation genetic testing for chromosomal structural rearrangement should be proposed after adequate explanation.

7.1 | Treatment of cases of RPLs with unknown etiology

Currently, there is no evidence-based treatment for RPL of unknown etiology. The ESHRE guidelines state that husband lymphocyte immunotherapy, which has been widely used in the past, is ineffective, has side effects, and should not be used.[92] Glucocorticoids, intralipid therapy, G-CSF, and endometrial scratching do not improve prognosis in cases of RPL with unknown etiology.[92,101]

Haas et al.[102] showed from the results of four RCTs that progesterone administration from early pregnancy tends to reduce the miscarriage rate in cases of RPL with a history of three or more miscarriages (RR 0.59: 95% CI: 0.34–1.01). However, these differences were not statistically significant. Intravaginal progesterone therapy may be effective in patients with a history of three or more miscarriages and genital bleeding at the time of the next pregnancy (Table 1). Transvaginal progesterone administration (400 mg progesterone daily until 16 weeks gestation) significantly increased the rate of live births (odds ratio 2.1, 95% CI: 1.0–4.4) in the above patients with RPL if started at less than 12 weeks gestation.[103] Progesterone increases the number of Treg cells, enhances Treg cell function, and suppresses inflammation,[37,40,101] which may reduce miscarriage. In addition, progesterone increases the production of progesterone inducible blocking factor and promotes the differentiation of uterine NK cells and M2Mφ,[15,104] which may play a role in maintaining pregnancy. Thus, progesterone replacement in early pregnancy may improve pregnancy outcomes in RPL. However, the optimization of patient selection, timing of administration, and whether oral or vaginal administration should be clarified.

A recent study reported that IVIG therapy early in pregnancy (4–5 weeks of gestation) improves live birth rates in cases of RPL of unknown etiology and a history of four or more miscarriages[105] (Table 1). IVIG therapy initiated after 6 weeks of gestation did not improve the live birth rate.[105] IVIG therapy has been reported to increase Treg cells, decrease NK cell activity, and neutralize unknown autoantibodies, and anti-inflammatory effects.[87,101,106] In a previously described case with an unknown etiology and a history of four or more miscarriages, IVIG therapy decreased NK cell activity at one-week post-treatment, but there was no difference in NK cell activity at 8 weeks of gestation between the treated and non-treated groups, indicating that IVIG therapy reduced NK cell activity only for a short period of time.[87] In contrast, effector Treg cells in the peripheral blood remained significantly higher than those in the non-treated group 1 week after IVIG administration and at 8 weeks of gestation, suggesting that IVIG therapy increased the number of Treg cells and may have contributed to the maintenance of pregnancy.[87]

Aspirin alone does not improve the prognosis of RPL of unknown etiology.[101] In contrast, a meta-analysis reported that low-molecular-weight heparin reduced the risk of miscarriage in patients with RPL and three or more prior miscarriages (Table 1). Five RCTs were analyzed and found that low-molecular-weight heparin significantly reduced the risk of miscarriage in patients with RPL with three or more prior miscarriages (RR 0.46: 95% CI: 0.35–0.61; $p = 0.001$).[101,107] Because heparin suppresses complement activity and inhibits leukocyte chemotaxis and activation,[101] these effects may reduce miscarriage. To date, there have been no reports that unfractionated heparin or combination therapy with low-dose aspirin and unfractionated heparin significantly improves live birth rates in patients with RPL of unknown etiology.[100] At present, unfractionated heparin therapy may be effective in RPL cases with three or more previous miscarriages and no thrombocytopenia[101] (Table 1).

In summary, intravaginal progesterone, IVIG therapy, or low-molecular-weight heparin may be effective in cases of RPL of unknown etiology. However, further clinical studies are needed to increase the number of patients with high levels of evidence (Table 1). Further clinical studies are needed to increase the number of cases and improve the level of evidence.

