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Investigation of Immune Cell Interactions to Promote Maternal and Fetal Health in a Healthy Pregnancy

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Abstract

Context: Pregnancy represents a unique physiological state characterized by profound immunological changes that facilitate the interaction between the maternal and fetal immune systems.

Evidence Acquisition: Key components of the immune system, including natural killer (NK) cells, macrophages (Mφ), regulatory T cells (Tregs), and cytokines, are crucial for maintaining maternal immune tolerance against the fetus.

Results: Decidual natural killer (dNK) cells are the most prevalent uterine natural killer cells (uNK), and their increase in early pregnancy indicates their direct relationship and vital role during this period. Trophoblast invasion, tissue regeneration, and immune regulation between the mother and fetus are key processes mediated by Mφs, necessary for a successful pregnancy. Cytokine balance is critical to prevent maternal immune rejection and ensure fetal survival. The Tregs are being investigated for their essential role in maintaining immune homeostasis. Overall, the interaction of immune cells such as NK cells, Mφs, Tregs, and cytokines is essential for successful pregnancy outcomes.

Conclusions: An imbalance among immune cells can lead to pregnancy complications such as preeclampsia and recurrent pregnancy loss (RPL). The purpose of this review is to investigate the function of NK cells, Mφs, Tregs, and cytokines in the context of healthy pregnancy.

Keywords: Maternal-Fetal Exchange, Pregnancy, Immune Tolerance, Immune System

1. Context

Pregnancy is a complex physiological state characterized by profound immune changes that facilitate successful fetal development. A successful pregnancy depends on accurate and coordinated communication between the fetus and the mother. Immune cells and cytokine signaling pathways play a prominent role as mediators of this communication (1). During pregnancy, the maternal immune system undergoes changes to tolerate the fetus. Natural killer (NK) cells are known as the key immune cells of the uterus during pregnancy, primarily covering the entire endometrium. In normal pregnancy, these cells do not exhibit cytotoxic properties and play a significant role in implantation and placental regulation (2, 3). Macrophages (Mφ), one of the main subsets of leukocytes in the decidua region of the uterus, perform a unique function in creating the immunological aspects of the interaction between mother and fetus due to their phenotypic flexibility (4, 5). Regulatory T cells (Tregs), a vital component of the T lymphocyte family, play a crucial role in maintaining immunological tolerance and regulating immune responses in both healthy and pathological processes. Research indicates that Tregs prevent the development of the maternal immune response against the fetus (6). Pregnancy and delivery are regulated by cytokines, and a disruption in the balance of these hormones can lead to difficulties such as autoimmune illnesses or microbial infections. This disorder may also cause recovery from autoimmune disease during pregnancy with recurrence after delivery (7, 8). Preeclampsia, spontaneous abortion, and intrauterine growth

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restriction (IUGR) are common pregnancy complications often resulting from abnormal placentation and impaired placental function. In preeclampsia, impaired trophoblast invasion and inadequate remodeling of maternal spiral arteries lead to reduced placental blood flow (9, 10). The imbalance between antiangiogenic factors such as sFlt-1 and sEng contributes to endothelial dysfunction, resulting in the clinical manifestations of preeclampsia (11). In cases of spontaneous abortion, defective trophoblast invasion and reduced HLA-G expression may trigger maternal natural killer (NK) cell activation, leading to fetal rejection (12). For IUGR, placental insufficiency and diminished uteroplacental blood flow deprive the fetus of oxygen and essential nutrients (13). These disorders not only increase the risk of fetal mortality but are also associated with long-term metabolic and cardiovascular complications in childhood and adulthood (14). Therefore, it is important to expand knowledge to understand the complexities of common immune regulatory pathways in pregnancy to improve and develop new strategies for the treatment of immune-based infertility. Understanding the interactions between NK cells, Tregs, and cytokines is crucial to elucidate the underlying mechanisms in successful reproductive processes and address pregnancy-related complications. This study investigates the function of NK cells, the vital role of Mφs during healthy pregnancy, and the function of cytokines and Tregs in balancing maternal and fetal immunity during healthy pregnancy.

2. Natural Killer Cells in Pregnancy: Supporting Fetal Growth and Maternal Health

Natural killer (NK) cells constitute approximately 15% of all circulating lymphocytes in humans. These cells possess inherent cytotoxic qualities and are characterized by their large size and granule-containing nature. The NK cells secrete inflammatory mediators such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), which contribute to cytotoxicity and the generation of inflammatory cytokines (15, 16). Phenotypically, NK cells are characterized by the expression of surface receptors cluster of differentiation (CD) 56 and CD16. Based on the concentration of CD56 antigen, NK cells can be divided into two groups: CD56dim and CD56bright. The CD56dim type exhibits high cytotoxicity in vitro, while the CD56bright type produces important immune cytokines such as IFN-γ (17). Typically, decidual natural killer (dNK) cells exhibit the CD56brightCD16- phenotype, whereas peripheral blood NK (pbNK) cells exhibit the CD56dimCD16+ phenotype (18). In humans, NK cells are exclusively found in the decidua region of the uterus; thus, dNK and uterine NK (uNK) cells are synonymous (19). The exact origin of uNK cells remains unknown. These cells may arise from the translocation of pbNK cells to the decidua and the presence of local hematopoietic precursor cells (HPC), as suggested by a study conducted on mice. The HPCs isolated from the decidua can develop into uNK cells in the presence of interleukin (IL) 15, which is the primary factor for uNK cell activation and maturation (19, 20). The population of blood leukocytes in the endometrium undergoes changes during menstruation, with the highest number observed in the secretory phase and the lowest in the proliferation phase. At the end of the secretory phase and the onset of pregnancy, the population of uNK cells increases rapidly, comprising about 70% of uterine leukocytes. This population reaches its peak in early pregnancy, but their numbers decrease as pregnancy progresses towards the trimester stage (21). The performance of NK cells is determined by the balance between signals received by activating and inhibitory receptors (22). The growth of the placenta and maintenance of the placental bed are crucial for fetal development throughout pregnancy, relying on fetal trophoblast invasion and the presence of immune cells such as uNK cells. It is evident that uNK cells are important for both trophoblast invasion and spiral artery regeneration, as they are frequently observed near regenerating spiral arteries and invading fetal trophoblast cells (23). During trophoblast invasion, trophoblasts separated from the embryo invade the decidua and uterine wall, leading to vascular changes in the uterine endometrium, known as decidualization. Meanwhile, uNK cells proliferate and settle at sites of trophoblast invasion. Extravillous trophoblasts (EVTs) derived from the fetus and maternal uNK cells regenerate maternal vessels and repair spiral arteries, allowing maternal blood to flow into the placental villi to supply nutrients and oxygen needed by the fetus (24, 25).

