Türk Kadın Sağlığı ve Neonatoloji Dergisi

To cite this article: Colak E,Zeyneloglu HB. The place of immunological factors in recurrent pregnancy loss and implantation failures. Turk J Womens Health Neonatol 2020; 2(3): 105-111

Review Article

The place of immunological factors in recurrent pregnancy loss and implantation failures

Tekrarlayan gebelik kaybı ve implantasyon başarısızlıklarında immünolojik faktörlerin yeri

*Eser Çolak[1], Hulusi Bülent Zeyneloğlu[2]

[1] Baskent University School of Medicine, Konya Medical and Research Center, Department of Gynecology and Obstetrics, Konya, Turkey

[2]Baskent University School of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Ankara, Turkey

Abstract

Despite recent advances in assisted reproductive methods and treatments in sustaining fetal viability, recurrent implantation failure (RIF) and recurring pregnancy loss (RPL) still pose significant problems in the context of in vitro fertilization (IVF). Recent studies focused on the role of immunological factors in the etiology of RIF and RPL. They demonstrated that infertile patients might suffer from dysregulated immune system cell activities, including CD4+ T helper (Th1, Th2, Th17, and Tregs), peripheral natural killer (pNK), uterine natural killer (uNK) cells. Researchers have investigated the use and efficacy of immunosuppressant drugs such as glucocorticoids, intravenous immunoglobulin, and TNF-α blockers in achieving successful implantation in infertile women but the efficacy of these treatments remains to be fully established. We conclude that, although the relationship between immunology and infertility is clear, there is still a long way to go to reach a thorough understanding.

Keywords: Infertility; Immunology; IVF; Recurrent pregnancy loss; Recurrent implantation failure

Corresponding author*: Eser Colak, Baskent University School of Medicine, Konya Medical and Research Center, Department of Gynecology and Obstetrics, Konya, Turkey
e-mail: dresercolak@hotmail.com
ORCID ID: 0000-0002-8184-7531
Received: 14.07.2020 Accepted: 28.09.2020
DOİ: 10.46969/ezh.768932

Öz

Canlı bir gebeliğin devamı için yardımcı üreme teknikleri ve tedavi yöntemlerinde gelişmeler sağlansa da, in vitro fertilizasyon (IVF) sonrası tekrarlayan implantasyon başarısızlığı (TİB) ve tekrarlayan gebelik kayıpları (TGK) büyük problem oluşturmaktadır. Son yıllarda yapılan çalışmalarda TİB ve TGK nedeni olarak immunolojik faktörlerin üzerinde durulmaktadır ve bu hasta gruplarında immun sistem hücreleri olan; CD4+ T-helper (Th1, Th2, Th17, Treg) ve periferik natural killer (pNK) ve uterin natural killer (uNK) hücrelerinin aktivitelerinde dengesizlikler gösterilmiştir. Bu nedenle, bu hasta gruplarında başarılı bir implantasyon ve gebelik devamı için glukokortikoidler, intravenöz immünoglobulin ve TNF-α blokerleri gibi immünosüpresan ilaçların kullanımı ve etkinliği araştırılmıştır. Ancak ilaçların etkinliği ile ilgili net bir sonuç elde edilmemiştir. Sonuç olarak, immunoloji ile infertilite ilişkisi aşikar olmasına rağmen, bu konuda daha katedilmesi gereken uzun bir yol vardır.

Anahtar kelimeler: İnfertilite; İmmunoloji; IVF; Tekrarlayan gebelik kaybı; Tekrarlayan implantasyon başarısızlıkları

1. Introduction

Infertility is defined as the inability of a couple to achieve a viable pregnancy after a year of unprotected regular sexual intercourse. Between 10 to 15% of couples of reproductive age suffer from infertility (1, 2). Despite recent advances in assisted reproductive methods and treatments in sustaining fetal viability, recurring implantation failure (RIF) and recurring pregnancy loss (RPL) still pose significant problems in the context of in vitro fertilization (IVF). RIF is defined as the failure of a woman under the age of 40 to achieve a viable pregnancy after the transfer of at least three fresh or frozen embryos (3). RPL is defined as three or more consecutive miscarriages that occur before 20 weeks of gestation (4). Previous studies investigated genetic causes and uterine structural anomalies, whereas recent studies focused on the role of immunological factors in both RIF and RPL.