8 | ACCURATE DIAGNOSIS OF THE INTRAUTERINE IMMUNE ENVIRONMENT AND ESTABLISHMENT OF PRECISE, PERSONALIZED TREATMENTS FOR EACH IMMUNOLOGICAL ABNORMALITY

The first thing to emphasize is that the immune environment of the uterus is different from that of peripheral blood. For example, $CD16^{-}CD56^{bright}$ NK cells predominate in the endometrium, whereas T cells predominate in peripheral blood, making it difficult to detect abnormalities in the intrauterine immune environment even if blood samples are taken from the peripheral blood, and the immune function is examined. For example, Treg cells are reduced in the decidua of miscarriage cases with normal fetal chromosomes but not in the peripheral blood.[108] Therefore, endometrial biopsy or examination of intrauterine lavage fluid is appropriate to diagnose the intrauterine immune environment (Figure 2). Lédée et al. studied NK cells by immunohistochemical staining and expression of mRNAs in the endometrium. Subsequently, IL-15/Fn-14 and IL-18/TWEAK mRNA ratios were determined.[109] Infertility or RPL cases were classified as over-immune-activation type, normal immune type, low immune activation type, mixed profile type, and individualized treatment. For the low immune activation type, endometrial scratching, luteal hCG supplementation, and seminal plasma exposure were performed. In cases of overactivation, immunotherapy was performed, such as exposure to high estrogens in the proliferative phase and hormone adaptation of the luteal phase. Good live birth rates were achieved with individualized treatments for each type.[109] Nevertheless, clinical trials with a higher level of evidence should be conducted in the future, as accurate diagnosis of the immune environment in the uterus and appropriate treatment are still in the pilot phase.

9 | CONCLUSION

Immune cells play important roles in implantation and subsequent placentation. Immune cells, especially Treg cells, immature DCs, and

M2Mφ, maintain pregnancy by suppressing immune activation and excessive inflammation. Numerous basic and clinical studies have shown that implantation failure and miscarriages occur when this balance is disturbed. Accurate evaluation of immune abnormalities in each case and optimal initiation of treatment just prior to conception or very early in pregnancy may lead to the treatment of RIF and RPL.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interests for this article.