2.1. Types of Decidual Natural Killer Cells Based on RNA Sequencing

Decidual tissue-resident markers such as CD49a and CD9 can be expressed by dNK cells, which can be categorized into three groups based on RNA sequencing: dNK1, dNK2, and dNK3. The dNK1 group expresses Beta-1,4-N-Acetyl-Galactosaminyltransferase 1 (B4GALNT1), CD39, and cytochrome P450 Family 26 Subfamily A Member 1 (CYP26A1). CD39 is a regulatory ecto-ATPase that helps shift the environmental equilibrium from pro-inflammatory to anti-inflammatory. Additionally, this group shows increased

expression of activating receptors KIR2DS1 and KIR2DS4, which bind to HLA-Cs in trophoblasts, as well as inhibitory killer cell immunoglobulin-like receptors KIR2DL1, KIR2DL2, and KIR2DL3, which also bind to HLA-Cs in trophoblasts. The dNK1 subgroup may interact with EVTs, as evidenced by the expression of leukocyte immunoglobulin-like receptor B1 (LILRB1), a receptor with a high affinity for the HLA-G dimer, and an active glycolytic metabolism.

The dNK2 group is indicated by the presence of Annexin A1 (ANXA1) and integrin subunit beta 2 (ITGB2) proteins, which express the activating receptors of NK cell Group 2 isoform C (NKG2C), NKG2E, and the inhibitory receptor NKG2A. The dNK3 group may express T-cell immunoreceptor with Ig, CD161, immunoreceptor tyrosine-based inhibition motif domains (TIGIT), CD103, and ITGB2, although they do not express CD127 intrinsic lymphocyte cell markers (15, 26, 27).

2.2. Utilizing the Function of Natural Killer Cells for Healthier Pregnancies

Natural killer (NK) cell receptors (NKR), such as the killer-cell immunoglobulin-like receptor (KIR), leukocyte immunoglobulin-like receptor B (LILRB), C-type lectin heterodimer family (NKG2, including NKG2A, NKG2C, and NKG2D), and natural cytotoxicity receptors (NCR), including NKp30, NKp44, and NKp46, are responsible for regulating the activity of these cells (28). Numerous studies have shown that dNK cells interact with HLA ligands, including HLA-G, HLA-C, and HLA-E, produced by extravillous trophoblasts (EVT), to reduce the cytotoxicity of dNK cells (29). The NK cells express KIR2DL4 and immunoglobulin-like transcript (ILT) 2 for HLA-G. During HLA-G binding to the membrane, this molecule interacts with KIR2DL4, ultimately leading to the inhibition of dNK cell-mediated cytolysis and suppression of their cytotoxic effects. HLA-G also plays a role in spiral artery regeneration and fetal development. HLA-E reduces NK cell toxicity through the inhibitory receptor NKG2A/CD94. HLA-C and HLA-G interact with their receptors on dNK1 to promote trophoblast invasion, vascular remodeling, and the maintenance of a high-immune-tolerance microenvironment for the fetus (21, 30, 31).

Additionally, dNK1 cells have higher levels of cytoplasmic granule proteins, including perforin 1, granulysin, granzyme (Gzm) A, and GzmB, which provide protection against placental infection and glycolysis-related enzymes (29). The function of dNK cells in the process of trophoblast invasion is regulated by various cytokines, such as IFN-γ, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), TGF-β, and IL-10; chemokines, such as CXC motif ligand 8 (CXCL8)/IL-8, CC chemokine ligand (CCL) 3/MIP1a, CCL4/MIP1b, CCL5/Rantes, CXCL10/IP-10, and CXCL12/SDF-1; and angiogenic factors, such as angiopoietin (Ang) 2, placental growth factor (PlGF), epidermal growth factor, and vascular endothelial growth factor A (VEGF-A). For instance, Ang-2, TNF, and TGF-β prevent trophoblast invasion, but released CXCL8 and CXCL10 bind to their receptors on invasive trophoblasts and promote trophoblast motility (25, 27).

3. The Critical Functions of Macrophage Roles in Healthy Pregnancy and Fetal Development

Macrophages play a crucial role during pregnancy in healing damaged tissues and blood vessels, facilitating trophoblast invasion, and maintaining tissue homeostasis. They also serve as the primary antigen-presenting cells (APCs) in the decidual region. After NK cells, Mφs are the second largest population of decidual leukocytes. Disruption of Mφ activity and changes in their polarity (differentiation into specific phenotypes) can lead to pregnancy disorders such as recurrent spontaneous abortion (RSA), premature birth, infertility, and preeclampsia (PE), which is also associated with intrauterine growth restriction (IUGR). During the embryo implantation stage, decidual macrophages (dMφs) exhibit an M1 (proinflammatory) phenotype. Following successful implantation, EVTs invade the uterine stroma, where both M1 and M2 (anti-inflammatory) Mφs are present. The M2 phenotype is considered dominant in most dMφs to prevent embryo rejection by the immune system (32-34). This is achieved by increasing the expression of CD206, CD209, and CD163, as well as the synthesis of TGF-β and IL-10. IL-10, IL-13, IL-4, and macrophage colony-stimulating factor (M-CSF) can activate M2 Mφs (4).

The dMφs produce IL-15, which promotes NK cell proliferation. CXCL16, produced by trophoblast cells, acts as an essential molecule in establishing M2 dMφ polarity, leading to the polarization of M2 Mφs. This results in the reduction of IL-15, inactivation of NK cells, decreased cytotoxicity, and the creation of a suitable environment for embryo development (35). Other immune molecules secreted by dMφs include prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase (36). Following a successful pregnancy, to prevent the activity of maternal T lymphocytes, the expression levels of CD80 and CD86, which are identification markers of M1 Mφs, decrease. M2a, M2b, M2c, and M2d are subgroups of M2 dMφs (34, 37).

dMφs are often derived from circulating monocytes and contribute to successful mating through the

secretion of cytokines and growth factors. They also protect the embryo against various infections and create immunological tolerance for semi-allogeneic embryos. Fibroblast growth factor (FGF), VEGF-A, keratinocyte growth factor (KGF), angiogenin (ANG), Ang-1, and Ang-2 are among the factors released by dMφs that contribute to spiral artery regeneration and angiogenesis (38). The dMφs limit decidual T cells' production of IFN-γ cytokines through interactions between programmed death-ligand 1 (PD-L1)/CD274 and programmed cell death protein 1 (PD-1)/CD274, resulting in the weakening and inhibition of the immune system due to inflammation (39) (Figure 1).

