CHAPTER II

REVIEW OF LITERATURE

2.1. Placental Microenvironment

The maternal decidua serves as the interface connecting maternal immune cells and fetal cells as shown in section 1.1. Decidual changes in the endometrial stroma are vital for successful implantation and placentation (1). During early pregnancy, the decidua is primarily composed of trophoblast cells of fetal origin and maternal decidual immune cells (DICs) (2). The majority of these DICs, ranging from 40% to 60%, are Natural Killer Cells (NK cells), followed by macrophages and some T cells (3).

2.1.1. Immune cells in decidua

2.1.1.1. Extravillous trophoblasts (EVTs)

The placenta plays a crucial role in maintaining a healthy pregnancy through a complex developmental process involving various cell types. One of these cell types is the extravillous trophoblasts (EVTs), which are derived from cytotrophoblasts (4-5). During early pregnancy, cytotrophoblasts infiltrate the uterine decidua and transform into EVTs. By the eighth week, these EVTs invade the decidua and establish direct communication with maternal uterine spiral arteries(6-7).EVTs via their interaction with decidual NK (dNK) cells they remodel the arterial walls and replace the endothelial cells, resulting in wider, adrenergic-insensitive, low-resistance conduits (8-9). This transformation allows optimal nutrient exchange and facilitates the transport of oxygen, nutrients, and metabolic support to the developing fetus(10-11). Disruptions in EVT-decidual immune cell communication (DIC) can lead to immune imbalances and maternal-fetal immune tolerance issues, potentially impacting pregnancy outcomes (12-14).The EVT expresses both HLA-C and non-classical HLA class I molecules, including HLA-E, HLA-F, and HLA-G (17-18). Although HLA-G expression is tissue restricted, it is abundantly expressed in trophoblast cells, mediating tolerance to the semi allogenic fetus and promoting angiogenesis andvascularization (19-20).

2.1.1.1.1. Human Leukocyte Antigen (HLA) ligands

of EVT2.1.1.1.1.1. HLA-G

The EVTs expresses both HLA-C and non-classical HLA class I molecules, including HLA-E, HLA-F, and HLA-G (21-2) . Although HLA-G expression is tissue restricted, it is abundantly expressed in trophoblast cells. HLA-G is the only known ligand for KIR 2DL4 receptors expressed on dNK cells (21,22).It mediates tolerance to the semi allogenic fetus and promotes angiogenesis and vascularization (23).

2.1.1.1.1.1.2. HLA-G isoforms

HLA-G has seven isoforms which include four membrane-bound (HLA-G1, -G2, -G3, and -G4) and three soluble (HLA-G5, -G6, and -G7) isoforms (24-25). However, there is a report of another soluble isoform called shedding HLA-G1 which is formed due to proteolytic cleavage of cell surface HLA-G1 by metalloproteinase 2 (MMP 2) (26-27). Distinctness of HLA-G isoforms lies in the presence of the number of extracellular immunoglobulin-like domains and whether intronic sequence encoded residues are included or not. All the seven isoforms have extracellularα1 domain (28-29).

The presence or absence of three extracellular domains - α1, α2, and α3 domains determines the isoforms. α1 and α3 domains are present in HLA-G2 but the α2 domain is deleted (29-30). Similarly, in HLA-G3 both α2 and α3 domains are absent and it contains only an α1 domain. HLA-G4 has both α1 and α2 domains but the α3 domain is absent. α1 and α2 domains form the antigen-presenting peptide-binding cleft in HLA-G1 and HLA-G5 molecules (31-35). HLA-G5 and HLA-G6 are the counterparts of HLA-G1 and HLA-G2 and are encoded by intron 4 (36-38). In HLA-G7, only α1 domain is present which is linked to two amino acid residues and is encoded by intron 2 (39-40).

Immunoregulatory functions of HLA-G is achieved via interaction of various activating and inhibitory receptors such as LILRB1 (ILT2/CD85j), LILRB2 (ILT4/CD85d), and KIR2DL4 (CD158d) expressed on the surface of immune cells (41-44).

HLA-G interacts with its receptors to mediate immune suppression through dysregulation of immune cell proliferation, differentiation, apoptosis cytotoxicity, cytokine signaling, chemotaxis and induction of regulatory cells and MDSCs or M2 type

macrophage (43-44). LILRB1 and LILRB2 receptors have 4 extracellular domains (D1–D4) and a transmembrane region of 23 amino acids and three immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails (45). The presence of these ITIM motifs is responsible for inhibitory characteristics and those LILR family receptors which lack these motifs possess an Arg residue in the transmembrane domain (46). Interaction of LILR receptors with their ligands results in the initiation of the inhibitory cascade by phosphorylating ITIMs and recruiting SHP phosphatases (47-48). Dimers formed by HLA-G bind on LILR receptors with a higher affinity compared to HLA-G monomers(49-51). These HLA-G dimers are also capable of binding to both the receptors simultaneously (52-54). Both LILRB1 and LILRB2 receptors can bind to both classical and non-classical HLA-I molecules. NK cells, T cells, B cells, MDSCs, DCs, and decidual macrophages mainly express LILRB1 or ILT2 receptor while LILRB2 or ILT4 expression is limited to monocytes, macrophages, neutrophils and DCs (55-58).