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REFERENCES

1. Erlebacher A. Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol. 2013;13:23–33. https://doi.org/10.1038/nri3361
2. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. Cell. 2012;150:29–38. https://doi.org/10.1016/j.cell.2012.05.031
3. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5:266–71. https://doi.org/10.1038/ni1037
4. Saito S, Nakashima A, Shima T, Tsuda S. Pregnancy depends on a delicate balance of immune activation and regulation. Explor Immunol. 2021;1:461–78. https://doi.org/10.37349/ei.2021.00031
5. Tafuri A, Alferink J, Möller P, Hämmerling GJ, Arnold B. T cell awareness of paternal alloantigens during pregnancy. Science. 1995;270:630–3. https://doi.org/10.1126/science.270.5236.630
6. Care AS, Diener KR, Jasper MJ, Brown HM, Ingman WV, Robertson SA. Macrophages regulate corpus luteum development during embryo implantation in mice. J Clin Invest. 2013;123:3472–87. https://doi.org/10.1172/JCI60561
7. Ono Y, Nagai M, Yoshino O, Koga K, Nawaz A, Hatta H, et al. CD11c+ M1-like macrophages (MΦs) but not CD206+ M2-like MΦ are involved in folliculogenesis in mice ovary. Sci Rep. 2018;8:8171. https://doi.org/10.1038/s41598-018-25837-3
8. Plaks V, Birnberg T, Berkutzki T, Sela S, BenYashar A, Kalchenko V, et al. Uterine DCs are crucial for decidua formation during embryo implantation in mice. J Clin Invest. 2008;118:3954–65. https://doi.org/10.1172/JCI36682
9. Ono Y, Yoshino O, Hiraoka T, Sato E, Fukui Y, Ushijima A, et al. CD206+ M2-like macrophages are essential for successful implantation. Front Immunol. 2020;11:557184. https://doi.org/10.3389/fimmu.2020.557184
10. Darrasse-Jèze G, Klatzmann D, Charlotte F, Salomon BL, Cohen JL. CD4+CD25+ regulatory/suppressor T cells prevent allogeneic fetus rejection in mice. Immunol Lett. 2006;102:106–9. https://doi.org/10.1016/j.imlet.2005.07.002
11. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, et al. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol. 2005;166:811–22. https://doi.org/10.1016/S0002-9440(10)62302-4
12. Sasaki Y, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod. 2004;10:347–53. https://doi.org/10.1093/molehr/gah044
13. Shima T, Sasaki Y, Itoh M, Nakashima A, Ishii N, Sugamura K, et al. Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice. J Reprod Immunol. 2010;85:121–9. https://doi.org/10.1016/j.jri.2010.02.006
14. Chen T, Darrasse-Jèze G, Bergot AS, Courau T, Churlaud G, Valdivia K, et al. Self-specific memory regulatory T cells protect embryos at implantation in mice. J Immunol. 2013;191:2273–81. https://doi.org/10.4049/jimmunol.1202413
15. Piccinni MP, Raghupathy R, Saito S, Szekeres-Bartho J. Cytokines, hormones and cellular regulatory mechanisms favoring successful reproduction. Front Immunol. 2021;12:717808. https://doi.org/10.3389/fimmu.2021.717808
16. Fujiwara H. Immune cells contribute to systemic cross-talk between the embryo and mother during early pregnancy in cooperation with the endocrine system. Reprod Med Biol. 2006;5(1):19–29. https://doi.org/10.1111/j.1447-0578.2006.00119.x
17. Yu N, Zhang B, Xu M, Wang S, Liu R, Wu J, et al. Intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) activated by HCG improves the implantation and pregnancy rates in patients with repeated implantation failure: a prospective randomized study. Am J Reprod Immunol. 2016;76(3):212–6. https://doi.org/10.1111/aji.12542
18. Yoshioka S, Fujiwara H, Nakayama T, Kosaka K, Mori T, Fujii S. Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in patients with repeated failure of IVF-embryo transfer. Hum Reprod. 2006;21(12):3290–4. https://doi.org/10.1093/humrep/del312
19. Boid JB, Hamilton WJ. The human placenta. London and Basingstoke: Machilan Press; 1975. p. 45–60.
20. Nakamura T, Shima T, Saeki A, Hidaka T, Nakashima A, Takikawa O, et al. Expression of indoleamine 2, 3-dioxygenase and the recruitment of Foxp3-expressing regulatory T cells in the development and progression of uterine cervical cancer. Cancer Sci. 2007;98:874–81. https://doi.org/10.1111/j.1349-7006.2007.00470.x
21. Saito S, Nishikawa K, Morii T, Narita N, Enomoto M, Ichijo M. Expression of activation antigens CD69, HLA-DR, interleukin-2 receptor-alpha (IL-2R alpha) and IL-2R beta on T cells of human decidua at an early stage of pregnancy. Immunology. 1992;75:710–2.
22. Nishikawa K, Saito S, Morii T, Hamada K, Ako H, Narita N, et al. Accumulation of CD16-CD56+ natural killer cells with high affinity interleukin 2 receptors in human early pregnancy decidua. Int Immunol. 1991;3:743–50. https://doi.org/10.1093/intimm/3.8.743
23. Saito S, Nishikawa K, Morii T, Enomoto M, Narita N, Motoyoshi K, et al. Cytokine production by CD16-CD56^{bright} natural killer cells in the human early pregnancy decidua. Int Immunol. 1993;5:559–63. https://doi.org/10.1093/intimm/5.5.559
24. Saito S, Kasahara T, Sakakura S, Umekage H, Harada N, Ichijo M. Detection and localization of interleukin-8 mRNA and protein in human placenta and decidual tissues. J Reprod Immunol. 1994;27:161–72. https://doi.org/10.1016/0165-0378(94)90001-9
25. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, et al. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. J Leukoc Biol. 2006;80:572–80. https://doi.org/10.1189/jlb.0406250

