ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
October 2015; 14(5):535-542.

The Relationship between *HLA-G* Gene Polymorphisms and Repeated Implantation Failure in Infertile Couples Undergoing Assisted Reproductive Technique

Zahra Kuroshli[1,2], Hamid Gourabi[1], Masood Bazrgar[1], Mohammad Hossein Sanati[1,3], and Masoud Zamani Esteki[4]

[1] *Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute, ACECR, Tehran, Iran*
[2] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[3] *Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*
[4] *Centre for Human Genetics, University Hospital Leuven & Department of Human Genetics, KU Leuven, Belgium*

Received: 22 September 2014; Received in revised form: 17 November 2014; Accepted: 9 December 2014

ABSTRACT

HLA-G is a tolerogenic molecule that expresses in cytotrophoblast cells and plays an important role in immune response suppression in maternal decidua. Interactions between the extracellular domains of the HLA-G protein with cell receptors of the immune system are well-known.

This study investigated the association between *HLA-G* gene polymorphism with repeated implantation failure (RIF). We used PCR followed by the sequencing technique for exons 2, 3, and 4, as well as intron 2 of the *HLA-G* gene in 100 couples with histories of two or more failed assisted reproductive technique (ART) attempts. The data were compared with the results of our previous study.

The results indicated that some alleles of the *HLA-G* gene such as: 0106, 010106, 01010106 and 0105N (null) alleles were significantly higher in the patient group compared to the control group ($p<0.05$). There were higher SNPs at the +482 T/C and +506 -/C positions in failed ART couples compared to controls ($p=0.03$; $p=0.01$, respectively).

HLA-G gene polymorphisms do not clearly affect the risk for implantation failure in most couples who undergo ART. However allelic variations, particularly in exons 3 and 4, and intron 2 of the *HLA-G* gene can lead to ART failure in human embryos.

Keywords: ART; *HLA-G* polymorphism; Implantation; RIF

INTRODUCTION

Embryonic implantation failure is one cause of infertility that can occur due to several factors. Considerable interest exists to determine the potential causes of repeated implantation failure (RIF) and strategies are used that may improve implantation in clinical practice, such as the assisted reproductive

Corresponding Author: Hamid Gourabi, PhD,
Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute, ACECR, Tehran, Iran. Tel: (+98 21) 2233 9947, Fax: (+98 21) 2356 2681, E-mail: Gourabi@royaninstitute.org

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

technique (ART).[1,2] Failure to achieve pregnancy, following 2–6 IVF cycles, in which more than 10 high-grade embryos have been transferred to the uterus is defined by various clinicians as RIF.[3] Successful embryo implantation depends on an appropriate function of the endometrium as well as a normal healthy embryo.[4] During pregnancy, the proper function of immunogenetic and immunological factors is more important; disruption in these may often lead to reproductive failure. The human leukocyte antigen (HLA) is the one of effective immunological complexes in human fertility and may have important roles in pregnancy outcome by influencing embryo cleavage; gamete, blastocyst, fetal and trophoblast development; implantation; and fetal survival.[5] HLA-G, as a member of non-classical HLA class I, displays limited polymorphisms along with seven different isoforms due to alternative splicing of the primary transcript, four membrane-anchored molecules (HLA-G1, G2,G3 and G4), three secreted isoforms (HLA-G5, G6 and G7), and a restricted expression profile.[6,7] The HLA-G protein represents several α-domains and an extracellular (heavy chain) with a non-covalent association to b2-microglobulin (b2-m).[8] The heavy chain is coded by the *HLA-G* gene located on the 6p21 region; it consists of eight exons and seven introns.[6] *HLA-G* expression has been detected during preimplantation development expression which includes unfertilized oocytes, early embryos (two-cell cleavage stage) and in blastocysts[9] with an asymmetric pattern.[10] Its expression is associated with an increase in developmental stage, from 35% in cleavage stage embryos to 100% in morulas and blastocysts.[11] The HLA-G protein is implicated in the modulation of maternal immune cell response[6,12,13] such as natural killer (NK) cells, T lymphocytes, and antigen presenting cells.[6,14,15] These functions are fulfilled through HLA-G extracellular domain interactions with leukocyte receptors, which include CD8, LILRB1 and LILRB2, and the killer cell immunoglobulin-like receptor KIR2DL4.[6,16,17] In early pregnancy, approximately 70% of the total leukocyte population in the decidua are comprised of uterine NK (uNK) cells.[18] Until after 20 weeks of gestation, this population of uNK cells does not decrease.[19] Several recent studies have shown a relationship between secretion of HLA-G in the embryo culture (EC) and pregnancy outcome. Recently, a German multi-center study has demonstrated that the proportion of sHLA-G in the EC increased with the embryonic developmental stage. There was a significant association between detection of sHLA-G in EC to pregnancy after ART. This might be considered a second parameter after the morphological scoring system for embryo selection.[20] Genetic polymorphisms in the 5` UTR and 3` UTR of the *HLA-G* gene are important. Significantly increased genotype frequency of +14-bp /+14-bp homozygotes have been reported in individuals with recurrent abortions compared with normal fertile controls.[21] Patient analysis on the genotype basis confirmed a significantly higher proportion of *HLA-G* 01013 and 01015N carriers in the RSA (Recurrent spontaneous abortion) group versus the fertile controls.[22] Another study showed an association between 725G SNP and sporadic miscarriage.[23] The current research assessed the association between *HLA-G* genotypes and RIF in infertile couples who underwent ART treatment compared with control couples.