KIR2DL4 or CD158d receptor is from killer cell immunoglobulin-like receptors (KIR) family and is expressed in all NK cell types(59-60). KIR2DL4 is distinct from other members of the KIR family with both activating and inhibitory properties. It contains a long cytoplasmic tail which is a characteristic of inhibitory receptors and a charged amino acid in the transmembrane domain similarly to activating KIR receptors (61-63). As per literature, IL-2 induces the expression of KIR2DL4 and its activation results in a weak cytotoxic activity with high IFN-γ production(64).

2.1.1.1.1.2. HLA-C

Class Ib HLA-C molecules expressed on EVT serve as ligands for KIR2DL1/L2/L3/S1/S2(65). And a balance of activating and inhibitory signalling by KIRs is crucial for optimal NK activation, for maintenance of pregnancy. HLA-C has two allotypes- HLA-C1 and HLA-C2, depending on the presence of asparagine (HLA-C1) or lysine (HLA- C2) at position 80 in the alpha 2 domain (66).

HLA-C2 acts as a cognate ligand for the inhibitory receptor KIR2DL1, delivering a robust inhibitory signal while also binding to the activating allele KIR2DS1 (67-68). The inhibitory signaling mediated by KIR2DL1 and HLA-C2 is counterbalanced by activating signaling mediated by HLA-C2 and KIR2DS1(69-71). On the other hand, HLA-C1 serves as the cognate ligand for inhibitory receptors KIR2DL2 and KIR2DL3, although with less effective inhibitory signaling compared to KIR2DL1(72-73). Furthermore, KIR2DL2 and, to a lesser extent, KIR2DL3 exhibit some cross-reactivity with C2 ligands.

2.1.1.2. decidual NK (dNK)

In humans, NK cells exist as peripheral NK cells (pNK cells) in the bloodstream and are widely distributed throughout the body. Additionally, there are tissue-resident NK (trNK) cells found in peripheral tissues, including the liver, lungs, skin, and uterus. A specific subset of trNK cells, known as decidual NK (dNK) cells, can be found in the endometrial decidua.Unlike the periphery, dNK exhibit CD56 bright CD16-KIR+ phenotype, lower cytotoxicity, and higher cytokine secretion. dNK is minimal cytotoxic and instead produce cytokines, growth factors, and angiogenic factors needed to appropriately remodel the maternal spiral arteries, promoting angiogenesis and attracting invasive trophoblasts to the decidua. Distinct subsets of dNK cells have been identified, encompassing different stages of pregnancy. dNK1 cells express killer cell immunoglobulin-like receptors (KIRs) and Leukocyte Immunoglobulin-Like Receptor B1 (ILT2). These receptors interact with HLA molecules, including HLA-C and HLA-G, expressed by extravillous trophoblasts (EVTs), facilitating trophoblast invasion and establishing an immune-tolerant microenvironment. dNK1 cells also possess cytoplasmic granule proteins involved in placental infection immunity and enzymes related to glycolysis. On the other hand, dNK2 cells express the chemokine receptor XCR1, which mediates the recruitment of EVTs and dendritic cells at the fetal-maternal interface, particularly attracting cDC1 cells through CCL5- CCR1 interactions. However, dNK3 cells are less prevalent in the decidua. The origin of dNK cells in the

uterus during pregnancy is still a topic of debate, with two main hypotheses proposing their recruitment from peripheral NK cells or differentiation within the uterus from local progenitor cells (30-32).

2.1.1.2.1 NK receptors: Killer Cell Immunoglobulin-Like Receptors (KIRs)

NK cells carry an array of activating and inhibitory receptors that recognize the ligands on target cells and control the cytolytic function (25). Activating receptors, NKG2D and members of natural cytotoxicity receptors (NCR) group facilitates allorecognition and cytotoxicity (26-28). While, inhibitory receptors-KIRs,CD94-NKG2A and co-receptors like CD96,TIGIT and PD1 complex mediate inhibition of effector function of NK cells.

KIR multigene family expressed on surface of dNK cells show high diversity with respect to gene content (genotype), allelic polymorphism and Copy number variations (CNVs) (39, 40). Encoded in the leukocyte receptor complex (LRC) on human chromosome 19q13.4, KIRs consist of fourteen genes [KIR2DL1–5,KIR3DL1–3, KIR2DS1–5, KIR3DS1] and two 2 pseudogenes (39). They inhibitory receptors have long cytoplasmic tails (L) containing immunoreceptor tyrosine-based inhibition motifs (ITIMs) which gives inhibitory signals (41, 42). However, they also bear immune-receptor tyrosine-based activating motifs (ITAMs) in short tail (S) receptors, resulting in transmission of an activating signal(43). Based on their gene content, KIRs are categorized into haplogroup-A and haplogroup-B. While haplogroup A is conserved and has higher inhibitory gene content, haplogroup B has variation with different combination of inhibitory and activating genes(44). KIR2DL4 is different from the other KIR family members as it only contains one ITIM instead of two and possesses an arginine in its transmembrane domain (45), suggesting its potential activating downstream signalling (46). KIR2DL4 expressed on dNK) cells has both an activating and inhibitory signaling (46, 47). It is present in endosomal compartment as well as on cell surface and upon interacting with HLAG, KIR2DL4 mediates endosomal signaling for the secretion of numerous cytokines and chemokines and growth factors essential for early placentation (48, 49). Aberrant expression or function of these receptors and ligands have been linked to pregnancy complications, including RSAB (48,50,51). Several recent studies have investigated the role of NK cells in early pregnancy failure and their interaction with HLA molecules.