26. Fu B, Zhou Y, Ni X, Tong X, Xu X, Dong Z, et al. Natural killer cells promote fetal development through the secretion of growth-promoting factors. Immunity. 2017;47:1100–1113.e6. https://doi.org/10.1016/j.immuni.2017.11.018
27. Li Q, Sharkey A, Sheridan M, Magistrati E, Arutyunyan A, Huhn O, et al. Human uterine natural killer cells regulate differentiation of extravillous trophoblast early in pregnancy. Cell Stem Cell. 2024;31:181–195.e9. https://doi.org/10.1016/j.stem.2023.12.013
28. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol. 2002;2:656–63. https://doi.org/10.1038/nri886
29. Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. Immunology. 2009;127:26–39. https://doi.org/10.1111/j.1365-2567.2008.03019.x
30. Lin XX, Xie YM, Zhao SJ, Liu CY, Mor G, Liao AH. Human leukocyte antigens: the unique expression in trophoblasts and their crosstalk with local immune cells. Int J Biol Sci. 2022;18:4043–52. https://doi.org/10.7150/ijbs.73616
31. King A, Burrows TD, Hiby SE, Bowen JM, Joseph S, Verma S, et al. Surface expression of HLA-C antigen by human extravillous trophoblast. Placenta. 2000;21:376–87. https://doi.org/10.1053/plac.1999.0496
32. Tsuda S, Zhang X, Hamana H, Shima T, Ushijima A, Tsuda K, et al. Clonally expanded decidual effector regulatory T cells increase in late gestation of normal pregnancy, but not in preeclampsia, in humans. Front Immunol. 2018;9:1934. https://doi.org/10.3389/fimmu.2018.01934
33. Tsuda S, Nakashima A, Shima T, Saito S. New paradigm in the role of regulatory T cells during pregnancy. Front Immunol. 2019;10:573. https://doi.org/10.3389/fimmu.2019.00573
34. Morita K, Tsuda S, Kobayashi E, Hamana H, Tsuda K, Shima T, et al. Analysis of TCR repertoire and PD-1 expression in decidual and peripheral CD8+ T cells reveals distinct immune mechanisms in miscarriage and preeclampsia. Front Immunol. 2020;11:1082. https://doi.org/10.3389/fimmu.2020.01082
35. Heikkinen J, Möttönen M, Alanen A, Lassila O. Phenotypic characterization of regulatory T cells in the human decidua. Clin Exp Immunol. 2004;136:373–8. https://doi.org/10.1111/j.1365-2249.2004.02441.x
36. Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, et al. Cutting edge: estrogen drives expansion of the $CD4^+CD25^+$ regulatory T cell compartment. J Immunol. 2004;173:2227–30. https://doi.org/10.4049/jimmunol.173.4.2227
37. Lee JH, Ulrich B, Cho J, Park J, Kim CH. Progesterone promotes differentiation of human cord blood fetal T cells into T regulatory cells but suppresses their differentiation into Th17 cells. J Immunol. 2011;187:1778–87. https://doi.org/10.4049/jimmunol.1003919
38. Schumacher A, Brachwitz N, Sohr S, Engeland K, Langwisch S, Dolaptchieva M, et al. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. J Immunol. 2009;182:5488–97. https://doi.org/10.4049/jimmunol.0803177
39. Huang X, Cai Y, Ding M, Zheng B, Sun H, Zhou J. Human chorionic gonadotropin promotes recruitment of regulatory T cells in endometrium by inducing chemokine CCL2. J Reprod Immunol. 2020;137:102856. https://doi.org/10.1016/j.jri.2019.102856
40. Green ES, Moldenhauer LM, Groome HM, Sharkey DJ, Chin PY, Care AS, et al. Regulatory T cells are paramount effectors in progesterone regulation of embryo implantation and fetal growth. JCI Insight. 2023;8:e162995. https://doi.org/10.1172/jci.insight.162995
41. Jiang TT, Chaturvedi V, Ertelt JM, Kinder JM, Clark DR, Valent AM, et al. Regulatory T cells: new keys for further unlocking the enigma of fetal tolerance and pregnancy complications. J Immunol. 2014;192:4949–56. https://doi.org/10.4049/jimmunol.1400498
