Chapter 6
Immune Factors in Recurrent Implantation Failure

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Abbreviations

ART	Assisted reproductive treatments
DET	Double embryo transfer
EVT	Invading extravillous trophoblast
HLA	Human leukocyte antigen
IVF	In vitro fertilization
KIR	Killer immunoglobulin-like receptor
NK cells	Natural killer cells
Pb	Peripheral blood
RIF	Recurrent implantation failure
RM	Recurrent miscarriage
SET	Single embryo transfer
uNK cells	Uterine natural killer cells

In the last decades, substantial progress has been made to improve the outcome of the assisted reproductive treatments (ART). Our knowledge about folliculogenesis, in vitro embryo culture and their chromosomal composition, as well as endometrial receptivity has undergone a huge improvement during the last few years. Despite this, a high percentage of embryos are still lost right after being transferred (50%) or a bit later, as early miscarriage or clinical miscarriage. A recent study [1] reported a 52% cumulative live birth rate (LBR) after transferring up to five embryos and 79% after 15 embryos had been transferred; but what happens to the rest of the embryos? There might be other factors that contribute to implantation failure or miscarriage, not just embryo aneuploidies—by far, the main contributor to implantation failure, such as endometrial factors, hydrosalpinges, infections or abnormal karyotypes, or even maternal tolerance to pregnancy. At the same time, due to

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repeated failed cycles even after gamete donation, we all have witnessed an increasing demand for immune tests and "immune treatments" from our patients. Although this patient's demand may be unjustified, we need to understand if there is a rationale behind to use it, or not, to explain them why not use it. The role of the immune system in recurrent miscarriage (RM) and recurrent implantation failure (RIF) is one of the most controversial issues in assisted reproduction [2]. The controversy, in part, is due to the fact that most of the previous studies about immune system implication in reproduction were focused on finding markers on peripheral blood [3, 4] and quick solutions using different lines of immunomodulation [5, 6]. The main reason that immune treatments have failed so far and immune tests (pbNK or uNK cell testing) have shown very weak or no predictive value is due to poor study design and great patient heterogeneity [2, 7].

Maternal tolerance, as we know, begins at the uterine level, and successful adaptation to the semiallogeneic fetus is more complicated than the initial concept suggested.

Natural Killer Cells

Peripheral blood natural killer (pbNK) cells have become an "immune study core" for women with recurrent miscarriage or RIF, based on the mistaken notion that they are causing reproductive failure by killing or "rejecting" the embryo.

Some reports had a general view [3] as natural killer (NK) cells from the peripheral blood (pbNK) and uterus (uNK) are merged together with the simple marker "NK cells" as the "main immune cells at the maternal–fetal interface." This is, from immunological point of view, an erroneous judgment as pbNK and uNK cells are completely different types of immune cells [8]. pbNK cells are cytotoxic and represent the first line of defense against viruses, tumors, and damaged cells, and they are not trained to "reject" or kill a healthy embryo(s).

In peripheral blood, there are two major types of NK cells [9]; 90% are CD56dim, CD16+, and 10% are CD56bright, CD162 [9, 10]. In contrast, uNK cells are CD56superbright, CD162 and differ radically from pbNK in other phenotype markers and functional assays [8, 11]. uNK killing in vitro assays is very weak compared with pbNK [12]. The uNK cells acquire their functional properties in utero as CD56+ cells do proliferate and differentiate in the specialized progesterone-dominated microenvironment of the secretory endometrium and early deciduas and seem to play an important role in controlling trophoblast invasion and the development of a healthy placenta [10].

Uterine NK (uNK) cells are small and sparse before ovulation, but rapidly proliferate and differentiate into large cells with prominent cytoplasmic granules soon after ovulation. uNK cells are the dominant immune cells in the uterine mucosa and account for 30% of cells in the stroma in the late secretory endometrium in humans [13]. During pregnancy uNK cells are abundant throughout deciduas, and they accumulate particularly at the site of placentation around the infiltrating trophoblast

cells in the decidua basalis [14]. They are also particularly prominent around spiral arteries and abundant early in gestation when the placenta is established.