3.1. The Role of HLA-G in Modulating Macrophage Function and Immune Tolerance During Pregnancy

In contrast to the conventional MHC-I molecules HLA-A, HLA-B, and HLA-C, HLA-G is a non-classical form of MHC-I that exhibits little variability. Membrane-bound isoforms are produced by transcription of HLA-G2, HLA-G3, and HLA-G4, while soluble isoforms are produced by transcription of HLA-G5, HLA-G6, and HLA-G1. Both pre-implanted embryos and EVTs express HLA-G. Soluble HLA-G (sHLA-G) is present in amniotic fluid, umbilical cord blood, maternal blood, and the culture media used for in vitro fertilization (IVF) embryos (30). KIR2DL4/CD158d, which serves as a receptor for HLA-G, is not expressed in Mφs, unlike in NK cells (21). The expression of inhibitory receptors ILT2/LILRB1 and ILT4/LILRB2 by Mφs located near invasive EVTs for HLA-G expressed by trophoblasts leads to negative intracellular signals, alters the function of secreted cytokines, and ultimately reduces the inflammatory response of the maternal immune system (34, 40, 41).

The decrease in M1 Mφ markers followed by an increase in M2 Mφ markers occurs due to the activation of Mφs by sHLA-G5, ultimately leading to an increase in the phagocytic activity of polarized Mφs. Regulating immune tolerance between the mother and fetus, as well as promoting placental growth, are important roles of this process (21). Phagocytosis of apoptotic bodies produced during the repair and regeneration of the decidual membrane and spiral artery is necessary for inducing tolerogenic immune responses, as it prevents endothelial activity and the recruitment of monocytes. Meanwhile, the phagocytic activity of Mφs against necrotic cells can lead to maternal inflammatory reactions against fetal antigens (38, 42).

Given that HLA-G expression occurs specifically during pregnancy, its vital role in reproduction, establishing immune tolerance, spiral artery regeneration, and fetal growth is well-established. Pregnancy complications caused by abnormal expression levels of HLA-G and its polymorphisms have been extensively studied in relation to their impact on pregnancy (30, 41). Polymorphisms in the regulatory regions of the HLA-G gene may influence its expression. HLA-G is highly expressed in invasive trophoblast cells of the placenta and is believed to play a role in pregnancy complications such as preeclampsia, RSA, IUGR, and preterm birth, all associated with immunological dysfunctions. These complications have been linked to low or undetectable levels of soluble HLA-G in maternal circulation (43, 44).

Current research increasingly focuses on using HLA-G as a promising therapeutic target. The dimeric form of HLA-G has attracted significant attention due to its high potential in disease treatment and improving pregnancy outcomes. However, developing effective therapeutic strategies requires a deeper understanding of the molecular and immunological mechanisms related to HLA-G and its interaction with different genotypes. Promising strategies include inducing HLA-G expression or using HLA-G-derived peptides to modulate the maternal immune response. These approaches may play a crucial role in preventing and managing pregnancy-related complications (41, 45).

4. The Role of Cytokines in Facilitating Maternal-Fetal Communication for a Healthy Pregnancy

The maternal immune system undergoes fundamental changes to protect a healthy pregnancy. Compared to the non-pregnant period, normal pregnancy is characterized by a slight increase in serum levels of both pro-inflammatory and anti-inflammatory cytokines (46). Cytokines facilitate implantation, placentation, and childbirth processes, while also maintaining maternal immune tolerance. Among the cytokines effective in successful pregnancy are IL-6 and TNF-α, which are necessary for regulating inflammatory responses. Dysfunction in cytokine expression during pregnancy can lead to complications such as PE, infection, intrauterine growth restriction (IUGR), and premature birth (1, 8, 47). Cytokines act as communication mediators between the blastocyst and the endometrium during implantation and can also support the placenta, enhance immunity, and promote the invasive and proliferative phenotypes of trophoblasts (8).

In contrast to Th2 cells, which produce anti-inflammatory cytokines like IL-10, IL-4, and IL-13 that contribute to wound healing and immunological tolerance, T helper cells (Th) type 1 are responsible for secreting pro-inflammatory cytokines IFN-γ and TNF-α