42. Robertson SA, Care AS, Moldenhauer LM. Regulatory T cells in embryo implantation and the immune response to pregnancy. J Clin Invest. 2018;128:4224–35. https://doi.org/10.1172/JCI122182
43. Care AS, Bourque SL, Morton JS, Hjartarson EP, Robertson SA, Davidge ST. Reduction in regulatory T cells in early pregnancy causes uterine artery dysfunction in mice. Hypertension. 2018;72:177–87. https://doi.org/10.1161/HYPERTENSIONAHA.118.10858
44. Shima T, Inada K, Nakashima A, Ushijima A, Ito M, Yoshino O, et al. Paternal antigen-specific proliferating regulatory T cells are increased in uterine-draining lymph nodes just before implantation and in pregnant uterus just after implantation by seminal plasma-priming in allogeneic mouse pregnancy. J Reprod Immunol. 2015;108:72–82. https://doi.org/10.1016/j.jri.2015.02.005
45. Rowe JH, Ertelt JM, Xin L, Way SS. Pregnancy imprints regulatory memory that sustains anergy to fetal antigen. Nature. 2012;490:102–6. https://doi.org/10.1038/nature11462
46. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive $foxp3^+$ regulatory T cells: more of the same or a division of labor? Immunity. 2009;30:626–35. https://doi.org/10.1016/j.immuni.2009.05.002
47. Araishi K, Shima T, Yasuda I, Tsuda S, Morita K, Yamaki-Ushijima A, et al. Dynamics of neuropilin1 (Nrp1)-positive thymus-derived and Nrp1-negative peripherally induced paternal antigen specific regulatory T cells in the uterus and spleen during pregnancy in mice. J Reprod Immunol. 2023;155:103792. https://doi.org/10.1016/j.jri.2022.103792
48. Moldenhauer LM, Schjenken JE, Hope CM, Green ES, Zhang B, Eldi P, et al. Thymus-derived regulatory T cells exhibit Foxp3 epigenetic modification and phenotype attenuation after mating in mice. J Immunol. 2019;203:647–57. https://doi.org/10.4049/jimmunol.1900084
49. Li Z, Liang X, Chen X, Chen Y, Wang F, Wang S, et al. The role of thymus- and extrathymus-derived regulatory T cells in maternal-fetal tolerance. Front Immunol. 2023;14:1109352. https://doi.org/10.3389/fimmu.2023.1109352
50. Jørgensen N, Persson G, Hviid TVF. The tolerogenic function of regulatory T cells in pregnancy and cancer. Front Immunol. 2019;10:911. https://doi.org/10.3389/fimmu.2019.00911
51. Muralidhara P, Sood V, Vinayak Ashok VV, Bansal K. Pregnancy and tumour: the parallels and differences in regulatory T cells. Front Immunol. 2022;13:866937. https://doi.org/10.3389/fimmu.2022.866937
52. Hadinedoushan H, Mirahmadian M, Aflatounian A. Increased natural killer cell cytotoxicity and IL-2 production in recurrent spontaneous abortion. Am J Reprod Immunol. 2007;58:409–14. https://doi.org/10.1111/j.1600-0897.2007.00524.x
53. Rao VA, Kurian NK, Rao KA. Cytokines, NK cells and regulatory T cell functions in normal pregnancy and reproductive failures. Am J Reprod Immunol. 2023;89:e13667. https://doi.org/10.1111/aji.13667
54. Mahajan D, Sharma NR, Kancharla S, Kolli P, Tripathy A, Sharma AK, et al. Role of natural killer cells during pregnancy and related complications. Biomolecules. 2022;12:68. https://doi.org/10.3390/biom12010068
55. Quenby S, Nik H, Innes B, Lash G, Turner M, Drury J, et al. Uterine natural killer cells and angiogenesis in recurrent reproductive failure. Hum Reprod. 2009;24:45–54. https://doi.org/10.1093/humrep/den348
56. Gamliel M, Goldman-Wohl D, Isaacson B, Gur C, Stein N, Yamin R, et al. Trained memory of human uterine NK cells enhances their