MATERIALS AND METHODS

Sample Preparation

The patient group consisted of 100 couples (mean age: 32.3 years) with a history of RIF. This group was selected from among Iranian patients who underwent ART treatment at Royan Institute. Of the 100 couples investigated, 90 couples underwent two and three procedures, 6 couples had four procedures and 4 couples had five or more procedures. Inclusion criteria for the study were as follows: two or more failed ART from the same partner, the absence of anatomical abnormalities of the uterus, normal results for lupus anticoagulants and cardiolipin antibodies, abnormal karyotypes, and patient informed consent. Only couples with idiopathic infertility were included in current study. Subsequently, we compared the obtained results with our last study of approximately 100 samples from healthy unrelated Iranian individuals (50 unrelated couples).[24]

DNA Extraction

Genomic DNA was isolated and purified from whole blood samples collected in 5% ethylene-diamide tetraacetic acid using the salting out procedure.[25] The concentration and purity of DNA was assessed by a biophotometer.

HLA-G Allele Typing

HLA-G genotyping was carried out with polymerase chain reaction (PCR) followed by sequencing analysis. Exons 2 and 3, and intron 2 were amplified with a primer pair: forward 5′- GGC TGA GAG GTC TAC AGG AGA T-3′ and reverse 5′-GCT CCC ACT CCA TGA GGT ATT-3′. Amplification of exon 4 was performed using the following primers: forward 5′-GTA TCT GGT TCA TTC TTA GGA TGG-3′ and reverse 5′-AAG ACT GCT CTG GGA AAG G-3′. The PCR products of exons 2 and 3, and intron 2 was 822 bp; for exon 4, it was 502 bp. The PCR program for exons 2 and 3, and intron 2 was: 95˚C for 10 min, 30 cycles at 94˚C for 1 min and 60˚C for 45 sec, followed by 72˚C for 45 sec. For exon 4 the program was: 95˚C for 5 min, 30 cycles at 94˚C for 1 min, and 59˚C for 45 sec, followed by 72˚C for 45 sec. The products were sequenced and PLINK software was used (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) for allelic haplotyping.

Statistical Analysis

Statistical analysis was performed with SPSS software (version 18.0; SPSS, Chicago, IL). Genotype frequency for the control[24] and patient groups was determined by Fisher's exact test. For all analyses, a p-value ≤0.05 was considered significant. We used the Fisher's exact test to evaluate each allele of *HLA-G* between the cases and controls, and to obtain odds ratios (OR) for the risk of ART failure and their 95% confidence intervals (CI).