2.2. Early pregnancy loss

Early pregnancy loss is a significant complication affecting around 10% of clinically confirmed pregnancies and is defined as the spontaneous abortion of a fetus before twenty weeks of gestation(52). Recurrent spontaneous abortion (RSAB), characterized by the repeated occurrence of two or more early pregnancy losses, is observed in approximately 2-3% of cases (53). While certain factors such as chromosomal abnormalities, hormonal imbalances, uterine abnormalities, autoantibodies, and immune dysfunction are recognized causes of early pregnancy loss, the underlying causes of approximately 40% of cases remain unidentified.

Immune dysfunction at the maternal-fetal interface has been implicated in early pregnancy failure, highlighting the complex nature of the immune response during pregnancy, where the maternal immune system must accept the allogenic fetus while maintaining the ability to mount an immune response against potential pathogens (53).

2.3 KIR-HLA disbalance in early pregnancy loss

Population studies have identified different KIR-HLA genotypes associated with pregnancy outcomes, with the prevalence of specific genotypes varying among populations (54-55). For instance, the Japanese population shows a high prevalence of the AA genotype with HLA-C1 allotypes, while Asian populations tend to have a higher frequency of activating genes in the KIR B haplogroup and HLA-C2 allotypes, which are favorable for successful pregnancies (56-57). The impact of immune factors on pregnancy outcomes has been the focus of several studies (57- 58). Maternal KIR2DS1 with paternal HLA-C2 has been reported as a protective factor , but conflicting results suggest that this combination poses a risk (59-60). Lower levels of KIR2DL1 and KIR2DS1 in peripheral NK cells have also been associated with adverse pregnancy outcomes (61-62). This highlights the co-evolutionary relationship between KIRs and HLA class I molecules , under balancing selection for reproduction and survival (63).

HLA-G is the only known ligand for KIR 2DL4 receptors expressed on dNK cells (64, 65) and mediates tolerance to the semi allogenic fetus and promotes angiogenesis and vascularization (65). Studies, including our own and those conducted by other researchers (66-67) have shown that HLA-G levels in the maternal serum as well as in early decidua and term placenta correlated positively with pregnancy outcome . The expression of HLA molecules on fetal trophoblasts has also been studied in the context of SAB. A study by Yang et al. (68) found that the expression of HLA-G was significantly decreased in the placenta of women with RSA compared to controls(80) while others have reported contradictory data on HLA-G levels (69) . Similarly, earlier reports on expression of HLA-C was significantly decreased in the women withRSA compared to controls (70).

2.3.3. Cytokine and Chemokine environment in early pregnancy loss

Chemokines are multifunctional molecules involved in intercellular communication and signal transduction. chemokine/chemokine receptor interactions dominate the trafficking of leukocytes, the mechanisms underlying the recruitment and maintenance of DICs most likely involve the expression and secretion of chemokines at the maternal–fetal interface (71-72).

Decidual cells produce various types of chemokines, such asCCL2, CXCL8, CX3CL1, CXCL10 and CXCL12, at significant levels (73-74). These chemokines are differently involved in the migration of peripheral NK (pNK) and decidual NK (dNK) cells into the decidua (75-76) Interestingly, CXCL12 andCX3CL1 preferentially attract CD161 pNK cells, while CXCL10 is essential for the recruitment of $CD56^{+}$CD162 pNK cells (77-78). Uterine expression of CXCL14 may also play a role in uterine NK-cell recruitment during the early pregnancy (79-80). However, a clear picture of the effects of CXCL14 on uterine NK-cell recruitment in the context of the uterus is still vague, since a report using knockout models indicates opposite effects of its chemoattractant roles on many types of leukocytes (81).

dNK cells are potent source of cytokines in the endometrium (82). The regulation of the endometrial immune system is crucial for successful implantation and maintenance of pregnancy, and this is achieved through the synchronized secretion of specific types of cytokines (83). The pro-inflammatory Th1 cell subtype secretes cytokines such as interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF-α), and interleukins IL-1, IL-2, IL-12, IL-15, and IL-18 (84). Conversely, the Th2 subtype secretes anti-inflammatory cytokines, including IL-4, IL-5, IL-6, IL-10, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF)(85). The balance between these cytokines, represented by the Th1/Th2 ratio, undergoes periodic changes throughout pregnancy, reflecting different stages and processes (85). Cytokines, such as IL-1, IL- 6, IL-8, TNF, matrix metalloproteinases (MMPs), and prostaglandins, also contribute to cervical effacement/dilatation and rupture of the membranes, leading to labor and delivery (86).