function in subsequent pregnancies. Immunity. 2018;48:951–962.e5. https://doi.org/10.1016/j.immuni.2018.03.030

57. Robillard PY, Dekker G, Scioscia M, Saito S. Progress in the understanding of the pathophysiology of immunologic maladaptation related to early-onset preeclampsia and metabolic syndrome related to late-onset preeclampsia. Am J Obstet Gynecol. 2022;226:S867–S875. https://doi.org/10.1016/j.ajog.2021.11.019
58. Yasuda I, Shima T, Moriya T, Ikebuchi R, Kusumoto Y, Ushijima A, et al. Dynamic changes in the phenotype of dendritic cells in the uterus and uterine draining lymph nodes after coitus. Front Immunol. 2020;11:557720. https://doi.org/10.3389/fimmu.2020.557720
59. Zhao Y, Zheng Q, Jin L. The role of B7 family molecules in maternal-fetal immunity. Front Immunol. 2020;11:458. https://doi.org/10.3389/fimmu.2020.00458
60. Wei R, Lai N, Zhao L, Zhang Z, Zhu X, Guo Q, et al. Dendritic cells in pregnancy and pregnancy-associated diseases. Biomed Pharmacother. 2021;133:110921. https://doi.org/10.1016/j.biopha.2020.110921
61. Miwa N, Hayakawa S, Miyazaki S, Myojo S, Sasaki Y, Sakai M, et al. IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-gamma increase in normal pregnancy but decrease in spontaneous abortion. Mol Hum Reprod. 2005;11:865–70. https://doi.org/10.1093/molehr/gah246
62. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science. 1998;281:1191–3. https://doi.org/10.1126/science.281.5380.1191
63. Chang RQ, Li DJ, Li MQ. The role of indoleamine-2,3-dioxygenase in normal and pathological pregnancies. Am J Reprod Immunol. 2018;79:e12786. https://doi.org/10.1111/aji.12786
64. Zong S, Li C, Luo C, Zhao X, Liu C, Wang K, et al. Dysregulated expression of IDO may cause unexplained recurrent spontaneous abortion through suppression of trophoblast cell proliferation and migration. Sci Rep. 2016;6:19916. https://doi.org/10.1038/srep19916
65. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:3887–95. https://doi.org/10.1002/j.1460-2075.1992.tb05481.x
66. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: past, present, and future. J Clin Invest. 2015;125:3384–91. https://doi.org/10.1172/JCI80011
67. Veras E, Kurman RJ, Wang TL, Shih IM. PD-L1 expression in human placentas and gestational trophoblastic diseases. Int J Gynecol Pathol. 2017;36:146–53. https://doi.org/10.1097/PGP.0000000000000305
68. Andrikopoulou A, Korakiti AM, Apostolidou K, Dimopoulos MA, Zagouri F. Immune checkpoint inhibitor administration during pregnancy: a case series. ESMO Open. 2021;6:100262. https://doi.org/10.1016/j.esmoop.2021.100262
69. Zhang Y, Wang H, Qiu P, Jiang J, Wu X, Mei J, et al. Decidual macrophages derived NO downregulates PD-L1 in trophoblasts leading to decreased Treg cells in recurrent miscarriage. Front Immunol. 2023;14:1180154. https://doi.org/10.3389/fimmu.2023.1180154
70. Bennett WA, Lagoo-Deenadayalan S, Whitworth NS, Brackin MN, Hale E, Cowan BD. Expression and production of interleukin-10 by human trophoblast: relationship to pregnancy immunotolerance. Early Pregnancy. 1997;3:190–8.
71. Yang D, Dai F, Yuan M, Zheng Y, Liu S, Deng Z, et al. Role of transforming growth factor-β1 in regulating fetal-maternal immune tolerance in normal and pathological pregnancy. Front Immunol. 2021;12:689181. https://doi.org/10.3389/fimmu.2021.689181
72. Robertson SA, Guerin LR, Bromfield JJ, Branson KM, Ahlström AC, Care AS. Seminal fluid drives expansion of the CD4+CD25+ T regulatory cell pool and induces tolerance to paternal alloantigens in mice. Biol Reprod. 2009;80:1036–45. https://doi.org/10.1095/biolreprod.108.074658