The number of pbNK and uNK cells shows great variability depending on the patient's clinical condition as infections, autoimmunity or tumors, day of the menstrual cycle, treatment condition (ovarian stimulation), stress, time of day, exercise, etc., and previous studies about NK cells and reproductive issues did not take this NK cell physiological variation into consideration.

Using "NK cells" to describe these two contrasting subsets of NK cells, pbNK and uNK, as a unique marker in women with infertility or disorders of pregnancy, contribute only to add more confusion about immune tests in ART.

The fetal cells in direct contact with the mother's immune system in the uterus are trophoblast cells, the layer surrounding the blastocyst [15, 16], and the mother's uterine immune system is dominated by uterine NK (uNK) cells [17], CD56bright CD16dim, which are distinct from peripheral blood NK cells and are the most abundant leukocyte population during the first trimester of human pregnancy [18].

The maternal and fetal circulations do not mix, although transient exchange of cells occurs, particularly during the trauma of delivery [10].

The successful maternal adaptation to the semiallogeneic fetus occurs in the uterus at the site of placentation. The key of the maternofetal tolerance process is the remodeling of the spiral arteries, with destruction of the media by invading extravillous trophoblast (EVT) cells. The EVT cells express major histocompatibility complex molecules: human leukocyte antigen (HLA), class I HLA-C and non-classical HLA-G and HLA-E antigens, whereas the class I antigens HLA-A and HLA-B and class II antigens are absent [19, 20]. Although, HLA-E and HLA-G are oligomorphic, the HLA-C molecules expressed by EVT cells are polymorphic, and ligands for killer immunoglobulin-like receptors (KIRs) are expressed by uNK cells [21]. The EVT invading into the maternal decidua are of fetal not maternal origin, and they express high levels of HLA-C, which is recognized by uterine NK (uNK) cells receptors, also known as KIR. Both polymorphic maternal KIRs and fetal HLA-C molecules are variable and specific to a particular pregnancy [10]. In any pregnancy, the maternal KIR genotype could be AA (non-activating KIRs), AB, BB, or Bx (1–10 activating KIRs) [22]; and the HLA-C ligands for KIRs are divided into two groups: HLA-C1 and HLA-C2. Of the two, C2 is a stronger ligand than C1 [23]. The A haplotypes contain mainly genes for inhibitory KIR, and B haplotypes have additional genes encoding activating KIR. The presence of activating KIR2DS1 (B haplotype) confers protection from pregnancy disorders [24], and its absence (A haplotype) increases the risk of pregnancy complications [14, 25].

Placentation is regulated by interactions (Fig. 6.1) between maternal killer immunoglobulin-like receptors (KIRs) expressed by the uNK and fetal HLA-C molecules expressed by EVT [26, 27]. Hiby et al. showed that invading EVTs are the principal site of HLA-C expression in the decidua basalis and that both maternal and paternal HLA-C allotypes are presented to KIRs [24, 28]. Insufficient invasion of the uterine lining by trophoblasts and vascular conversion in the decidua are thought to be the primary defect in disorders such as recurrent miscarriage (RM), preeclampsia, and fetal growth restriction (FGR) [29], and this process is regulated