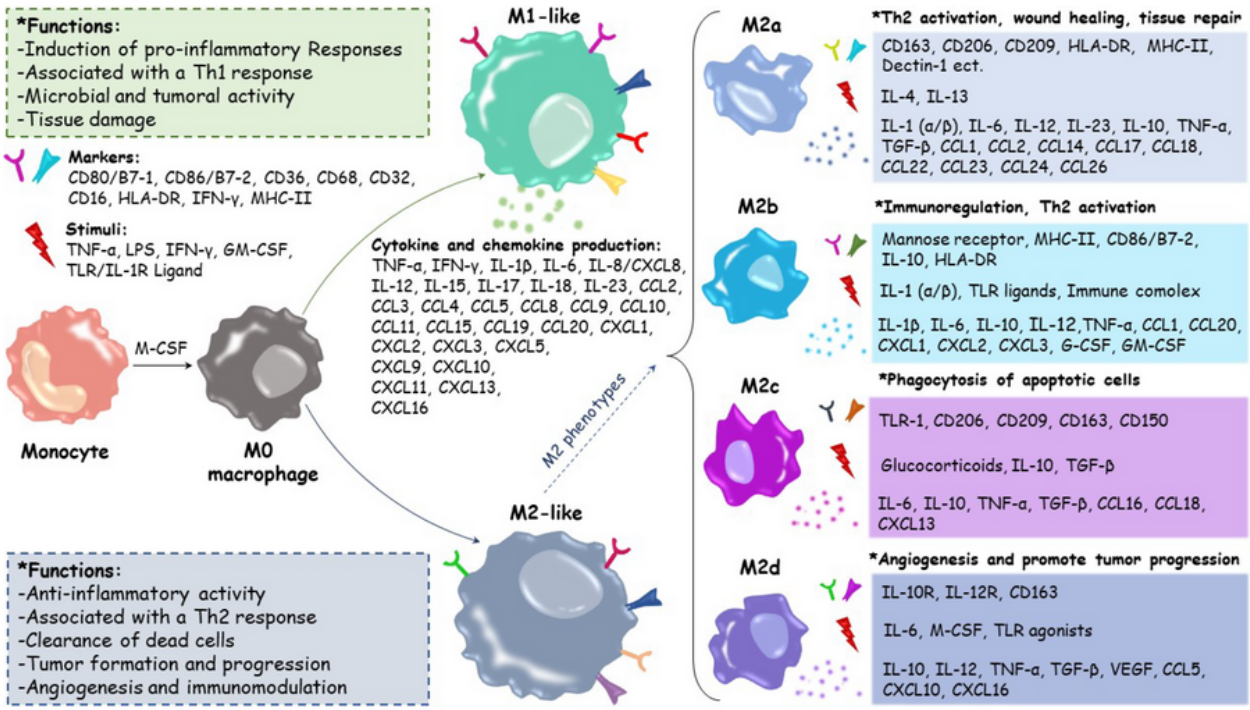

Figure 1. Macrophage polarization, key features and their functional roles

(8, 48, 49). IL-1 and its family members, as representatives of pro-inflammatory factors, regulate some inflammatory diseases, including preterm birth (50). Emerging cytokines such as IL-35, IL-37, and IL-38 are involved in human pregnancy. IL-35 is an inhibitory cytokine necessary for increasing immune tolerance and preventing fetal rejection by the mother. High levels of IL-35 during pregnancy help reduce inflammatory responses and are associated with successful pregnancy. IL-37, similar to IL-35, has anti-inflammatory effects that can prevent pregnancy complications such as PE. IL-38 also regulates inflammation and potentially maintains a balanced immune environment during pregnancy (31, 51, 52).

IL-35 and IL-37 are involved in many inflammatory diseases, autoimmune disorders, malignancies, infectious diseases, and sepsis due to their anti-inflammatory and immunomodulatory effects (53). IL-35, often produced by CD4+ forkhead box protein P3 (FOXP3)+ Treg cells, was first identified in 2007. The activity of these cytokines is necessary for the suppressive properties of the Tregs population. Activated B cells, tolerogenic dendritic cells (DCs), and monocytes can produce and secrete this cytokine. IL-35 and TGF-β are responsible for increasing immunosuppressive factors in the first trimester of pregnancy, with IL-35 being the main immunosuppressive factor in the second and third trimesters. Consequently, there is a considerable drop in IL-35 levels during recurrent pregnancy loss (RPL) (51). IL-35 plays a crucial role in maintaining maternal-fetal tolerance during pregnancy. This cytokine is produced by trophoblast cells and promotes Th2 polarization, essential for successful pregnancy. IL-35 increases the production of IL-4 and IL-10 by Th cells, creating an environment conducive to fetal tolerance (54).

The IL-1 family includes IL-37, identified in 2000. This cytokine's molecular weight ranges from 17 to 25 kilodaltons, and its gene is located on chromosome 2. IL-37 expression is typically modest but significantly rises in response to inflammation (50). In normal pregnancies, baseline levels of IL-37 are consistently expressed in chorionic villous tissue and the umbilical cord (55). In patients with preeclampsia, the level of IL-37 protein in the placenta shows more than a fivefold increase compared to normal pregnancies (56). IL-38 is naturally expressed in very low amounts; however, disruption of IL-38 expression may cause many

autoimmune diseases. IL-38 expression has been observed in embryonic tissues and adult tissues, such as the cardiovascular, respiratory, digestive, and reproductive systems (52). In women affected by preeclampsia, reduced levels of IL-37 and IL-38 lead to overactivation of Th17 cells and disruption of placental angiogenesis. These alterations contribute to placental dysfunction and exacerbate the disease (57-59).

5. Critical Functions of Regulatory T Cells in Supporting Healthy Pregnancy Outcomes

Regulatory T cells are a specific subset of T cells vital for building tolerance and preserving immunological homeostasis, particularly in pregnant women. Tregs function as potent immune suppressors to reduce inflammation and shield the fetus from immune system rejection by the mother. They perform these processes by suppressing the activity of effector T cells through classical mechanisms such as direct cell contact or by secreting certain cytokines (6). The FOXP3 transcription factor, identified in 2003 after extensive studies on mice (60), is the main gene for Treg differentiation, and its stable expression is characteristic of Tregs (61).

Among human Treg cell subsets, effector Treg cells (eTreg), which include CD4+CD45RA-FOXP3high, have high suppressive properties, while naïve Treg cells (nTreg), which include CD4+CD45RA+FOXP3low, possess less suppressive properties. The subset of effective Treg cells in late pregnancy is the most dominant type compared to peripheral and decidual blood Tregs (62, 63). The Treg cell count in the uterus rises dramatically in the middle to late stages of pregnancy. Late in pregnancy, there are fewer Treg cells specific to the paternal antigen in the uterine draining lymph nodes, indicating that during mid- to late pregnancy, Treg cells specific to paternal/fetal antigens migrate from the uterine draining lymph nodes to the pregnant uterus (64).

According to one study, in addition to the rise in Treg cells during pregnancy, Tregs accumulate in the uterus and draining lymph nodes each time a female mouse approaches estrus. Pregnancy-related hormones such as progesterone and estrogen, which change throughout the estrous cycle, may contribute to Treg accumulation (65). During estrus and early pregnancy, Tregs enter the uterus via chemokines such as CCL1, CCL4, CCL17, and CCL22. Approximately 70% of CD4+CD25+ Treg cells in pregnant mice express CC chemokine receptor (CCR) 5, which identifies CCL4. Additionally, the interaction of CCR8 with CCL1 can enhance the immune suppressive function of Treg cells by inducing the expression of FOXP3, IL-10, TGF-β, and the production of granzyme B (GzmB) (60).