73. Koelman CA, Coumans AB, Nijman HW, Doxiadis II, Dekker GA, Claas FH. Correlation between oral sex and a low incidence of preeclampsia: a role for soluble HLA in seminal fluid? J Reprod Immunol. 2000;46:155–66. https://doi.org/10.1016/s0165-0378(99)00062-5
74. Shima T, Nakashima A, Yasuda I, Ushijima A, Inada K, Tsuda S, et al. Uterine CD11c+ cells induce the development of paternal antigen-specific Tregs via seminal plasma priming. J Reprod Immunol. 2020;141:103165. https://doi.org/10.1016/j.jri.2020.103165
75. Chen S, Zhang J, Huang C, Lu W, Liang Y, Wan X. Expression of the T regulatory cell transcription factor FoxP3 in peri-implantation phase endometrium in infertile women with endometriosis. Reprod Biol Endocrinol. 2012;10:34. https://doi.org/10.1186/1477-7827-10-34
76. Moldenhauer LM, Foyle KL, Wilson JJ, Wong YY, Sharkey DJ, Green ES, et al. A disrupted FOXP3 transcriptional signature underpins systemic regulatory T cell insufficiency in early pregnancy failure. iScience. 2024;27:108994. https://doi.org/10.1016/j.isci.2024.108994
77. Ghaebi M, Abdolmohammadi-Vahid S, Ahmadi M, Eghbal-Fard S, Dolati S, Nouri M, et al. T cell subsets in peripheral blood of women with recurrent implantation failure. J Reprod Immunol. 2019;131:21–9. https://doi.org/10.1016/j.jri.2018.11.002
78. Crawford G, Ray A, Gudi A, Shah A, Homburg R. The role of seminal plasma for improved outcomes during in vitro fertilization treatment: review of the literature and meta-analysis. Hum Reprod Update. 2015;21:275–84. https://doi.org/10.1093/humupd/dmu052
79. Saccone G, Di Spiezio Sardo A, Ciardulli A, Caissutti C, Spinelli M, Surbek D, et al. Effectiveness of seminal plasma in in vitro fertilisation treatment: a systematic review and meta-analysis. BJOG. 2019;126:220–5. https://doi.org/10.1111/1471-0528.15004
80. Liu S, Wei H, Li Y, Diao L, Lian R, Zhang X, et al. Characterization of dendritic cell (DC)-10 in recurrent miscarriage and recurrent implantation failure. Reproduction. 2019;158:247–55. https://doi.org/10.1530/REP-19-0172
81. Blois SM, Kammerer U, Alba Soto CA, Tometten MC, Shaikly V, Barrientos G, et al. Dendritic cells: key to fetal tolerance? Biol Reprod. 2007;77:590–8. https://doi.org/10.1095/biolreprod.107.060632
82. Yunna C, Mengru H, Lei W, Weidong C. Macrophage M1/M2 polarization. Eur J Pharmacol. 2020;877:173090. https://doi.org/10.1016/j.ejphar.2020.173090
83. Wen Q, Kong Y, Zhao HY, Zhang YY, Han TT, Wang Y, et al. G-CSF-induced macrophage polarization and mobilization may prevent acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2019;54:1419–33. https://doi.org/10.1038/s41409-019-0449-9
84. Busnelli A, Somigliana E, Cirillo F, Baggiani A, Levi-Setti PE. Efficacy of therapies and interventions for repeated embryo implantation failure: a systematic review and meta-analysis. Sci Rep. 2021;11:1747. https://doi.org/10.1038/s41598-021-81439-6
85. Liu M, Yuan Y, Qiao Y, Tang Y, Sui X, Yin P, et al. The effectiveness of immunomodulatory therapies for patients with repeated implantation failure: a systematic review and network meta-analysis. Sci Rep. 2022;12:18434. https://doi.org/10.1038/s41598-022-21014-9
86. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online. 2014;28:14–38. https://doi.org/10.1016/j.rbmo.2013.08.011
87. Yamada H, Deguchi M, Saito S, Takeshita T, Mitsui M, Saito T, et al. High doses of intravenous immunoglobulin stimulate regulatory T