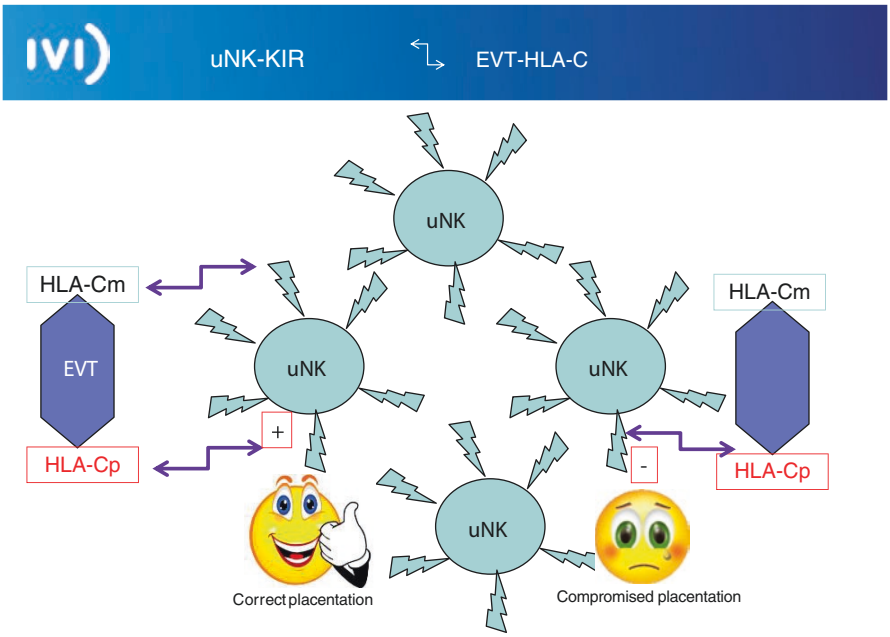

Fig. 6.1 KIR and fetal HLA-C interaction on own oocyte pregnancies. *HLA-Cm* maternal HLA-C; *HLA-Cp* paternal HLA-C. Red color: *nonself* HLA-C (own oocytes), *EVT* invading extravillous trophoblast, *uNK* uterine NK cells

by interaction between maternal KIRs expressed by the uNK and their ligand HLA-C expressed by EVT.

Pregnancies are at increased risk of recurrent miscarriage, preeclampsia, or FGR in mothers who are homozygous for KIR haplotype A (KIR AA) when the fetus has more HLA-C2 genes than the mother and the additional fetal HLA-C2 alleles are of paternal origin [24]. Protection from preeclampsia is likely to be mediated by the activating KIR2DS1 (B haplotype), which also binds HLA-C2.

Thus, depending on the particular KIR-HLA-C interactions, the trophoblast cell invasion will be regulated.

Hiby et al. [27] and Faridi and Agrawal [30] have reported differences in outcomes of medically unassisted pregnancies, increased risk of RM, preeclampsia, and FGR, in mothers with KIR AA carrying a fetus with paternal HLA-C2, and this finding suggests that placentation is deficient when there is a very strong inhibitory signal to uNK cells mediated via the KIR A haplotype gene. Hiby et al. [24, 26, 27, 31, 32] performed larger cohort studies that analyzed both maternal and paternal genotypes, with a large control group, and demonstrated a clear difference between the KIR and HLA-C genotypes in patients with disorders such as RM, preeclampsia, and FGR. Epidemiological studies provide clear evidence that selection for human reproductive success has adapted to the KIR and HLA-C genes and could be responsible for maintaining balanced polymorphisms between the HLA-C1 and HLA-C2 groups and the A and B KIR haplotypes [17, 28, 33, 34].

But What Happens in ART?

Assisted pregnancies differ from medically unassisted pregnancies, in those patients who receive sometimes more than one embryo per transfer, and also donor oocytes, sperm donor or embryo donation, are often used.

After double embryo transfer (DET), the expression of more than one paternal HLA-C per trophoblast cell is induced. In oocyte donor cycles, an increasingly demanded treatment due to advanced maternal age, the oocyte maternal HLA-C, which is genetically different from the mother's receptor, behaves as a paternal HLA-C, and this induces that more nonself HLA antigens are presented to the mother's KIR (per transfer) compared with "normal" pregnancies. After DET in an oocyte donor cycle, the expression of two nonself or "paternal" HLA-C in the EVT (per embryo) is present in the decidua basalis. The trophoblast antigen presentation (HLA-C) to uNK KIRs happens much more frequently than in natural pregnancy, because the embryo transfer is performed even monthly in RIF patients.