The field of reproductive immunology established by Madewar who, in 1953, asked why the semi-allogeneic fetus is not rejected by the maternal immune system (5). Pregnancy is a unique condition that allows the toleration of the embryo, a semi-allogeneic tissue, by the maternal immune system. In order for the mother to be able to tolerate the father's alloantigens, the maternal immune system must develop a balance between tolerance and immunity. Immune imbalance contributes to endometrial implantation failure and miscarriage. Research suggests that infertile patients may suffer from dysregulated immune system cell activities, including CD4+ T helper (Th1, Th2, Th17, and Tregs), peripheral natural killer (pNK), uterine natural killer (uNK) cells (6). These cells and their cytokines participate in blastocyst implantation (**Figure 1**).

This review aims to discuss studies demonstrating an association between NK and T cell counts and ratios, and infertility.

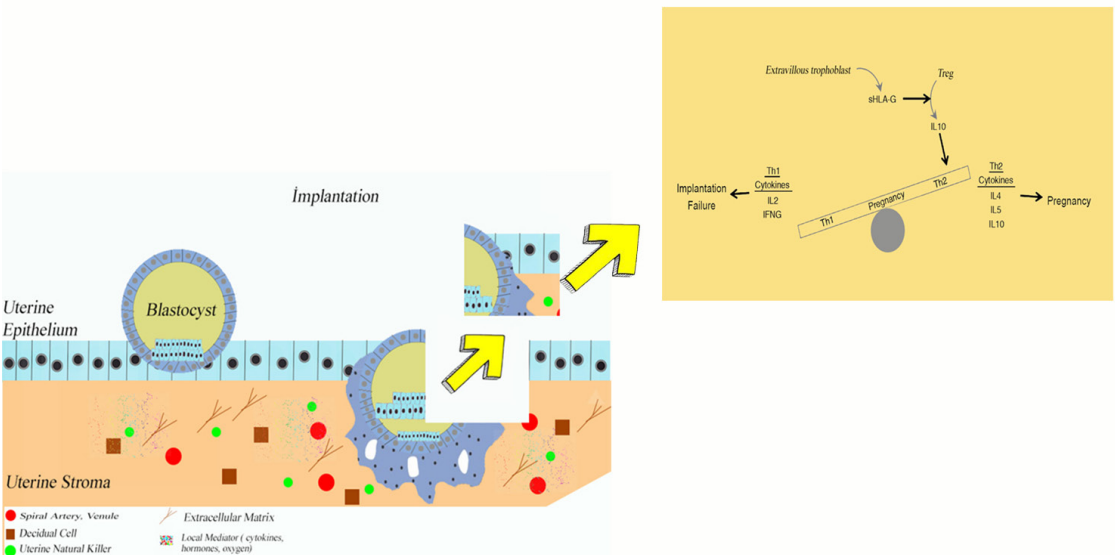

Figure 1. Hormones, oxygen, cytokines, growth factors, and immune system cells effective in blastocyst implantation and placenta formation

2. Methods

Using online databases, we carried out a systematic literature review on the role of immunologic factors in both RIF and recurring pregnancy loss (RPL). The major relevant studies were retrieved mainly from PubMed, Google Scholar, MEDLINE and Web of Science using the keywords 'immunologic factors and infertility', 'immunologic factors and recurrent implantation failure', 'immunologic factors and recurrent pregnancy loss' and 'treatment of immunologic problems for recurrent implantation failure and recurrent pregnancy loss'. Only English-language publications were included in this review.