One of the critical cytokines secreted by dNK cells is transforming growth factor-beta (TGF-β), which plays a crucial role in promoting angiogenesis and vascular remodeling in the placenta (88-90). TGF-β also regulates the proliferation and differentiation of trophoblast cells and promotes their invasion into the maternal endometrium (91-93). Additionally, dNK cells secrete various chemokines that recruit other immune cells to the maternal-fetal interface, including macrophages and T cells (94-95)

2.3.4 Autoantibodies in early pregnancy loss.

Autoantibodies in pathological pregnancies are implicated in NK cell imbalance, which plays a crucial role in maintaining immune balance at the maternal-fetal interface (96-97). Thrombotic antiphospholipid syndrome (APS), characterized by persistent antiphospholipid antibodies (aPLs) including anti-β2 glycoprotein I (anti-β2GPI) and anticardiolipin antibodies, is associated with adverse pregnancy outcomes (98-99). Abnormal expression of aPLs can induce inflammatory reactions and vascular endothelial damage, leading to microvascular thrombus formation. Anti-β2GPI antibodies inhibit trophoblastic cell autophagy and activate inflammasomes, amplifying the inflammatory response and potentially resulting in fetal rejection(100-102).

Notably, occurrence of both aCL and aβ2GPI antibodies have been associated with pregnancy complications (98-99,103). However, with conflicting findings. While some studies have not found a significant correlation between these antibodies and pregnancy loss, aCL has been identified as the most prevalent among the obstetric population with adverse pregnancies (103). Elevated levels of APLA have been observed history of multiple pregnancy failures as comparedto single pregnancy loss (95-97).

Additionally, the presence of antinuclear autoantibodies (ANAs) targeting cell nucleus components is associated with increased risks of complications like preeclampsia and fetal growth restriction (101). ANAs may impact embryo quality and development, reducing pregnancy and implantation rates (103).

2.4. References

1. Warning JC, McCracken SA, Morris JM. A balancing act: mechanisms by which the fetus avoids rejection by the maternal immune system. Reproduction.;141(6):715. 2011
2. Le Bouteiller P, Bensussan A. Up-and-down immunity of pregnancy in humans. F1000Research.;6. 2017
3. Schumacher A, Sharkey DJ, Robertson SA, Zenclussen AC. Immune cells at the fetomaternal interface: how the microenvironment modulates immune cells to foster fetal development. The Journal of Immunology.201(2):325-34. 2018
4. Sargent I, Wilkins T, Redman C. Maternal immune responses to the fetus in early pregnancy and recurrent miscarriage. The Lancet.332(8620):1099-1041988.
5. Ghaebi M, Nouri M, Ghasemzadeh A, Farzadi L, Jadidi-Niaragh F, Ahmadi M, et al. Immune regulatory network in successful pregnancy and reproductive failures. Biomedicine &Pharmacotherapy.88:61-73. 2017
6. Alves C, Rapp A. Spontaneous Abortion: StatPearls Publishing, Treasure Island (FL); 2022 2022.
7. Hertz-Picciotto I, Samuels SJ. Incidence of early loss of pregnancy. The New England journal of medicine.;319(22):1483-4. 1988
8. Prager S, Dalton VK, Allen RH. Early pregnancy loss. Obstetrics and Gynecology.;132(5):E197-E207. 2018
9. Fu YY, Ren CE, Qiao PY, Meng YH. Uterine natural killer cells and recurrent spontaneous abortion. American Journal of Reproductive Immunology.;86(2):e13433. 2021
10. Coulam CB, Stern JJ, Bustillo M. Ultrasonographic findings of pregnancy losses after treatment for recurrent pregnancy loss: intravenous immunoglobulin versus placebo. Fertility andsterility.;61(2):248-51. 1994
11. Vaquero E, De Carolis C, Valensise H, Romanini C, Lazzarin N,Moretti C. Mild Thyroid Abnormalities and Recurrent Spontaneous Abortion: Diagnostic and Therapeutical Approach 1. American Journal of Reproductive Immunology.43(4):204-8. 2000

12. Toth B, Jeschke U, Rogenhofer N, Scholz C, Würfel W, Thaler CJ, et al. Recurrent miscarriage: current concepts in diagnosis and treatment. Journal of reproductive immunology;85(1):25-32. . 2010
13. Rotondo J, Bosi S, Bazzan E, Di Domenico M, De Mattei M, Selvatici R, et al. Methylenetetrahydrofolate reductase gene promoter hypermethylation in semen samples of infertile couples correlates with recurrent spontaneous abortion. Human reproduction.;27(12):3632-8. 2012
14. Rotondo JC, Selvatici R, Di Domenico M, Marci R, Vesce F, Tognon M, et al. Methylation loss at H19 imprinted gene correlates with methylenetetrahydrofolate reductase gene promoter hypermethylation in semen samples from infertile males. Epigenetics.8(9):990-7. 2013
15. 15.Rotondo JC, Lanzillotti C, Mazziotta C, Tognon M, Martini F. Epigenetics of male infertility: the role of DNA methylation. Frontiers in Cell and Developmental Biology. 1;9:689624. 202
16. 16.Griebel CP, Halvorsen J, Golemon TB, Day AA. Management of spontaneous abortion.American family physician.;72(7):1243-50. 2005
17. Fukuta K, Yoneda S, Yoneda N, Shiozaki A, Nakashima A, Minamisaka T, et al. Risk factors for spontaneous miscarriage above 12 weeks or premature delivery in patients undergoing cervical polypectomy during pregnancy. BMC Pregnancy and Childbirth.;20(1):1-9. 2020
18. Jeve YB, Davies W. Evidence-based management of recurrent miscarriages. Journal ofhuman reproductive sciences.;7(3):159. 2014
19. Hsu P, Nanan RKH. Innate and adaptive immune interactions at the fetal–maternal interface in healthy human pregnancy and pre-eclampsia. Frontiers in immunology.;5:125. 2014
20. Glover LE, Crosby D, Thiruchelvam U, Harmon C, Chorcora CN, Wingfield MB, et al. Uterine natural killer cell progenitor populations predict successful implantation in women with endometriosis-associated infertility. American Journal of Reproductive Immunology.;79(3):e12817. 2018