In human populations, pregnancy disorders are predicted to reduce the frequency of group A KIR, HLA-C2, or both, and this selection is thought to have originated during human evolution [21, 33, 34]. An inverse correlation between the frequencies of the KIR AA haplotype and HLA-C2 has been observed. Populations with the highest frequency of KIR AA (Japanese and Koreans) have the lowest HLA-C2 frequencies, whereas populations with the lowest frequency of KIR AA (Aboriginal Australians and Asian Indians) have the highest HLA-C2 frequencies. Natural selection seems to have driven an allele-level group A KIR haplotype and HLA-C1 ligand to an unusually high frequency in the Japanese, such that the detrimental KIR AA-HLA-C2 combination does not significantly affect pregnancy outcomes in Japanese and Korean populations [35].

This correlation provides evidence that selection for human reproductive success has adapted to the KIR and HLA-C genes and could be responsible for maintaining balanced polymorphisms between the HLA-C1 and HLA-C2 groups and the A and B KIR haplotypes [21, 28, 33, 34] However, this natural human evolution is not taken into consideration nowadays during ART. Furthermore, donor oocytes are often used in ART, and the literature describes higher maternal morbidity in egg donation pregnancies (pregnancy-induced hypertension, preeclampsia, FGR) [36] and preterm birth compared with pregnancies with own oocyte ART [37–39]. Although part of this increased frequency of complications may be due to the main indication for oocyte donation, which is advanced maternal age, recent age-matched data confirmed this higher frequency of unwanted events, and immunology maladaptation could be the reason [38, 40].

The increased expression of paternal HLA-C after DET could be associated with more pregnancy disorders than single embryo transfer (SET) in mothers with an inhibitory KIR haplotype (AA). A recent study [41] analyzed pregnancy, miscarriage, and LBR/cycle according to KIR haplotype and categorized by DET or SET. A higher rate of early miscarriage after DET when the patient's own oocytes were used occurred in those with the KIR AA (22.8%), followed by those with a KIR AB (16.7%), when compared with mothers with a KIR BB (11.1%) ($p = 0.03$).

A significantly decreased LBR/cycle after DET of donated oocytes was observed in mothers with a KIR AA haplotype (7.5%) compared with those with a KIR AB (26.4%) and KIR BB (21.5%)($p = 0.006$) [41].

The decreased LBR after DET in donor oocyte cycles in mothers KIR AA may be due to increased expression of nonself HLA-C (paternal and oocyte donor HLA-C). In this case, four "paternal" HLA-C would exist per trophoblast cells after DET: one coming from the father and another one coming from the donor, per embryo transferred, as the oocyte donor HLA-C behaves as "paternal" or non-self HLA-C. Expressing four "paternal" HLA-C is more likely to find at least one paternal or oocyte donor HLA-C2 than in own oocytes and SET, and probably implantation or placentation failure occurs in mothers who are KIR AA.

No other report has studied the impact of KIR-HLA-C on donor oocyte cycles. The authors speculate that completing a normal pregnancy was possible only for mothers with the KIR AA haplotype who carry a baby with a least one nonself HLA-C1 (nonself HLA-C1). Recently, they performed a prospective study [42] including 30 women with unknown etiology of RIF and RM and their oocyte donor-assisted reproductive cycles. All women had KIR AA genotype and their partners HLA-C2 genes. They had 54 embryo transfer cycles (82.76% DET; 17.24% SET) with unknown HLA-C oocyte donors and 28 cycles with HLA-C1C1 donors (21.05% DET; 78.95% SET). Pregnancy, miscarriage, and LBR/cycle after embryo transfer (ET) with unknown oocyte donor HLA-C and after transfers with HLA-C1C1 oocyte donor were studied.

Higher pregnancy rate per cycle after HLA-C1C1 oocyte donor transfer (85.71%) compared with unknown HLA-C oocyte donor cycles (31.48%) was observed in the same patients KIR AA with HLA-C2 partners ($p < 0.0001$). Higher miscarriage rate per cycle after unknown HLA-C oocyte donor transfer (94.44%) compared with HLA-C1C1 oocyte donor transfer (8.33%) was observed ($p < 0.0001$).

Significantly increased LBR per cycle was observed after ET with HLA-C1C1 oocyte donor (82.14%) compared with the LBR in the same KIR AA patients and HLA-C2 partners after cycles with unknown HLA-C oocyte donor (0%) ($p < 0.0001$).