3. Natural killer cells

A regular functioning immune system is essential for the successful implantation of an embryo (7). Natural killer (NK) cells play a central role in the innate immune system (8, 9). The pNK and uNK cells are phenotypically and functionally different cell types and are distinguished according to their receptor expression intensity, particularly surface CD56 (10). A minority of pNK cells (10%) resemble uNK cells whereas 90% are CD56dim (i.e. highly cytotoxic) and CD16+, while 80% of uNK cells are CD56bright (less cytotoxic) and CD16- (11). The reason why peripheral NK cells are more cytotoxic is twofold: first, the type of surface CD56, and second, the downregulation of CD16 expression by uNK cells, which in turn triggers NK cell antibody-dependent cell-mediated cytotoxicity (11, 12). Hence, uNK cells play a more prominent regulatory role in early implantation. The initiation of embryo implantation is associated with the production of progesterone and interleukin 15 (IL-15) and the stimulation of uNK cells during the luteal phase of the menstrual cycle (11, 13). These cells promote vascular remodelling via endothelial growth factor and are also responsible for trophoblast remodelling and cytokine secretion (10, 14, 15).

The literature contains different methods for NK cell determination, including an endometrial biopsy or lavage sampling, menstrual blood sampling, or placental/decidual sampling (16, 17). NKT cells constitute an important subgroup of lymphocytes that demonstrate both T cell and NK cell-like characteristics (18). NKT cells have two distinct functions in immune responses: pro-inflammatory and tolerogenic (19), leading researchers to speculate that NKT cells may play an important role in implantation and immune intolerance to the fetus (20). Zhou et al. investigated whether peripheral blood NKT-like cells were in any way associated with IVF treatment outcome and showed that patients with higher peripheral NKT-like cell counts had better IVF outcomes (i.e. higher pregnancy and live birth rates). Yuan et al. found elevated pNK cells to be correlated with unexplained recurrent spontaneous abortions (18).

In contrast, one study reported that pNK cell counts were not significantly different but that uNK cells were reduced in patients with RIF (21). In a comparative analysis, Hosseini et al. evaluated NK cell subsets in peripheral and menstrual blood samples of recurring spontaneous abortion patients and found more prominent population differences in NK cell subgroups in the peripheral blood (17). Also, several studies that investigated the efficacy of uNK cells in predicting implantation success after IVF conclude that uNK cell count was not effective in predicting IVF success (22, 23). In reference to these studies, it could be argued that immunomodulatory treatments may be unnecessary in the context of IVF treatments and may only be applied for carefully selected patients. Some studies similarly report no difference in uNK (CD56 and CD16) counts of fertile and infertile women (24), and also that elevated CD16 levels may predict poor prognosis (25). Kofod et al. emphasized that increased CD56 and decreased CD16 levels may predict viable pregnancy outcome in IVF treatment (26). Multiple studies did not find a significant correlation between pNK cell level and pregnancy loss and IVF outcome. On the other hand, the available results on uNK cell levels are contradictory. Regardless, recent studies associate elevated uNK cells with improved IVF outcomes (**Table 1**). Further studies are needed to elucidate the impact of NK cells on pregnancy loss and IVF outcomes.

4. T-helper cells

Cytokines play an important role in successful implantation and the continuation of pregnancy. CD4 T helper cells differentiate into two major subtypes as Th1 and Th2 that are distinguished based on the cytokines they secrete. Th1 cells typically secrete pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-2 (IL-2) (27) whereas Th2 cells secrete anti-inflammatory cytokines, such as IL-4, IL-6, and IL-10, and appear to have immunosuppressive effects that are important for the healthy continuation of pregnancy (28). The balance of pro- and anti-inflammatory cytokines depends entirely on the ratio of Th1 and Th2 cells (29). It is possible to measure cytokine levels from serum, plasma, placenta, or endometrial lavage samples (30).

Although the available results on Th cells and cytokines are contradictory, the general opinion is that Th2 dominance is required for successful implantation and pregnancy. Most of these studies included RIF and RPL patients and emphasized the ratios of Th1/Th2 and their cytokines.