FOXP3-HLA-G+ Tregs can express HLA-G and secrete soluble HLA-G (sHLA-G), which exerts immunoregulatory effects on a wide variety of immune cells by interacting with inhibitory receptors such as ILT2 (66, 67). Studies show that Tregs suppress NK cell cytolytic function via TGF-β (68). TGF-β also suppresses the functions of NK cells, DCs, and Mφs. The Tregs interact with DCs via CTLA-4 and LAG3 (69). Fetal trophoblast cells express and secrete several immunosuppressive molecules that play an important role in balancing Tregs (60, 70). Galectins have been shown to be important in inhibiting the maternal immune system by modifying some trophoblast regulatory processes, including promoting Treg cell development and inducing T cell death (67).

In addition to suppressing inflammatory responses to create a suitable environment for fetal tolerance, Tregs prevent complications such as spontaneous abortion and PE (65, 70). Deficiencies in the Treg population are associated with reproductive disorders, highlighting their importance in pregnancy outcomes (71).

6. Conclusions

The interaction between NK cells, Mφs, cytokines, and Tregs forms a complex network essential for establishing and maintaining a healthy pregnancy. Understanding these interactions provides valuable insights into the immunological mechanisms that support maternal and fetal health. At the maternal-fetal interface, the innate immune system—which includes NK cells and Mφs—plays a crucial role in regulating trophoblast invasion, vascular remodeling, and immunological control. Additionally, Tregs of the adaptive immune system are essential for preventing the rejection of semi-allogeneic embryos and ensuring maternal and fetal tolerance. The proper development of pregnancy depends on the cytokine-mediated balance of pro- and anti-inflammatory effects.

Selecting embryos with high HLA-G expression in IVF increases the chances of pregnancy success. Modulating regulatory cytokines or inhibiting inflammatory signals can improve dNK cell function in cases of recurrent miscarriage. The use of M2-polarization inducing factors such as IL-10 or TGF-β can reduce embryo rejection in immune infertility. Investigating future treatment strategies can lead to improvements in maternal and fetal health, increased immune tolerance, and reduced risks related to pregnancy complications.

Footnotes

Authors' Contribution: Study concept and design: M. P.; Acquisition of data: M. P.; Analysis and interpretation of data: MP; Drafting of the manuscript: M. P.; Critical revision of the manuscript for important intellectual content: M. P.; Statistical analysis: M. P.; Administrative, technical, and material support: M. P.; Study supervision: M. P.

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References

1. Yockey LJ, Iwasaki A. Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. *Immunity*. 2018;**49**(3):397-412. [PubMed ID: 30231982]. [PubMed Central ID: PMC6152841]. https://doi.org/10.1016/j.immuni.2018.07.017.
2. 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**(2). e13667. [PubMed ID: 36480305]. https://doi.org/10.1111/aji.13667.
3. Sharma S. Natural killer cells and regulatory T cells in early pregnancy loss. *Int J Dev Biol*. 2014;**58**(2-4):219-29. [PubMed ID: 25023688]. [PubMed Central ID: PMC4306453]. https://doi.org/10.1387/ijdb.140109ss.
4. Sun F, Wang S, Du M. Functional regulation of decidual macrophages during pregnancy. *J Reprod Immunol*. 2021;**143**:103264. [PubMed ID: 33360717]. https://doi.org/10.1016/j.jri.2020.103264.
5. Nagamatsu T, Schust DJ. The contribution of macrophages to normal and pathological pregnancies. *Am J Reprod Immunol*. 2010;**63**(6):460-71. [PubMed ID: 20163399]. https://doi.org/10.1111/j.1600-0897.2010.00813.x.
6. Huang N, Chi H, Qiao J. Role of Regulatory T Cells in Regulating Fetal-Maternal Immune Tolerance in Healthy Pregnancies and Reproductive Diseases. *Front Immunol*. 2020;**11**:1023. [PubMed ID: 32676072]. [PubMed Central ID: PMC7333773]. https://doi.org/10.3389/fimmu.2020.01023.
7. Meyyazhagan A, Kuchi Bhotla H, Pappuswamy M, Tsibizova V, Al Qasem M, Di Renzo GC. Cytokine see-saw across pregnancy, its related complexities and consequences. *Int J Gynaecol Obstet*. 2023;**160**(2):516-25. [PubMed ID: 35810391]. https://doi.org/10.1002/ijgo.14333.
8. Munro SK, Balakrishnan B, Lissaman AC, Gujral P, Ponnampalam AP. Cytokines and pregnancy: Potential regulation by histone deacetylases. *Mol Reprod Dev*. 2021;**88**(5):321-37. [PubMed ID: 33904218]. https://doi.org/10.1002/mrd.23430.
9. Guerby P, Tasta O, Swiader A, Pont F, Bujold E, Parant O, et al. Role of oxidative stress in the dysfunction of the placental endothelial nitric oxide synthase in preeclampsia. *Redox Biol*. 2021;**40**:101861. [PubMed ID: 33548859]. [PubMed Central ID: PMC7873691]. https://doi.org/10.1016/j.redox.2021.101861.
10. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. *Nat Rev Nephrol*. 2019;**15**(5):275-89. [PubMed ID: 30792480]. [PubMed Central ID: PMC6472952]. https://doi.org/10.1038/s41581-019-0119-6.
11. Rana S, Burke SD, Karumanchi SA. Imbalances in circulating angiogenic factors in the pathophysiology of preeclampsia and related disorders. *Am J Obstet Gynecol*. 2022;**226**(2S):S1019-34. [PubMed ID: 33096092]. [PubMed Central ID: PMC8884164]. https://doi.org/10.1016/j.ajog.2020.10.022.
12. Luo F, Yue J, Li L, Mei J, Liu X, Huang Y. Narrative review of the relationship between the maternal-fetal interface immune tolerance and the onset of preeclampsia. *Ann Transl Med*. 2022;**10**(12):713. [PubMed ID: 35845477]. [PubMed Central ID: PMC9279811]. https://doi.org/10.21037/atm-22-2287.
13. Chassen S, Jansson T. Complex, coordinated and highly regulated changes in placental signaling and nutrient transport capacity in IUGR. *Biochim Biophys Acta Mol Basis Dis*. 2020;**1866**(2):165373. [PubMed ID: 30684642]. [PubMed Central ID: PMC6650384]. https://doi.org/10.1016/j.bbadis.2018.12.024.
14. Neiger R. Long-Term Effects of Pregnancy Complications on Maternal Health: A Review. *J Clin Med*. 2017;**6**(8). [PubMed ID: 28749442]. [PubMed Central ID: PMC5575578]. https://doi.org/10.3390/jcm6080076.
15. 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**(1). [PubMed ID: 35053216]. [PubMed Central ID: PMC8773865]. https://doi.org/10.3390/biom12010068.
16. Bequet Y, Lashley E, Goddijn M, van der Hoorn MP. The role of uterine natural killer cells in recurrent pregnancy loss and possible treatment options. *Fertil Steril*. 2023;**120**(5):945-7. [PubMed ID: 37640099]. https://doi.org/10.1016/j.fertnstert.2023.08.949.
17. Rodrigues MN, Favaron PO, Dombrowski JG, Souza RMD, Miglino MA. Role of natural killer (NK) cells during pregnancy: A review. *Open J Animal Sci*. 2013;**3**(2):138-44. https://doi.org/10.4236/ojas.2013.32021.
18. Shojaei Z, Jafarpour R, Mehdizadeh S, Bayatipoor H, Pashangzadeh S, Motallebnezhad M. Functional prominence of natural killer cells and natural killer T cells in pregnancy and infertility: A comprehensive review and update. *Pathol Res Pract*. 2022;**238**:154062. [PubMed ID: 35987030]. https://doi.org/10.1016/j.prp.2022.154062.
19. Bos M, Colucci F. A New Look at Immunogenetics of Pregnancy: Maternal Major Histocompatibility Complex Class I Educates Uterine Natural Killer Cells. *Int J Mol Sci*. 2024;**25**(16). [PubMed ID: 39201555]. [PubMed Central ID: PMC11354926]. https://doi.org/10.3390/ijms25168869.
20. Faas MM. Uterine natural killer cells and successful pregnancy: from mouse experiments to human physiology. *Explor Immunol*. 2022:518-39. https://doi.org/10.37349/ei.2022.00065.
21. Mao J, Feng Y, Zhu X, Ma F. The Molecular Mechanisms of HLA-G Regulatory Function on Immune Cells during Early Pregnancy. *Biomolecules*. 2023;**13**(8). [PubMed ID: 37627278]. [PubMed Central ID: PMC10452754]. https://doi.org/10.3390/biom13081213.
22. Moffett A, Shreeve N. Local immune recognition of trophoblast in early human pregnancy: controversies and questions. *Nat Rev Immunol*. 2023;**23**(4):222-35. [PubMed ID: 36192648]. [PubMed Central ID: PMC9527719]. https://doi.org/10.1038/s41577-022-00777-2.
23. Faas MM, de Vos P. Uterine NK cells and macrophages in pregnancy. *Placenta*. 2017;**56**:44-52. [PubMed ID: 28284455]. https://doi.org/10.1016/j.placenta.2017.03.001.
24. Wells AI, Coyne CB. Uterine NK cell education: Learning the ropes in pregnancy. *Immunity*. 2021;**54**(6):1102-4. [PubMed ID: 34107267].