21 Yang F, Zheng Q, Jin L. Dynamic Function and Composition Changes of Immune Cells During Normal and Pathological Pregnancy at the Maternal-Fetal Interface. Frontiers in Immunology. 2019-October-18;10. English,2019.

22. Gomez-Lopez N, Guilbert LJ, Olson DM. Invasion of the leukocytes into the fetal-maternal interface during pregnancy. Journal of leukocyte biology.;88(4):625-33. 2010

23. Van der Zwan A, Van Unen V, Beyrend G, Laban S, Van der Keur C, Kapsenberg HJ, et al. Visualizing dynamic changes at the maternal-fetal interface throughout human pregnancy by mass cytometry. Frontiers in Immunology.11:571300. 2020

24. Krop J, van der Zwan A, Ijsselsteijn ME, Kapsenberg H, Luk SJ, Hendriks SH, et al. Imaging mass cytometry reveals the prominent role of myeloid cells at the maternal-fetal interface. Iscience.;25(7):104648. 2022

25. Moretta L, Pietra G, Vacca P, Pende D, Moretta F, Bertaina A, et al. Human NK cells: From surface receptors to clinical applications. Immunology letters.;178:15-9. 2016

26. Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, et al. Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annual review of immunology.;19(1):197-223. 2001

27. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. science. 1999;285(5428):727-9.

28. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. Nature Reviews Immunology.2(12):957-65. 2002

29. Quatrini L, Della Chiesa M, Sivori S, Mingari MC, Pende D, Moretta L. Human NK cells, their receptors and function. European journal of immunology. 2021;51(7):1566-79.

30. Boulenouar S, Doisne J-M, Sferruzzi-Perri A, Gaynor LM, Kieckbusch J, Balmas E, et al. The residual innate lymphoid cells in NFIL3-deficient mice support suboptimal maternal adaptations to pregnancy. Frontiers in immunology.;7:43. 2016

31. Redhead ML, Portilho NA, Felker AM, Mohammad S, Mara DL, Croy BA. The transcription factor NFIL3 is essential for normal placental and embryonic development but not for uterine natural killer (UNK) cell differentiation in mice. Biology of reproduction. 2016;94(5):101, 1-16. 32.Sojka DK, Yang L,
32. Yokoyama WM. Uterine natural killer cells. Frontiers inimmunology.;10:960. 2019
33. Campbell JJ, Qin S, Unutmaz D, Soler D, Murphy KE, Hodge MR, et al. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. The Journal of Immunology.;166(11):6477-82. 2001
34. Inngjerdingen M, Damaj B, Maghazachi AA. Expression and regulation of chemokine receptors in human natural killer cells. Blood, The Journal of the American Society of Hematology.;97(2):367-75. 2001
35. Maghazachi AA. Role of chemokines in the biology of natural killer cells. The Chemokine System in Experimental and Clinical Hematology.:37-58,2010.
36. Castriconi R, Carrega P, Dondero A, Bellora F, Casu B, Regis S, et al. Molecular mechanisms directing migration and retention of natural killer cells in human tissues. Frontiers in immunology.;9:2324. 2018
37. Moffett-King A. Natural killer cells and pregnancy. Nature Reviews Immunology.;2(9):656- 632002.
38. 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. Frontiers in Immunology.:1684. 2021
39. Bruijnesteijn J, De Groot NG, Bontrop RE. The genetic mechanisms driving diversification of the KIR gene cluster in primates. Frontiers in Immunology.;11:5828042020.
40. Phukan S, Sarmah N, Sarma H, Dutta A, Mattaparthi VSK, Baruah MN, et al. Higher affinity binding alleles and copy number variation of inhibitory KIR2DL1 gene influence the immune surveillance in head and neck squamous cell carcinoma in the population of Assam, North-East India. Human Gene.;34:2010862022.