RESULTS

HLA-G Alleles in Couples

The frequency and percentage of *HLA-G* alleles among the cases and controls, along with additional details are shown in Tables 1 and 2 (polymorphisms of HLA-G were in Hardy-Weinberg equilibrium for all groups). An overall analysis of failed ART couples compared with controls showed a significant difference between the two groups ($p \leq 0.05$). These differences included an increase of G*0106 (p=0.002; OR=3.98; 95% CI=1.54-10.28); G*01010106 (p=0.004; OR=2.35; 95% CI=1.4-78.6); G*010106 (p=0.000; OR=13.83; 95% CI=1.86-102.7); and G*0105 N alleles (p=0.01; OR=0) in the failed ART group compared with the control group. We observed the G*0105N allele only in the patient group (29/400; 3%). There were statistically significant differences observed in the control group for G*010101 (p=0.001; OR=0.54; 95% CI=0.39-0.77); G*010108 (p=0.04; OR= -1.63; 95% CI=0.04-1.02); and G*010403 (p=0.01; OR=0.51; 95% CI=0.3-0.88) allele frequencies (Table 2). According to previous studies, the G*0105N allele was associated with significantly lower serum concentrations of the soluble isoform of HLA-G.[26]

***HLA-G* Alleles in Men and Women**

A comparison of *HLA-G* allele frequencies in men and women from both groups revealed that 13 (6.5%)

Table 1. Distribution of HLA-G allele frequencies in 100 couples with history of ART failure.

HLA-G alleles	Failed ART couples 2- 4 unsuccessful ART, n (%)	Failed ART couples ≥ 5 unsuccessful ART, n (%)
010101	151 (39.3)	0
0106	35 (9.1)	2 (12.5)
01010106	19 (4.9)	1(6.2)
010102	64 (16.7)	0
010103	8 (2.1)	2 (12.5)
010105	6 (1.6)	1 (6.2)
010106	24 (6.2)	2 (12.5)
010107	14 (3.6)	1 (6.2)
010108	2 (5)	0
0103	7 (1.8)	2 (12.5)
0105N	9 (2.3)	3 (18.8)
010401	5 (1.3)	1 (6.2)
010403	31 (8.1)	1 (6.2)
010404	9 (2.3)	0
All alleles	384	16

Table 2. Distribution of HLA-G allele frequencies in 100 couples with history of ART failure and 50 healthy couples ($p \leq 0.05$; CI=95%).[24]

HLA-G alleles	Failed ART couples, n (%)	Healthy couples, n (%)	*P*- value	OR[a] (95% CI[b])
010101	151 (37.8)	105 (52.5)	0.001	0.54 (0.39-0.77)
0106	37 (9.2)	5 (2.5)	0.002	3.98 (1.54-10.28)
01010106	20 (5)	1 (0.5)	0.004	2.35 (1.4-78.6)
010102	64 (16)	32 (16)	1	1 (0.63-1.59)
010103	10 (2.5)	1 (0.5)	0.11	5.1 (0.65-40.14)
010105	7 (1.8)	4 (2)	1	-0.14 (0.25-3.01)
010106	26 (6.5)	1 (0.5)	0	13.83 (1.86-102.7)
010107	15 (3.8)	6 (3)	0.81	1.26 (0.48-3.3)
010108	2 (0.5)	5 (2.5)	0.04	-1.63 (0.04-1.02)
0103	9(2.2)	3 (1.5)	0.76	1.51 (0.4-5.65)
0105 N	12 (3)	0 (0)	0.01	0 (0-0)
010401	6 (1.5)	3 (1.5)	1	1 (0.25-4.04)
010403	32 (8)	29 (14.5)	0.01	0.51 (0.3-0.88)
010404	9 (2.2)	5 (2.5)	1	0.9 (0.3-2.7)
All alleles	400	200		

[a]CI, confidence interval.

[b]Odds ratios were generated from two-by-two tables, and statistical significance was assessed using the Fisher exact test.

higher in failed ART men compared to fertile men (p=0.04; OR=6.88; 95% CI=1.88-53.38). The G*010101 allele was observed in 67 (33.5%) failed ART men and 54 (54%) healthy men. The frequency of this allele differed between the two groups with a shift toward the control group (p=0.001; OR=0.43; 95% CI=0.26-0.7; Table 3). On the other hand, in women who underwent ART (21n; 10.5%), the G*0106 allele was clearly increased compared with healthy women (1n; 1%; p=0.002; OR=11.61; 95% CI=1.53-87.64).

There was a high frequency of the G*010106 allele amongst infertile women (13n; 6.5%). This allele was not detected in healthy women (p=0.006; OR=0). Other data showed increased G*010403 allele frequency in healthy women (19n; 19%) compared to failed ART women (12n; 6%; p=0.001; OR=0.27; 95% CI=0.13-0.59; Table 4).