https://doi.org/10.1016/j.immuni.2021.05.009.

25. 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**(2):181-195 e9. [PubMed ID: 38237587]. https://doi.org/10.1016/j.stem.2023.12.013.
26. Liu Y, Gao S, Zhao Y, Wang H, Pan Q, Shao Q. Decidual Natural Killer Cells: A Good Nanny at the Maternal-Fetal Interface During Early Pregnancy. *Front Immunol*. 2021;**12**:663660. [PubMed ID: 34054831]. [PubMed Central ID: PMC8149889]. https://doi.org/10.3389/fimmu.2021.663660.
27. Jabrane-Ferrat N. Features of Human Decidual NK Cells in Healthy Pregnancy and During Viral Infection. *Front Immunol*. 2019;**10**:1397. [PubMed ID: 31379803]. [PubMed Central ID: PMC6660262]. https://doi.org/10.3389/fimmu.2019.01397.
28. Feyaerts D, Benner M, Comitini G, Shadmanfar W, van der Heijden OWH, Joosten I, et al. NK cell receptor profiling of endometrial and decidual NK cells reveals pregnancy-induced adaptations. *Front Immunol*. 2024;**15**:1353556. [PubMed ID: 38571943]. [PubMed Central ID: PMC10987737]. https://doi.org/10.3389/fimmu.2024.1353556.
29. Zhang X, Wei H. Role of Decidual Natural Killer Cells in Human Pregnancy and Related Pregnancy Complications. *Front Immunol*. 2021;**12**:728291. [PubMed ID: 34512661]. [PubMed Central ID: PMC8426434]. https://doi.org/10.3389/fimmu.2021.728291.
30. Xu X, Zhou Y, Wei H. Roles of HLA-G in the Maternal-Fetal Immune Microenvironment. *Front Immunol*. 2020;**11**:592010. [PubMed ID: 33193435]. [PubMed Central ID: PMC7642459]. https://doi.org/10.3389/fimmu.2020.592010.
31. Bai Y, Liang J, Liu W, Wang F, Li C. Possible roles of HLA-G regulating immune cells in pregnancy and endometrial diseases via KIR2DL4. *J Reprod Immunol*. 2020;**142**:103176. [PubMed ID: 32711226]. https://doi.org/10.1016/j.jri.2020.103176.
32. Jena MK, Nayak N, Chen K, Nayak NR. Role of Macrophages in Pregnancy and Related Complications. *Arch Immunol Ther Exp (Warsz)*. 2019;**67**(5):295-309. [PubMed ID: 31286151]. [PubMed Central ID: PMC7140981]. https://doi.org/10.1007/s00005-019-00552-7.
33. Krop J, Tian X, van der Hoorn ML, Eikmans M. The Mac Is Back: The Role of Macrophages in Human Healthy and Complicated Pregnancies. *Int J Mol Sci*. 2023;**24**(6). [PubMed ID: 36982375]. [PubMed Central ID: PMC10049527]. https://doi.org/10.3390/ijms24065300.
34. Ning F, Liu H, Lash GE. The Role of Decidual Macrophages During Normal and Pathological Pregnancy. *Am J Reprod Immunol*. 2016;**75**(3):298-309. [PubMed ID: 26750089]. https://doi.org/10.1111/aji.12477.
35. Wang XQ, Zhou WJ, Hou XX, Fu Q, Li DJ. Trophoblast-derived CXCL16 induces M2 macrophage polarization that in turn inactivates NK cells at the maternal-fetal interface. *Cell Mol Immunol*. 2018;**15**(12):1038-46. [PubMed ID: 29588487]. [PubMed Central ID: PMC6269500]. https://doi.org/10.1038/s41423-018-0019-x.
36. Pourfridoni M, Askarpour H. COVID-19 and the increase in schizophrenia incidence in the future: A hypothesis and a serious warning. *Health Sci Rep*. 2023;**6**(1). e978. [PubMed ID: 36479392]. [PubMed Central ID: PMC9721364]. https://doi.org/10.1002/hsr2.978.
37. Bezemer RE, Faas MM, van Goor H, Gordijn SJ, Prins JR. Decidual macrophages and Hofbauer cells in fetal growth restriction. *Front Immunol*. 2024;**15**:1379537. [PubMed ID: 39007150]. [PubMed Central ID: PMC11239338]. https://doi.org/10.3389/fimmu.2024.1379537.
38. Vishnyakova P, Elchaninov A, Fatkhudinov T, Sukhikh G. Role of the Monocyte-Macrophage System in Normal Pregnancy and Preeclampsia. *Int J Mol Sci*. 2019;**20**(15). [PubMed ID: 31357698]. [PubMed Central ID: PMC6696152]. https://doi.org/10.3390/ijms20153695.