It has been demonstrated that the ratios of pro-inflammatory and anti-inflammatory cytokines (i.e. IFN-γ/IL-4, IFN-γ/IL-10, IFN-γ/TGF-β1, IL-6/IL-10, IL-6/TGF-β1, IL-1β/TGF-β1, and TNF-α/TGF-β) in the peripheral blood are increased in RIF patients. These results suggest that the Th1/Th2 ratio may predict prognosis in IVF (31). Kalu et al. similarly demonstrated that Th2 dominance is associated with improved IVF outcomes

Table 1. Studies investigating expression of uNK and pNK cells in samples from different anatomical locations in the context of infertility.

STUDY	SAMPLE TYPE	METHOD	VARIABLES	RESULT
Hosseini et al., 2014 (21)	Uterine and peripheral	Menstrual blood-peripheral blood-flow cytometry	CD56, CD16	uNK counts were higher in RPL patients; no statistical difference in pNK counts
Tohma et al., 2020 (25)	Uterine and peripheral	Uterine lavage- peripheral blood-flow cytometry	CD56, CD16	uNK counts were lower in RIF patients; no statistical difference in pNK counts
Donoghue et al., 2019 (26)	Uterine	Endometrial biopsy-immunohistochemistry	CD56, CD16	No added benefit in predicting the success or failure of implantation after IVF
Kofod et al., 2017 (30)	Uterine	Endometrial biopsy-immunohistochemistry	CD56, CD16	Better IVF outcomes with increased CD56 levels
Giuliani et al., 2014 (29)	Uterine	Endometrial biopsy-immunohistochemistry	CD56, CD16	Higher populations of CD16 may result in increased risk of infertility disorders; no statistical difference in CD56
Matteo et al., 2007 (28)	Uterine	Endometrial biopsy-flow cytometry	CD56, CD16	No differences between infertile and fertile women
Zhou et al., 2013 (24)	Peripheral	Peripheral blood-flow cytometry	CD56, CD16, CD3	Elevated CD3, CD56, and CD16 levels are associated with increased pregnancy and live birth rates in IVF
Yuan et al., 2015 (22)	Peripheral	Peripheral blood-flow cytometry	CD56, CD16, CD3	Elevated CD3, CD56, and CD16 levels are associated with RPL

uNK: Uterine natural killer cells, pNK: Peripheral natural killer cells, IVF: In vitro fertilization, RPL: Recurrent pregnancy loss, RIF: Recurrent implantation failure

Table 2. Studies investigating expression of Th1-Th2 cells and pro/anti-inflammatory cytokines in samples from different anatomical locations in the context of infertility.

STUDY	SAMPLE TYPE	VARIABLES	RESULT
Yuan et al., 2015 (22)	Decidual	Th1 and Th2 cells and pro/anti-inflammatory cytokines	Higher IFN-gamma expression, increased decidual Th1/Th2 ratio, and decreased IL-4 and IL-10 in patients with RPL
Liang et al., 2015 (35)	Plasma	Th1 and Th2 cells and pro/anti-inflammatory cytokines	The ratios of pro-inflammatory and anti-inflammatory cytokines were higher in the RIF patients
Kalu et al., 2008 (36)	Plasma	Th1 and Th2 cells and pro/anti-inflammatory cytokines	The Th1/Th2 ratio was higher in RIF patients
Comba et al., 2015 (34)	Plasma and endometrial tissue	Pro/anti-inflammatory cytokines	Higher IL-12, IL-18, and INF-γ and lower LIF and MIF in RPL patients
Kwak-kim et al., 2003 (37)	Plasma	Th1 and Th2 cells and pro/anti-inflammatory cytokines	The Th1/Th2, INF-γ/IL-4, and TNF-α/IL-4 ratios were higher in RIF and RPL patients
Lee et al., 2013 (38)	Plasma	Th1 and Th2 cells and pro/anti-inflammatory cytokines	The Th1/Th2 ratio was higher in RPL patients