41. Cianga VA, Rusu C, Pavel-Tanasa M, Dascalescu A, Danaila C, Harnau S, et al. Combined flow cytometry natural killer immunophenotyping and KIR/HLA-C genotyping reveal remarkable differences in acute myeloid leukemia patients, but suggest an overall impairment of the natural killer response. Frontiers in Medicine.;10. 2023

42 Dębska-Zielkowska J, Moszkowska G, Zieliński M, Zielińska H, Dukat-Mazurek A, Trzonkowski P, et al. KIR receptors as key regulators of NK cells activity in health and disease. Cells.;10(7):1777. 2021

43. Blunt MD, Khakoo SI. Activating killer cell immunoglobulin-like receptors: detection, function and therapeutic use. International Journal of Immunogenetics.;47(1):1-12. 2020 44.Martin AM, Kulski JK, Gaudieri S, Witt CS, Freitas EM, Trowsdale J, et al. Comparative genomic analysis, diversity and evolution of two KIR haplotypes A and B. Gene.;335:121-31. 2004

44. Rajagopalan S, Long EO. KIR2DL4 (CD158d): an activation receptor for HLA-G. Frontiers in immunology.3:2582012.

45. Kikuchi-Maki A, Yusa S-i, Catina TL, Campbell KS. KIR2DL4 is an IL-2-regulated NK cell receptor that exhibits limited expression in humans but triggers strong IFN-γ production. The Journal of Immunology.171(7):3415-25. 2003

46. Kikuchi-Maki A, Catina TL, Campbell KS. Cutting edge: KIR2DL4 transduces signals into human NK cells through association with the Fc receptor γ protein. The Journal of Immunology.;174(7):3859-63. 2005

47. Rajagopalan S. Endosomal signaling and a novel pathway defined by the natural killer receptor KIR2DL4 (CD158d). Traffic.11(11):1381-902010.

48. Zhao Y, Wang H, Pan Q. Decidual Natural Killer Cells: A Good Nanny at the Maternal-Fetal Interface During Early Pregnancy. Immune Regulations in Reproductive Organs and Organ Transplant. 2022.

49. Moffett A, Chazara O, Colucci F. Maternal allo-recognition of the fetus. Fertility and sterility.;107(6):1269-72. 2017

50. 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.;127(1):26-392009.

51. Moffett A, Loke C. Immunology of placentation in eutherian mammals. Nature Reviews Immunology.;6(8):584-94.2006

52. Xu X, Zhou Y, Wei H. Roles of HLA-G in the maternal-fetal immune microenvironment. Frontiers in immunology.;11:5920102020.

53. 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. Journal of Reproductive Immunology.;142:103176. 2020

54. Basak S, Srinivas V, Mallepogu A, Duttaroy AK. Curcumin stimulates angiogenesis through VEGF and expression of HLA-G in first-trimester human placental trophoblasts. Cell Biology International.44(5):1237-512020.

55. Nilsson LL, Hviid TVF. HLA Class Ib-receptor interactions during embryo implantation and early pregnancy. Human Reproduction Update.28(3):435-542022.

56. Bora M, Sarmah N, Das B, Baruah MN, Deka G, Hazarika SG, et al. A comparative study on regulation of HLA-G expression in bad obstetric history and in head and neck squamous cell carcinoma from Northeast India. Human Immunology. 83(5):453-7. 2022.

57. Craenmehr MH, Nederlof I, Cao M, Drabbels JJ, Spruyt-Gerritse MJ, Anholts JD, et al. Increased HLA-G expression in term placenta of women with a history of recurrent miscarriage despite their genetic predisposition to decreased HLA-G levels. International Journal of Molecular Sciences.20(3):625. 2019

58.Wang L, Wang X, Chen X, Wu D, Cen H, Mao D, et al. The relationship between hsa_circ_0051326 and HLA-G expression in the blood of patients with pre-eclampsia. Ginekologia Polska. 2021.

59.Tronik-Le Roux D, Verine J, Jacquier A, Stanciu R, Renard J, Schenowitz C, et al. Tumor Heterogeneity Uncovered by HLA-G Isoforms Expression.

60. Persson G, Stæhr CS, Klok FS, Lebech M, Hviid TVF. Evidence for a shift in placental HLA- G allelic dominance and the HLA-G isoform profile during a healthy pregnancy and preeclampsia. Biology of Reproduction.105(4):846-58. 2021
61. Wuerfel FM, Huebner H, Häberle L, Gass P, Hein A, Jud SM, et al. HLA-G and HLA-F protein isoform expression in breast cancer patients receiving neoadjuvant treatment. Scientific Reports.;10(1):15750. 2020
62. Yang X, Yang Y, Yuan Y, Liu L, Meng T. The roles of uterine natural killer (NK) cells and KIR/HLA-C combination in the development of preeclampsia: a systematic review. BioMed research international. 2020
63. Papúchová H, Meissner TB, Li Q, Strominger JL, Tilburgs T. The dual role of HLA-C in tolerance and immunity at the maternal-fetal interface. Frontiers in immunology.;10:2730. 2019
64. Piekarska K, Radwan P, Tarnowska A, Radwan M, Wilczyński JR, Malinowski A, et al. ERAP/HLA-C and KIR Genetic Profile in Couples with Recurrent Implantation Failure. International Journal of Molecular Sciences.;23(20):12518. 2022
65. Gwozdowicz S, Nestorowicz K, Graczyk-Pol E, Szlendak U, Rogatko-Koros M, Mika- Witkowska R, et al. KIR specificity and avidity of standard and unusual C1, C2, Bw4, Bw6 and A3/11 amino acid motifs at entire HLA: KIR interface between NK and target cells, the functional and evolutionary classification of HLA class I molecules. International journal of immunogenetics.;46(4):217-31. 2019
66. Parker EL, Silverstein RB, Verma S, Mysorekar IU. Viral-immune cell interactions at the maternal-fetal interface in human pregnancy. Frontiers in immunology.11:5220472020.
67. Sun H, Martin TG, Marra J, Kong D, Keats J, Macé S, et al. Individualized genetic makeup that controls natural killer cell function influences the efficacy of isatuximab immunotherapy in patients with multiple myeloma. Journal for immunotherapy of cancer.;9(7). 2021
68. Pollock NR, Harrison GF, Norman PJ. Immunogenomics of killer cell immunoglobulin-like receptor (KIR) and HLA class I: coevolution and consequences

for human health. The Journal of Allergy and Clinical Immunology: In Practice.;10(7):1763-75. 2022