cell and suppress natural killer cell in women with recurrent pregnancy loss. J Reprod Immunol. 2023;158:103977. https://doi.org/10.1016/j.jri.2023.103977
88. Kaufman GN, Massoud AH, Dembele M, Yona M, Piccirillo CA, Mazer BD. Induction of regulatory T cells by intravenous immunoglobulin: a bridge between adaptive and innate immunity. Front Immunol. 2015;6:469. https://doi.org/10.3389/fimmu.2015.00469
89. Ahmadi M, Aghdam SA, Nouri M, Babaloo Z, Farzadi L, Ghasemzadeh A, et al. Intravenous immunoglobulin (IVIG) treatment modulates peripheral blood Th17 and regulatory T cells in recurrent miscarriage patients: non randomized, open-label clinical trial. Immunol Lett. 2017;192:12–9. https://doi.org/10.1016/j.imlet.2017.10.003
90. McAlpine SM, Roberts SE, Heath JJ, Käsermann F, Issekutz AC, Issekutz TB, et al. High dose intravenous IgG therapy modulates multiple NK cell and T cell functions in patients with immune dysregulation. Front Immunol. 2021;12:660506. https://doi.org/10.3389/fimmu.2021.660506
91. Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent pregnancy loss. Nat Rev Dis Primers. 2020;6:98. https://doi.org/10.1038/s41572-020-00228-z
92. ESHRE Recurrent Pregnancy Loss Guideline Development Group. Guideline on the management of recurrent pregnancy loss. 2023.
93. Morita K, Ono Y, Takeshita T, Sugi T, Fujii T, Yamada H, et al. Risk factors and outcomes of recurrent pregnancy loss in Japan. J Obstet Gynaecol Res. 2019;45:1997–2006. https://doi.org/10.1111/jog.14083
94. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010;63:601–10. https://doi.org/10.1111/j.1600-0897.2010.00852.x
95. Makhseed M, Raghupathy R, Azizieh F, Omu A, Al-Shamali E, Ashkanani L. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. Hum Reprod. 2001;16:2219–26. https://doi.org/10.1093/humrep/16.10.2219
96. Wang W, Sung N, Gilman-Sachs A, Kwak-Kim J. T helper (Th) cell profiles in pregnancy and recurrent pregnancy losses: Th1/Th2/Th9/Th17/Th22/Tfh cells. Front Immunol. 2020;11:2025. https://doi.org/10.3389/fimmu.2020.02025
97. Sato T, Sugiura-Ogasawara M, Ozawa F, Yamamoto T, Kato T, Kurahashi H, et al. Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. Hum Reprod. 2019;34:2340–8. https://doi.org/10.1093/humrep/dez229
98. Guo Z, Xu Y, Zheng Q, Liu Y, Liu X. Analysis of chromosomes and the T helper 17 and regulatory T cell balance in patients with recurrent spontaneous abortion. Exp Ther Med. 2020;19:3159–66. https://doi.org/10.3892/etm.2020.8537
99. Inada K, Shima T, Nakashima A, Aoki K, Ito M, Saito S. Characterization of regulatory T cells in decidua of miscarriage cases with abnormal or normal fetal chromosomal content. J Reprod Immunol. 2013;97:104–11. https://doi.org/10.1016/j.jri.2012.12.001
100. Yao Y, Xu XH, Jin L. Macrophage polarization in physiological and pathological pregnancy. Front Immunol. 2019;10:792. https://doi.org/10.3389/fimmu.2019.00792
101. Genest G, Almasri W, Banjar S, Beauchamp C, Buckett W, Dzineku F, et al. Immunotherapy for recurrent pregnancy loss: a reappraisal. Fertil Steril Rev. 2022;3:24–41. https://doi.org/10.1016/j.xfnr.2021.11.002
102. Haas DM, Hathaway TJ, Ramsey PS. Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. Cochrane Database Syst Rev. 2019;2019:CD003511. https://doi.org/10.1002/14651858.CD003511.pub4
103. Stephenson MD, McQueen D, Winter M, Kliman HJ. Luteal start vaginal micronized progesterone improves pregnancy success in women with recurrent pregnancy loss. Fertil Steril. 2017;107:684–690.e2. https://doi.org/10.1016/j.fertnstert.2016.11.029
104. Raghupathy R, Al-Mutawa E, Al-Azemi M, Makhseed M, Azizieh F, Szekeres-Bartho J. Progesterone-induced blocking factor (PIBF) modulates cytokine production by lymphocytes from women with recurrent miscarriage or preterm delivery. J Reprod Immunol. 2009;80:91–9. https://doi.org/10.1016/j.jri.2009.01.004
105. Yamada H, Deguchi M, Saito S, Takeshita T, Mitsui M, Saito T, et al. Intravenous immunoglobulin treatment in women with four or more recurrent pregnancy losses: a double-blind, randomised, placebo-controlled trial. eClinicalMedicine. 2022;50:101527. https://doi.org/10.1016/j.eclinm.2022.101527
106. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. N Engl J Med. 2012;367:2015–25. https://doi.org/10.1056/NEJMra1009433
107. Wang G, Zhang R, Li C, Chen A. Evaluation of the effect of low molecular weight heparin in unexplained recurrent pregnancy loss: a meta-analysis of randomized controlled trials. J Matern Fetal Neonatal Med. 2022;35:7601–8. https://doi.org/10.1080/14767058.2021.1957819
108. Inada K, Shima T, Ito M, Ushijima A, Saito S. Helios-positive functional regulatory T cells are decreased in decidua of miscarriage cases with normal fetal chromosomal content. J Reprod Immunol. 2015;107:10–9. https://doi.org/10.1016/j.jri.2014.09.053
109. Lédée N, Petitbarat M, Prat-Ellenberg L, Dray G, Cassuto GN, Chevrier L, et al. Endometrial immune profiling: a method to design personalized care in assisted reproductive medicine. Front Immunol. 2020;11:1032. https://doi.org/10.3389/fimmu.2020.01032

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