uNK: Uterine natural killer cells, pNK: Peripheral natural killer cells, IVF: In vitro fertilization, RPL: Recurrent pregnancy loss, RIF: Recurrent implantation failure

among RIF patients (32). IFN-γ/IL-4 and TNF-α/IL-4 and Th1/Th2 rates were reported to be increased in RIF and RPL patients (33, 34). Results of decidual and endometrial samples were comparable to those of plasma samples. In RPL patients had elevated decidual IFN-γ levels, and reduced decidual IL-4 and IL-10 levels (18). Another study reported elevated IL-12, IL-18, and IFN-γ levels and reduced leukemia inhibitory factor (LIF) and migration inhibitory factor (MIF) levels in the plasma and endometrial samples of RPL patients (30) (**Table 2**).

5. Human leukocyte antigen (HLA)

The MHC cluster located on chromosome 6 encodes HLA molecules, including HLA Classes I, II and III. HLA Class I proteins are divided into classical antigens (HLA-A, HLA-B and HLA-C) and nonclassical HLA Class Ib antigens, including HLA E, F and G (35, 36). The HLA Class II region contains the HLA-DR, HLA-DQ and HLA-DP loci. HLA and NK cells are the most prominent immunological factors in fertility and might play a crucial role in the incidence and establishment of pregnancy. They can influence pregnancy-

related processes and actors, including embryonic aggregation, gametes, blastocyst formation, fetal growth, trophoblasts, transplantation, and embryonic survival (37).

HLA class I molecules are expressed in all nucleated cells whereas HLA Class II molecules are expressed only in antigen-presenting cells (e.g. dendritic cells, macrophages, B cells) and some non-APC cells (T cells, endothelial cells). On the other hand, trophoblasts have been shown to express the non-classical HLA-G molecule (38). HLA-C molecules, and to a limited degree, the HLA-A, HLA-B, HLA-G and HLA-E molecules, appear to play an important role in pregnancy by binding to the "activating/inhibitory" ligands on the NK and T cell surfaces (39).

The interaction between the "killer immunoglobulin-like receptor (KIR)" molecules on maternal immune cells (uNK/NK and cytotoxic T cells) and the paternal antigens [Human Leukocyte Antigens (HLA)] can determine the course of the pregnancy (38). The literature on the relationship between HLA and non-classical HLA molecules and pregnancy indicates that polymorphic HLA-G derivatives are associated with recurrent pregnancy loss (40).

6. Immunomodulatory Treatment

Researchers have investigated the use and efficacy of immunosuppressant drugs such as glucocorticoids, intravenous immunoglobulin, and TNF-α blockers in achieving successful implantation in infertile women but the efficacy of these treatments remains to be fully established (41).

6.1. Intravenous immunoglobulin

A systematic review and meta-analysis by Li et al. found that intravenous immunoglobulin (IVIG) was associated with increased implantation, clinical pregnancy, and live birth rates in women with unexplained infertility or IVF/ICSI failure when compared to a placebo (42). However, a 2014 Cochrane review, including eight randomized studies on 303 women with recurrent pregnancy loss, concluded that IVIG treatment did not improve live birth rates (43).

6.2. Corticosteroids

The efficacy or utility of corticosteroids in RIF patients is not clearly established (44). Meta-analyses emphasize that corticosteroids do not increase live birth rates in RIF patients (45). However, some studies report better IVF outcomes when corticosteroids are combined with heparin, aspirin, or progesterone (46, 47). It is not clearly understood how these treatments affect immune cells (Th1, Th2, NK) or cytokine release. Further studies that investigate pre- and post-treatment changes in immune cells and cytokines are needed.

6.3. TNF-α blockers

TNF-α blockers (e.g. adalimumab) inhibit TNF-α and reduce the inflammatory response. Despite studies indicating increased pregnancy rates when aspirin or IVIG treatments are combined with TNF-α blockers, this treatment does not significantly increase live birth rates (48). These drugs are thought to act by altering cytokine ratios. It was reported that Th1/Th2 and TNF-α/IL-10 ratios were reduced after TNF-α blocker treatment (49).