69. Yang X, Meng T. Killer‐cell immunoglobulin‐like receptor/human leukocyte antigen‐C combination and 'great obstetrical syndromes'. Experimental and Therapeutic Medicine.;22(4):1-10. 2021

70. Kniotek M, Roszczyk A, Zych M, Szafarowska M, Jerzak M. Differences in the expression of KIR, ILT inhibitory receptors, and VEGF production in the induced decidual NK cell cultures of fertile and RPL women. BioMed research international. 2021

71. Wilczyńska K, Wiśniewski A, Malinowski A, Barcz E, Wilczyński JR, Kuśnierczyk P, et al. ERAP, KIR and HLA-C gene interaction in susceptibility to recurrent spontaneous abortion in the Polish population. Human Immunology.;80(5):344-8. 2019

72. Huo, R., Guo, Q., Hu, J., Li, N., Liu, H., Zhang, Z., Mi, L., Peng, X., Zhang, L. and Xu, K., Natural killer cells in obstetric antiphospholipid syndrome. Chinese Medical Journal, 135(07),.790-792, 2022.

73. Wang, D., Lv, W., Zhang, S. and Zhang, J., 2019. Advances in the research on anticardiolipin antibody. Journal of immunology research, 2019.

74. Carp, H. J. A., & Shoenfeld, Y. Recurrent spontaneous abortions in antiphospholipid syndrome: natural killer cells—an additional mechanism in a multi factorial process. Rheumatology, 46(10), 1517-1519. 200).

75. Zuo, Y., Shi, H., Li, C., & Knight, J. S. Antiphospholipid syndrome: a clinical perspective. Chinese Medical Journal, 133(08), 929-940. 2020

76. Manukyan, G., Martirosyan, A., Slavik, L., Margaryan, S., Ulehlova, J., Mikulkova, Z., . & Kriegova, E.). Anti-domain 1 β2 glycoprotein antibodies increase expression of tissue factor on monocytes and activate NK Cells and CD8+ cells in vitro. Autoimmunity Highlights, 11, 1-9. 2020

77.Yan, H., Li, B., Su, R., Gao, C., Li, X., & Wang, C. Preliminary Study on the Imbalance Between Th17 and Regulatory T Cells in Antiphospholipid Syndrome. Frontiers in Immunology, 13, 873644,2022.

78. Sun, S., Li, C., Kou, X., Chen, C., Guo, F., & Zhao, A. Association of prednisone and antinuclear antibodies with pregnancy outcomes in women with unexplained recurrent pregnancy loss. International Journal of Gynecology & Obstetrics, 154(3), 492-499. 2021.

79. Chen, S., Yang, G., Wu, P., Sun, Y., Dai, F., He, Y., ... & Shi, G. August). Antinuclear antibodies positivity is a risk factor of recurrent pregnancy loss: a meta-analysis. In Seminars in Arthritis and Rheumatism (Vol. 50, No. 4, pp. 534-543). WB Saunders. 2020,

80. Molazadeh, M., Karimzadeh, H., & Azizi, M. R. Prevalence and clinical significance of antinuclear antibodies in Iranian women with unexplained recurrent miscarriage. Iranian Journal of Reproductive Medicine, 12(3), 221. 2014

81. Carp, H. J. A., & Shoenfeld, Y. Recurrent spontaneous abortions in antiphospholipid syndrome: natural killer cells—an additional mechanism in a multi factorial process. Rheumatology, 46(10), 1517-1519. 2007.

82. Fan, J., Zhong, Y., & Chen, C. Combined treatment of prednisone and aspirin, starting before ovulation induction, may improve reproductive outcomes in ANA-positive patients. American Journal of Reproductive Immunology, 76(5), 391-395. 2016.

83. Li, Y., Wang, Y., Lan, Y., Zhang, J., Liang, Y., & Wang, S. Antinuclear antibodies in follicular fluid may reduce efficacy of in vitro fertilization and embryo transfer by invading endometrium and granular cells. American Journal of Reproductive Immunology, 84(4), e13289.2020)

84. Hou R, Huang R, Zhou Y, Lin D, Xu J, Yang L, et al. Single-cell profiling of the microenvironment in decidual tissue from women with missed abortions. Fertility and Sterility.;119(3):492-5032023.

85. Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. American journal of reproductive immunology.;77(6):e12643,2017.

86. Liu, T., Guo, X., Liao, Y., Liu, Y., Zhu, Y., & Chen, X. (2022). Correlation between the presence of antinuclear antibodies and recurrent pregnancy loss: a mini review.