This new findings show that the maternal KIR haplotype and fetal HLA-C have an impact on the live birth rate after IVF cycles, especially when donor oocyte and DET are used. Expressing four paternal HLA-C in the EVT cells after DET with donor oocytes is more likely to result in at least one nonself HLA-C2 (even by HLA-C2 allelic frequency on Caucasian population) than with one's own oocyte after SET, and implantation or placentation failure probably occurs in mothers with the KIR AA haplotype. Therefore, selecting HLA-C1, among oocyte and/or sperm donors for patients undergoing egg donation and who express inhibitory KIR haplotypes, could be more efficient and safer. The authors assume that it is a small sample and that is the first report observing differences in LBR by oocyte donor HLA-C in mothers KIR AA with HLA C2 partners. Apart from the statistical significance, the association strength is noticeably high, allowing greater confidence in the findings; however, larger studies are needed and should be replicated by other groups prior to final acceptance of this theory into clinical practice.

The new concept is emerging, and the evidence points to important physiological roles for uNK cells in healthy placentation as well as to abnormal uNK cell function in pregnancy disorders.

The combination of maternal KIR haplotype and parental donor HLA-C could predict which couple can benefit for the selection of SET/DET, or donor selection by HLA-C in ART, in order to increase the LBR/cycle, and it would facilitate the reduction of embryos that are being transferred, facilitating the increase of SET. Therefore, selecting HLA-C1, among oocyte and/or sperm donors for patients undergoing egg donation ART and inhibitory KIR, could be more efficient and safer as identified by epidemiological studies [10, 24, 43]

Other Immune Maternal Cells

The uterine mucosa is the major site where fetal placental cells are directly in contact with maternal tissues, rich in uNK cells, but also contains maternal T cells, effector T cells, and regulatory T cells. The trophoblast cells invading maternal decidua are allogeneic and could be potential targets for T cells. Although the interactions between uNK cells and EVT are clear, how effector T cells might interact with the trophoblast cells is still unclear [44].

Maternal T cells are not immunologically inert, as shown by the presence of fetal-specific T cells and T-cell-dependent humoral responses specific for the rhesus D antigen in rhesus-negative women or for paternally derived allogeneic HLA molecules in multiparous women [27, 45, 46]. Moreover, many studies in mice and humans have described mechanisms that favor T-cell tolerance in the deciduas, among which the expression of receptors that bind the trophoblast HLA-G on decidual antigen-presenting cells, preventing them from being immunogenic [27, 47].

The previous studies have been performed on mice and human spontaneous pregnancies, and questions also remain regarding the mechanisms by which effector T cells cause fetal loss especially in ART in which the fetal antigen presentation happens much more frequently than in natural pregnancy (even monthly), especially in recurrent miscarriage and RIF patients.

Conclusions

A new concept is emerging that the uterine immune system uses NK cell allorecognition to regulate placentation and control the maternofetal interface. In ART, these new insights [41, 42] could have an impact on the selection of SET in patients with recurrent miscarriage or RIF and a KIR AA haplotype. Also, although data are still premature and need to be validated, they may have clinical significance, helping with oocyte and/or sperm donor selection according to HLA-C in patients with

recurrent miscarriage or RIF and a KIR AA haplotype as HLA-C1/C1 donors are predicted to be safer and C2/C2 males or oocyte donors may be more “dangerous” as identified by epidemiological studies [24, 43].

This is a new concept and, based on it, it is reasonable to think that the use of different lines of immune therapies (such as prednisolone, intravenous immunoglobulin, intralipid, tumor necrosis factor-a blockers), given in different fertility clinics to decrease the NK cells’ activity in infertile women, has to be reconsidered because the scientific principle of the maternofetal tolerance has been misunderstood.