6.4. Tacrolimus

The immunosuppressive activity of tacrolimus is achieved by the inhibition of calcineurin, resulting in reduced inflammatory cytokines, including TNF-α, IL-1β, and IL-6 (50). Nakagawa et al. applied tacrolimus therapy to patients with a history of RIF starting two days before embryo transfer until a positive pregnancy test. They reported increased pregnancy and live birth rates in patients who received treatment (51). On the other hand, it was observed that patients with reduced Th1 levels did not have significantly different clinical pregnancy or live birth rates. However, pregnancies were more likely to progress into the second trimester in patients who were treated with tacrolimus and had lower Th1 levels (52). A study of 100 pregnant women with a history of organ transplantation and tacrolimus therapy reported that four fetuses developed congenital anomalies such as meningomyelocele, urogenital defects, hearing defects, hypospadias, multicystic dysplastic kidneys, and cleft palate (53). Further and more extensive and detailed studies are needed concerning tacrolimus treatment applications.

7. Conclusion

In conclusion, numerous articles have recently been published on the relationship between immunology and infertility, resulting in a better understanding of the effects of immunology on implantation failure and recurrent miscarriage. However, there are still many unknowns, and, as of yet, there are no effective treatment methods for any potential immunological problems. Therefore, well-designed randomized controlled trials are needed to determine relevant immunological conditions and to develop treatment modalities for potential problems.

Declaration of Interest

The authors report no conflict of interest.

Ethical approval: This review protocol was approved after obtaining the necessary permissions by the obstetrics and gynecology department of Baskent University. Ethics committee approval was not sought as the study is a review of the previous works of literature.

References

1. Stanford JB. What is the true prevalence of infertility? Fertil Steril 2013; 99:1201-1202.
2. Dahl M, Hviid TVF. Human leucocyte antigen class Ib molecules in pregnancy success and early pregnancy loss. Hum Reprod Update 2012; 18:92-109.