Frontiers in Endocrinology, 13, 873286.

87. Ticconi, C., Inversetti, A., Logruosso, E., Ghio, M., Casadei, L., Selmi, C., & Di Simone,N. (2023). Antinuclear antibodies positivity in women in reproductive age: from infertility to adverse obstetrical outcomes–A meta-analysis. Journal of Reproductive Immunology, 103794.
88. Liu, T., Guo, X., Liao, Y., Liu, Y., Zhu, Y., & Chen, X. (2022). Correlation between the presence of antinuclear antibodies and recurrent pregnancy loss: a mini review. Frontiers in Endocrinology, 13, 873286.
89. Sun, S., Li, C., Kou, X., Chen, C., Guo, F., & Zhao, A. (2021). Association of prednisone and antinuclear antibodies with pregnancy outcomes in women with unexplained recurrent pregnancy loss. International Journal of Gynecology & Obstetrics, 154(3), 492-499.
90. Ticconi, C., Inversetti, A., Logruosso, E., Ghio, M., Casadei, L., Selmi, C., & Di Simone,N. (2023). Antinuclear antibodies positivity in women in reproductive age: from infertility to adverse obstetrical outcomes–A meta-analysis. Journal of Reproductive Immunology, 103794.
91. Fukushige, M., Lu, X., Satoh, M., Oda, M., Ohba, T., & Katoh, T. (2023). Association between antinuclear antibody positivity and chemical exposure among pregnant Japanese women: A cross-sectional study based on the Japan environment and Children's study. International Journal of Hygiene and Environmental Health, 248, 114094.
92. Hou, Y., Song, Z., Zhong, Y., Peng, Z., Cai, X., Xie, Y., ... & Li, C. (2023). The Pregnancy Outcomes of Patients with Antinuclear Antibodies or Antiphospholipid Antibodies Positive Undergo In Vitro Fertilization.
93. Gao, R., Deng, W., Meng, C., Cheng, K., Zeng, X., & Qin, L. (2021). Combined treatment of prednisone and hydroxychloroquine may improve outcomes of frozen embryo transfer in antinuclear antibody-positive patients undergoing IVF/ICSI treatment. Lupus, 30(14), 2213-2220.
94. Cavalcante, M. B., Cavalcante, C. T. D. M. B., Sarno, M., da Silva, A. C. B., & Barini, R. (2020). Antinuclear antibodies and recurrent miscarriage: systematic review and meta-analysis. American Journal of Reproductive Immunology, 83(3),

e13215.

95. Scarrone, M., Canti, V., Vanni, V. S., Bordoli, S., Pasi, F., Quaranta, L., ... & Rovere- Querini, P. (2022). Treating unexplained recurrent pregnancy loss based on lessons learned from obstetric antiphospholipid syndrome and inherited thrombophilia: A propensity-score adjusted retrospective study. Journal of Reproductive Immunology, 154, 103760.
96. Ambati, A., Zuo, Y., & Knight, J. S. (2023). An update on inflammation in antiphospholipid syndrome. Current Opinion in Rheumatology, 35(2), 89-97.
97. Salmon, J. (2021). 24 The role of complement and complement inhibition in APS pregnancy.
98. Tabacco, S., Giannini, A., Garufi, C., Botta, A., Salvi, S., Del Sordo, G., ... & De Carolis, S. (2019). Complementemia in pregnancies with antiphospholipid syndrome. Lupus, 28(13), 1503-1509.
99. Fierro, J. J., Velasquez, M., Cadavid, A. P., & de Leeuw, K. (2022). Effects of anti-beta 2-glycoprotein 1 antibodies and its association with pregnancy-related morbidity in antiphospholipid syndrome. American Journal of Reproductive Immunology, 87(1), e13509.
100. Tao, J. J., Adurty, S., & Desancho, M. T. (2021). Management and Outcomes of Women with Antiphospholipid Antibodies or Antiphospholipid Syndrome during Pregnancy. Blood, 138,1018.
101. Su, Z., Huang, Z., Zhao, J., Li, M., Hu, J., Zeng, X., ... & Yang, B. (2022). Detection of IgA antiphospholipid antibodies does not improve thrombotic antiphospholipid syndrome classification: a two-center study. Clinical and Applied Thrombosis/Hemostasis, 28, 10760296221081129.
102. Selmi, C., De Santis, M., Battezzati, P. M., Generali, E., Lari, S. A., Ceribelli, A., ... & Zuin, M. (2020). Anti-phospholipid antibody prevalence and association with subclinical atherosclerosis and atherothrombosis in the general population. International journal of cardiology, 300, 209-213.
103. Li, Y., Wang, Y., Lan, Y., Zhang, J., Liang, Y., & Wang, S. (2020). Antinuclear antibodies in follicular fluid may reduce efficacy of in vitro fertilization and embryo transfer by invading endometrium and granular cells. *American Journal of*

Reproductive Immunology, *84*(4), e13289.

104. Lu, Y., Yan, J., Liu, J., Tan, J., Hong, Y., Wei, D., ... & Sun, Y. (2020). Prednisone for patients with recurrent implantation failure: study protocol for a double-blind, multicenter, randomized, placebo-controlled trial. *Trials*, *21*, 1-7.