39. Yin T, Li X, Li Y, Zang X, Liu L, Du M. Macrophage plasticity and function in cancer and pregnancy. *Front Immunol*. 2023;**14**:1333549. [PubMed ID: 38274812]. [PubMed Central ID: PMC10808357]. https://doi.org/10.3389/fimmu.2023.1333549.
40. Zhuang B, Shang J, Yao Y. HLA-G: An Important Mediator of Maternal-Fetal Immune-Tolerance. *Front Immunol*. 2021;**12**:744324. [PubMed ID: 34777357]. [PubMed Central ID: PMC8586502]. https://doi.org/10.3389/fimmu.2021.744324.
41. Lynge Nilsson L, Djurisic S, Hviid TV. Controlling the Immunological Crosstalk during Conception and Pregnancy: HLA-G in Reproduction. *Front Immunol*. 2014;**5**:198. [PubMed ID: 24860568]. [PubMed Central ID: PMC4026753]. https://doi.org/10.3389/fimmu.2014.00198.
42. Chen Q, Guo F, Jin HY, Lau S, Stone P, Chamley L. Phagocytosis of apoptotic trophoblastic debris protects endothelial cells against activation. *Placenta*. 2012;**33**(7):548-53. [PubMed ID: 22504042]. https://doi.org/10.1016/j.placenta.2012.03.007.
43. Hviid TV. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. *Hum Reprod Update*. 2006;**12**(3):209-32. [PubMed ID: 16280356]. https://doi.org/10.1093/humupd/dmi048.
44. Cecati M, Giannubilo SR, Emanuelli M, Tranquilli AL, Saccucci F. HLA-G and pregnancy adverse outcomes. *Med Hypotheses*. 2011;**76**(6):782-4. [PubMed ID: 21376476]. https://doi.org/10.1016/j.mehy.2011.02.017.
45. Rouas-Freiss N, Goncalves RM, Menier C, Dausset J, Carosella ED. Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. *Proc Natl Acad Sci U S A*. 1997;**94**(21):11520-5. [PubMed ID: 9326642]. [PubMed Central ID: PMC23525]. https://doi.org/10.1073/pnas.94.21.11520.
46. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine*. 2014;**70**(2):134-40. [PubMed ID: 25082648]. [PubMed Central ID: PMC4254150]. https://doi.org/10.1016/j.cyto.2014.06.018.
47. Spence T, Allsopp PJ, Yeates AJ, Mulhern MS, Strain JJ, McSorley EM. Maternal Serum Cytokine Concentrations in Healthy Pregnancy and Preeclampsia. *J Pregnancy*. 2021;**2021**:6649608. [PubMed ID: 33680514]. [PubMed Central ID: PMC7925069]. https://doi.org/10.1155/2021/6649608.
48. Dutta S, Sengupta P, Liew FF. Cytokine landscapes of pregnancy: mapping gestational immune phases. *Gynecol Obstetrics Clin Med*. 2024;**4**(1). https://doi.org/10.1136/gocm-2024-000011.
49. Raghupathy R. Cytokines and pregnancy complications: modulation for prevention and treatment. *Explor Immunol*. 2022:414-27. https://doi.org/10.37349/ei.2022.00059.
50. Wang L, Liu Z, Huang D, Ran Y, Zhang H, He J, et al. IL-37 Exerts Anti-Inflammatory Effects in Fetal Membranes of Spontaneous Preterm Birth via the NF-kappaB and IL-6/STAT3 Signaling Pathway. *Mediators Inflamm*. 2020;**2020**:1069563. [PubMed ID: 32733162]. [PubMed Central ID: PMC7369678]. https://doi.org/10.1155/2020/1069563.
51. Yue CY, Zhang B, Ying CM. Elevated Serum Level of IL-35 Associated with the Maintenance of Maternal-Fetal Immune Tolerance in Normal Pregnancy. *PLoS One*. 2015;**10**(6). e0128219. [PubMed ID: 26042836]. [PubMed Central ID: PMC4456370]. https://doi.org/10.1371/journal.pone.0128219.
52. Wang M. The Role of IL-37 and IL-38 in Obstetrics Abnormalities. *Front Med (Lausanne)*. 2021;**8**:737084. [PubMed ID: 34513891]. [PubMed Central ID: PMC8429600]. https://doi.org/10.3389/fmed.2021.737084.
53. Bello RO, Chin VK, Abd Rachman Isnadi MF, Abd Majid R, Atmadini Abdullah M, Lee TY, et al. The Role, Involvement and Function(s) of Interleukin-35 and Interleukin-37 in Disease Pathogenesis. *Int J Mol Sci*. 2018;**19**(4). [PubMed ID: 29641433]. [PubMed Central ID: PMC5979316]. https://doi.org/10.3390/ijms19041149.