3. Coughlan C, Ledger W, Wang Q, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online 2014; 28: 14-38.
4. Ehrlich S, Williams PL, Missmer SA, et al. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. Environ Health Perspect 2012; 120: 978-983.
5. Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 1953; 7: 320-338.
6. Lee SK, Kim CJ, Kim DJ, Kang JH. Immune cells in the female reproductive tract. Immune Netw 2015; 15: 16-26.
7. Garcia-Velasco JA. Introduction: Immunology and assisted reproductive technology in the 21st century. Fertil Steril 2017; 107: 1267-1268.
8. Azargoon A, Mirrasouli Y, Shokrollahi Barough M, Barati M, Kokhaei P. The State of Peripheral Blood Natural Killer Cells and Cytotoxicity in Women with Recurrent Pregnancy Loss and Unexplained Infertility. Int J Fertil Steril 2019; 13:12-17.
9. Tang AW, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod 2011; 26:1971-1980.
10. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol 2002; 2:656-663.
11. Moffett A, Shreeve N. First do no harm: uterine natural killer (NK) cells in assisted reproduction. Hum Reprod 2015; 30:1519-1525.
12. Souza SS, Ferriani RA, Santos CMP, Voltarelli JC. Immunological evaluation of patients with recurrent abortion. J Reprod Immunol 2002; 56:111-121.
13. Björkström NK, Ljunggren HG, Michaëlsson J. Emerging insights into natural killer cells in human peripheral tissues. Nat Rev Immunol 2016; 16:310-320.
14. Kalkunte SS, Mselle TF, Norris WE, Wira CR, Sentman CL, Sharma S. Vascular endothelial growth factor C facilitates immune tolerance and endovascular activity of human uterine NK cells at the maternal-fetal interface. J Immunol 2009; 182:4085-4092.
15. Faas MM, de Vos P. Uterine NK cells and macrophages in pregnancy. Placenta 2017; 56: 44-52.
16. King K, Smith S, Chapman M, Sacks G. Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. Hum Reprod 2010; 25:52-58.
17. Hosseini S, Zarnani AH, Asgarian-Omran H, et al. Comparative analysis of NK cell subsets in menstrual and peripheral blood of patients with unexplained recurrent spontaneous abortion and fertile subjects. J Reprod Immunol 2014; 103:9-17.
18. Yuan J, Li J, Huang SY, Sun X. Characterization of the subsets of human NKT-like cells and the expression of Th1/Th2 cytokines in patients with unexplained recurrent spontaneous abortion. J Reprod Immunol 2015; 110:81-88.
19. Boyson JE, Aktan I, Barkhuff DA, Chant A. NKT cells at the maternal-fetal interface. Immunol Invest 2008; 37:565-582.
20. Zhou J, Zhao X, Wang Z, Wang J, Sun H, Hu Y. High circulating CD3+CD56+CD16+ natural killer-like T cell levels predict a better IVF treatment outcome. J Reprod Immunol 2013; 97:197-203.
21. Tohma YA, Musabak U, Gunakan E, Akilli H, Onalan G, Zeyneloglu HB. The Role of Analysis of NK Cell Subsets in Peripheral Blood and Uterine Lavage Samples in Evaluation of Patients with Recurrent Implantation Failure. J Gynecol Obstet Hum Reprod 2020; 101793.
22. Donoghue JF, Paiva P, Teh WT, et al. Endometrial uNK cell counts do not predict successful implantation in an IVF population. Hum Reprod 2019; 34:2456-2466.
23. Seshadri S, Sunkara SK. Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. Hum Reprod Update 2014; 20: 429-438.
24. Matteo MG, Greco P, Rosenberg P, et al. Normal percentage of CD56bright natural killer cells in young patients with a history of repeated unexplained implantation failure after in vitro fertilization cycles. Fertil Steril 2007; 88:990-993.
25. Giuliani E, Parkin KL, Lessey BA, Young SL, Fazleabas AT. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. Am J Reprod Immunol 2014; 72:262-269.
26. Kofod L, Lindhard A, Bzorek M, Eriksen JO, Larsen LG, Hviid TVF. Endometrial immune markers are potential predictors of normal fertility and pregnancy after in vitro fertilization. Am J Reprod Immunol 2017; 78: e12684.
27. Daher S, Denardi KAG, Blotta MHSL, et al. Cytokines in recurrent pregnancy loss. J Reprod Immunol 2004; 62:151-157.
28. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss 3rd JF, Petraglia F. Inflammation and pregnancy. Reprod Sci 2009; 16: 206-215.
29. Salamonsen LA, Hannan NJ, Dimitriadis E. Cytokines and chemokines during human embryo implantation: roles in implantation and early placentation. Semin Reproduct Med 2007; 25:437-444.
30. Comba C, Bastu E, Dural O, et al. Role of inflammatory mediators in patients with recurrent pregnancy loss. Fertil Steril 2015; 104:1467-1474.
31. Liang PY, Diao LH, Huang CY, et al. The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. Reprod Biomed Online 2015; 31:823-826.
32. Kalu E, Bhaskaran S, Thum MY, et al. Serial estimation of Th1:th2 cytokines profile in women undergoing in-vitro fertilization-embryo transfer. Am J Reprod Immunol 2008; 59:206-211.
33. Kwak-Kim JYH, Chung-Bang HS, Ng SC, et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. Hum Reprod 2003; 18:767-773.