References

1. Garrido N, Bellver J, Remohi J, Simon C, Pellicer A. Cumulative live-birth rates per total number of embryos needed to reach newborn in consecutive in vitro fertilization (IVF) cycles: a new approach to measuring the likelihood of IVF success. Fertil Steril. 2011;96(1):40–6.
2. Alecsandru D, Garcia-Velasco JA. Immune testing and treatment: still an open debate. Hum Reprod. 2015;30(8):1994.
3. Sacks G. Enough! Stop the arguments and get on with the science of natural killer cell testing. Hum Reprod. 2015;30(7):1526–31.
4. 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(8):1971–80.
5. Moffett A, Shreeve N. Reply: First do no harm: continuing the uterine NK cell debate. Hum Reprod. 2015;31(1):218–9.
6. Moffett A, Shreeve N. First do no harm: uterine natural killer (NK) cells in assisted reproduction. Hum Reprod. 2015;30(7):1519–25.
7. Alecsandru D, Garcia-Velasco JA. Immunology and human reproduction. Curr Opin Obstet Gynecol. 2015;27(3):231–4.
8. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med. 2003;198(8):1201–12.
9. Caligiuri MA. Human natural killer cells. Blood. 2008;112(3):461–9.
10. Moffett A, Colucci F. Uterine NK cells: active regulators at the maternal-fetal interface. J Clin Invest. 2014;124(5):1872–9.
11. King A, Balendran N, Wooding P, Carter NP, Loke YW. CD3- leukocytes present in the human uterus during early placentation: phenotypic and morphologic characterization of the CD56++ population. Dev Immunol. 1991;1(3):169–90.
12. King A, Birkby C, Loke YW. Early human decidual cells exhibit NK activity against the K562 cell line but not against first trimester trophoblast. Cell Immunol. 1989;118(2):337–44.
13. Bulmer JN, Lash GE. Human uterine natural killer cells: a reappraisal. Mol Immunol. 2005;42(4):511–21.
14. Xiong S, Sharkey AM, Kennedy PR, Gardner L, Farrell LE, Chazara O, et al. Maternal uterine NK cell-activating receptor KIR2DS1 enhances placentation. J Clin Invest. 2013;123(10):4264–72.
15. Moffett A, Loke C. Immunology of placentation in eutherian mammals. Nat Rev Immunol. 2006;6(8):584–94.
16. Moffett A, Loke C. Implantation, embryo-maternal interactions, immunology and modulation of the uterine environment – a workshop report. Placenta. 2006;27(Suppl A):S54–5.
17. Parham P. NK cells and trophoblasts: partners in pregnancy. J Exp Med. 2004;200(8):951–5.
18. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol. 2002;2(9):656–63.