54. Lombardelli L, Logiodice F, Kullolli O, Haller H, Agostinis C, Bulla R, et al. At Embryo Implantation Site IL-35 Secreted by Trophoblast, Polarizing T Cells towards IL-35+ IL-10+ IL-4+ Th2-Type Cells, Could Favour Fetal Allograft Tolerance and Pregnancy Success. *Int J Mol Sci*. 2022;**23**(9). [PubMed ID: 35563316]. [PubMed Central ID: PMC9103079]. https://doi.org/10.3390/ijms23094926.
55. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. 2011;**11**(2):98-107. [PubMed ID: 21233852]. https://doi.org/10.1038/nri2925.
56. Southcombe JH, Redman CW, Sargent IL, Granne I. Interleukin-1 family cytokines and their regulatory proteins in normal pregnancy and pre-eclampsia. *Clin Exp Immunol*. 2015;**181**(3):480-90. [PubMed ID: 25693732]. [PubMed Central ID: PMC4557384]. https://doi.org/10.1111/cei.12608.
57. Satiroglu O, Gurlek B, Durakoglugil ME, Duman H, Erdogan T, Cetin M, et al. The role of serum interleukin-37 levels, inflammation and blood pressure in patients with preeclampsia. *Clin Exp Hypertens*. 2020;**42**(7):669-74. [PubMed ID: 32476486]. https://doi.org/10.1080/10641963.2020.1772813.
58. Geldenhuys J, Rossouw TM, Lombaard HA, Ehlers MM, Kock MM. Disruption in the Regulation of Immune Responses in the Placental Subtype of Preeclampsia. *Front Immunol*. 2018;**9**:1659. [PubMed ID: 30079067]. [PubMed Central ID: PMC6062603]. https://doi.org/10.3389/fimmu.2018.01659.
59. de Graaf DM, Teufel LU, Joosten LAB, Dinarello CA. Interleukin-38 in Health and Disease. *Cytokine*. 2022;**152**:155824. [PubMed ID: 35220115]. https://doi.org/10.1016/j.cyto.2022.155824.
60. Jorgensen N, Persson G, Hviid TVF. The Tolerogenic Function of Regulatory T Cells in Pregnancy and Cancer. *Front Immunol*. 2019;**10**:911. [PubMed ID: 31134056]. [PubMed Central ID: PMC6517506]. https://doi.org/10.3389/fimmu.2019.00911.
61. Headen K, Jakaite V, Mesaric VA, Scotta C, Lombardi G, Nicolaides KH, et al. The Role of Regulatory T Cells and Their Therapeutic Potential in Hypertensive Disease of Pregnancy: A Literature Review. *Int J Mol Sci*. 2024;**25**(9). [PubMed ID: 38732104]. [PubMed Central ID: PMC11084408]. https://doi.org/10.3390/ijms25094884.
62. Shigeta N, Kumasawa K, Tanaka A, Badger Wing J, Nakamura H, Sakaguchi S, et al. Dynamics of effector and naive Regulatory T cells throughout pregnancy. *J Reprod Immunol*. 2020;**140**:103135. [PubMed ID: 32339846]. https://doi.org/10.1016/j.jri.2020.103135.
63. Tsuda S, Nakashima A, Shima T, Saito S. New Paradigm in the Role of Regulatory T Cells During Pregnancy. *Front Immunol*. 2019;**10**:573. [PubMed ID: 30972068]. [PubMed Central ID: PMC6443934]. https://doi.org/10.3389/fimmu.2019.00573.
64. Saito S. Reconsideration of the Role of Regulatory T Cells during Pregnancy: Differential Characteristics of Regulatory T Cells between the Maternal-Fetal Interface and Peripheral Sites and between Early and Late Pregnancy. *Med Princ Pract*. 2022;**31**(5):403-14. [PubMed ID: 36195068]. [PubMed Central ID: PMC9801372]. https://doi.org/10.1159/000527336.
65. Leber A, Teles A, Zenclussen AC. Regulatory T cells and their role in pregnancy. *Am J Reprod Immunol*. 2010;**63**(6):445-59. [PubMed ID: 20331584]. https://doi.org/10.1111/j.1600-0897.2010.00821.x.
66. Wang W, Zhao Y, Zhou X, Sung N, Chen L, Zhang X, et al. Dynamic changes in regulatory T cells during normal pregnancy, recurrent pregnancy loss, and gestational diabetes. *J Reprod Immunol*. 2022;**150**:103492. [PubMed ID: 35149275]. https://doi.org/10.1016/j.jri.2022.103492.
67. Krop J, Heidt S, Claas FHJ, Eikmans M. Regulatory T Cells in Pregnancy: It Is Not All About FoxP3. *Front Immunol*. 2020;**11**:1182. [PubMed ID: 32655556]. [PubMed Central ID: PMC7324675]. https://doi.org/10.3389/fimmu.2020.01182.
68. Roy S, Barnes PF, Garg A, Wu S, Cosman D, Vankayalapati R. NK cells lyse T regulatory cells that expand in response to an intracellular pathogen. *J Immunol*. 2008;**180**(3):1729-36. [PubMed ID: 18209070]. https://doi.org/10.4049/jimmunol.180.3.1729.
69. Arce-Sillas A, Alvarez-Luquin DD, Tamaya-Dominguez B, Gomez-Fuentes S, Trejo-Garcia A, Melo-Salas M, et al. Regulatory T Cells: Molecular Actions on Effector Cells in Immune Regulation. *J Immunol Res*. 2016;**2016**:1720827. [PubMed ID: 27298831]. [PubMed Central ID: PMC4889823]. https://doi.org/10.1155/2016/1720827.
70. Ruocco MG, Chaouat G, Florez L, Bensussan A, Klatzmann D. Regulatory T-cells in pregnancy: historical perspective, state of the art, and burning questions. *Front Immunol*. 2014;**5**:389. [PubMed ID: 25191324]. [PubMed Central ID: PMC4139600]. https://doi.org/10.3389/fimmu.2014.00389.
71. Gomez-Lopez N, Arenas-Hernandez M, Romero R, Miller D, Garcia-Flores V, Leng Y, et al. Regulatory T Cells Play a Role in a Subset of Idiopathic Preterm Labor/Birth and Adverse Neonatal Outcomes. *Cell Rep*. 2020;**32**(1):107874. [PubMed ID: 32640239]. [PubMed Central ID: PMC7396155]. https://doi.org/10.1016/j.celrep.2020.107874.