34. Lee SK, Na BJ, Kim JY, et al. Determination of clinical cellular immune markers in women with recurrent pregnancy loss. Am J Reprod Immunol 2013; 70:398-411.
35. Choudhury SR, Knapp LA. Human reproductive failure I: immunological factors. Hum Reprod Update 2001; 7:113-134.
36. Matter TF, Sharif F A. HLA-G and HLA-E Gene polymorphisms in idiopathic recurrent spontaneous abortion women in Gaza strip-Palestine. Int J Reprod Contracept Obstet Gynecol 2013; 2:277-283.
37. Afkhami F, Khaniani MS, Farzadi L, Paknejad Z, Derakhshan SM. The HLA-G 14-bp insertion/deletion polymorphism in recurrent spontaneous abortion among Iranian women. Iran J Allergy Asthma Immunol 2014; 13:364-369.
38. Gregori S, Amodio G, Quattrone F, Panina-Bordignon P. HLA-G Orchestrates the Early Interaction of Human Trophoblasts with the Maternal Niche. Front Immunol 2015; 6:128.
39. Moffett A, Chazara O, Colucci F, Johnson MH. Variation of maternal KIR and fetal HLA-C genes in reproductive failure: too early for clinical intervention. Reprod Biomed Online 2016; 33:763-769.
40. Lynge Nilsson L, Djurisic S, Hviid TV. Controlling the Immunological Crosstalk during Conception and Pregnancy: HLA-G in Reproduction. Front Immunol 2014; 5:198.
41. Hviid MM, Macklon N. Immune modulation treatments—where is the evidence? Fertil Steril 2017; 107:1284-1293.
42. Li J, Chen Y, Liu C, Hu Y, Li L. Intravenous immunoglobulin treatment for repeated IVF/ICSI failure and unexplained infertility: a systematic review and a meta-analysis. Am J Reprod Immunol 2013; 70:434-447.
43. Wong LF, Porter TF, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev. 2014; 2014(10):CD000112.
44. Tang AW, Alfirevic Z, Turner MA, Drury JA, Small R, Quenby S. A feasibility trial of screening women with idiopathic recurrent miscarriage for high uterine natural killer cell density and randomizing to prednisolone or placebo when pregnant. Hum Reprod 2013; 28:1743-1752.
45. Boomsma CM, Keay SD, Macklon NS. Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. Cochrane Database Syst Rev 2012; (6):CD005996.
46. Fawzy M, Shokeir T, El-Tatongy M, Warda O, El-Refaiey AAA, Mosbah A. Treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage: a randomized placebo-controlled study. Arch Gynecol Obstet 2008; 278:33-38.
47. Gomaa MF, Elkholy AG, El-Said MM, Abdel-Salam NE. Combined oral prednisolone and heparin versus heparin: the effect on peripheral NK cells and clinical outcome in patients with unexplained recurrent miscarriage. A double-blind placebo randomized controlled trial. Arch Gynecol Obstet 2014; 290:757-762.
48. Winger EE, Reed JL, Ashoush S, Ahuja S, El-Toukhy T, Taranissi M. Treatment with adalimumab (Humira®) and intravenous immunoglobulin improves pregnancy rates in women undergoing IVF. Am J Reprod Immunol 2009; 61:113-120.
49. Winger EE, Reed JL, Ashoush S, El-Toukhy T, Ahuja S, Taranissi M. Degree of TNF-α/IL--10 cytokine elevation correlates with IVF success rates in women undergoing treatment with adalimumab (Humira) and IVIG. Am J Reprod Immunol 2011; 65:610-618.
50. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther Drug Monit 1995; 17:584-591.
51. Nakagawa K, Kwak-Kim J, Ota K, et al. Immunosuppression with tacrolimus improved reproductive outcome of women with repeated implantation failure and elevated peripheral blood TH1/TH2 cell ratios. Am J Reprod Immunol 2015; 73:353-361.
52. Nakagawa K, Kwak-Kim J, Kuroda K, Sugiyama R,Yamaguchi K. Immunosuppressive treatment using tacrolimus promotes pregnancy outcome in infertile women with repeated implantation failures. Am J Reprod Immunol 2017; 78:e12682.
53. Kainz A, Harabacz I, Cowlrick IS, Gadgil S, Hagiwara D. Analysis of 100 pregnancy outcomes in women treated systemically with tacrolimus. Transpl Int 2000; 13 Suppl 1:S299-300.