19. King A, Hiby SE, Gardner L, Joseph S, Bowen JM, Verma S, et al. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors–a review. Placenta. 2000;21(Suppl A):S81–5.
20. Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. Immunology. 2009;127(1):26–39.
21. Parham P, Moffett A. Variable NK cell receptors and their MHC class I ligands in immunity, reproduction and human evolution. Nat Rev Immunol. 2013;13(2):133–44.
22. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. Immunity. 1997;7(6):753–63.
23. Winter CC, Gumperz JE, Parham P, Long EO, Wagtmann N. Direct binding and functional transfer of NK cell inhibitory receptors reveal novel patterns of HLA-C allotype recognition. J Immunol. 1998;161(2):571–7.
24. Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, et al. Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. J Clin Invest. 2010;120(11):4102–10.
25. Wang S, Li YP, Ding B, Zhao YR, Chen ZJ, Xu CY, et al. Recurrent miscarriage is associated with a decline of decidual natural killer cells expressing killer cell immunoglobulin-like receptors specific for human leukocyte antigen C. J Obstet Gynaecol Res. 2014;40(5):1288–95.
26. Hiby SE, Regan L, Lo W, Farrell L, Carrington M, Moffett A. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. Hum Reprod. 2008;23(4):972–6.
27. Hiby SE, Walker JJ, O'Shaughnessy KM, Redman CW, Carrington M, Trowsdale J, et al. Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. J Exp Med. 2004;200(8):957–65.
28. Hiby SE, Ashrafian-Bonab M, Farrell L, Single RM, Balloux F, Carrington M, et al. Distribution of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands in two Iranian populations. Immunogenetics. 2010;62(2):65–73.
29. Arck PC, Hecher K. Fetomaternal immune cross-talk and its consequences for maternal and offspring's health. Nat Med. 2013;19(5):548–56.
30. Faridi RM, Agrawal S. Killer immunoglobulin-like receptors (KIRs) and HLA-C allorecognition patterns implicative of dominant activation of natural killer cells contribute to recurrent miscarriages. Hum Reprod. 2011;26(2):491–7.
31. Hiby SE, Apps R, Chazara O, Farrell LE, Magnus P, Trogstad L, et al. Maternal KIR in combination with paternal HLA-C2 regulate human birth weight. J Immunol. 2014;192(11):5069–73.
32. Hiby SE, Ashrafian-Bonab M, Farrell L, Single RM, Balloux F, Carrington M, et al. Distribution of killer cell immunoglobulin-like receptors (KIR) and their HLA-C ligands in two Iranian populations. Immunogenetics. 2009;62(2):65–73.
33. Adams EJ, Parham P. Species-specific evolution of MHC class I genes in the higher primates. Immunol Rev. 2001;183:41–64.
34. Rajalingam R, Parham P, Abi-Rached L. Domain shuffling has been the main mechanism forming new hominoid killer cell Ig-like receptors. J Immunol. 2004;172(1):356–69.
35. Saito S, Takeda Y, Sakai M, Nakabayahi M, Hayakawa S. The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men. J Reprod Immunol. 2006;70(1-2):93–8.
36. Savasi VM, Mandia L, Laoreti A, Cetin I. Maternal and fetal outcomes in oocyte donation pregnancies. Hum Reprod Update. 2016;22:620.
37. Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-induced hypertension: a meta-analysis and case series. Dtsch Arztebl Int. 2011;108(3):23–31.
38. Masoudian P, Nasr A, de Nanassy J, Fung-Kee-Fung K, Bainbridge SA, El Demellawy D. Oocyte donation pregnancies and the risk of preeclampsia or gestational hypertension: a systematic review and metaanalysis. Am J Obstet Gynecol. 2015;214(3):328–39.

39. Blazquez A, Garcia D, Rodriguez A, Vassena R, Figueras F, Vernaeve V. Is oocyte donation a risk factor for preeclampsia? A systematic review and meta-analysis. J Assist Reprod Genet. 2016;33(7):855–63.
40. Jeve YB, Potdar N, Opoku A, Khare M. Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies. Int J Gynaecol Obstet. 2016;133(2):156–8.
41. Alecsandru D, Garrido N, Vicario JL, Barrio A, Aparicio P, Requena A, et al. Maternal KIR haplotype influences live birth rate after double embryo transfer in IVF cycles in patients with recurrent miscarriages and implantation failure. Hum Reprod. 2014;29(12):2637–43.
42. Alecsandru D, Barrio A, Aparicio P, Aparicio M, García-Velasco JA, editors. Maternal killer-cell immunoglobulin-like receptor (KIR) and fetal HLA-C compatibility in ART-oocyte donor influences live birth rate. ESHRE. 2016.
43. Skjaerven R, Vatten LJ, Wilcox AJ, Ronning T, Irgens LM, Lie RT. Recurrence of pre-eclampsia across generations: exploring fetal and maternal genetic components in a population based cohort. BMJ. 2005;331(7521):877.
44. Nancy P, Erlebacher A. T cell behavior at the maternal-fetal interface. Int J Dev Biol. 2014;58(2-4):189–98.
45. James E, Chai JG, Dewchand H, Macchiarulo E, Dazzi F, Simpson E. Multiparity induces priming to male-specific minor histocompatibility antigen, HY, in mice and humans. Blood. 2003;102(1):388–93.
46. van Kampen CA, Versteeg-vd Voort Maarschalk MF, Langerak-Langerak J, Roelen DL, Claas FH. Kinetics of the pregnancy-induced humoral and cellular immune response against the paternal HLA class I antigens of the child. Hum Immunol. 2002;63(6):452–8.
47. Apps R, Gardner L, Moffett A. A critical look at HLA-G. Trends Immunol. 2008;29(7):313–21.