Table 3. Distribution of *HLA-G* allele frequencies in 100 men from couples with history of ART failure and 50 healthy men ($p \leq 0.05$; CI=95%) [24]

HLA-G alleles	Failed ART men, n (%)	healthy men, n (%)	*P*- value	OR[a] (95% CI[b])
010101	67 (33.5)	54 (54)	0.001	0.43 (0.26-0.7)
0106	16 (8)	4 (4)	0.23	2.09 (0.68-6.41)
01010106	12 (6)	1 (1)	0.07	1.84 (0.81-49.3)
010102	35 (17.5)	15 (15)	0.63	1.2 (0.62-2.32)
010103	5 (2.5)	1 (1)	0.67	2.54 (0.29-22.02)
010105	3 (1.5)	2 (2)	1	-0.29 (0.12-4.53)
010106	13 (6.5)	1 (1)	0.04	6.88 (1.88-53.38)
010107	10 (5)	2 (2)	0.35	2.58 (0.55-12)
010108	2 (1)	3 (3)	0.33	-1.11 (0.05-1.99)
0103	3 (1.5)	3 (3)	0.4	0.49 (0.01-2.49)
0105 N	5 (2.5)	0 (0)	0.17	0
010401	4 (2)	2 (2)	1	1 (0.18-5.56)
010403	20 (10)	10 (10)	1	1 (0.45-2.23)
010404	5 (2.4)	2 (2)	1	1.25 (0.24-6.56)
All alleles	200	100		

[a]CI, confidence interval.

[b]Odds ratios were generated from two-by-two tables, and statistical significance was assessed using the Fisher exact test.

Table 4. Distribution of *HLA-G* allele frequencies in 100 women from couples with history of ART failure and 50 healthy women ($p \leq 0.05$; CI=95%).[24]

HLA-G alleles	Failed ART women, n (%)	Healthy women, n (%)	*P*- value	OR[a] (95% CI[b])
010101	84 (42)	51 (51)	0.14	0.7 (0.43-1.12)
0106	21 (10.5)	1 (1)	0.002	11.61 (1.53-87.64)
01010106	8 (4)	0 (0)	0.057	0
010102	29 (14.5)	17 (17)	0.61	0.83 (0.43-1.6)
010103	5 (2.5)	0 (0)	0.17	0
010105	4 (2)	2 (2)	1	1 (0.18-5.56)
010106	13 (6.5)	0 (0)	0.006	0
010107	5 (2.5)	4 (2)	0.49	0.62 (0.16-2.34)
010108	0 (0)	2 (2)	0.11	0
0103	6 (3)	0 (0)	0.18	0
0105 N	7 (3.5)	0 (0)	1	0
010401	2 (1)	1 (1)	1	1 (0.9-11.16)
010403	12 (6)	19 (19)	0.001	0.27 (0.13-0.59)
010404	4 (2)	3 (3)	0.69	0.67 (0.15-0.03)
All alleles	200	100		

[a]CI, confidence interval.

[b]Odds ratios were generated from two-by-two tables, and statistical significance was assessed using the Fisher exact test.

Investigation of *HLA-G* Homozygosity in Couples

The previous study significantly indicated higher frequency in HLA homozygosity among couples with unexplained infertility.[27] Our data showed the following frequency of homozygous carriers: G*0106 (ART: 8.7%, control: 0%); G*010102 (ART: 18.8%, control: 8.8%); G*01010106 (ART: 5.8%, control: 0%); and G*010106 (ART: 5.8%, control: 0%) alleles. This difference was not statistically significant. We could not explain the cause for the difference in frequency of the G*010102 allele between the failed ART and control groups. However, since the G*0106, G*01010106, and G*010106 alleles were significantly higher in the ART group, we suggested that homozygosity in these alleles could lead to ART failure in couples (Table 5).

Table 5. Distribution of failed ART couples frequencies, homozygote for HLA-G alleles in 100 couples with history of ART failure and 50 healthy couples ($p \leq 0.05$; CI=95%).[24]

HLA-G alleles	Homozygote Failed ART couples, n (%)	Homozygote healthy couples, n (%)	*P*- value	OR[a] (95% CI[b])
010101	34 (49.3)	24 (70.6)	0.06	0.4 (0.17-0.97)
0106	6 (8.7)	0 (0)	0.17	0
01010106	4 (5.8)	0 (0)	0.03	0
010102	13 (18.8)	3 (8.8)	0.25	2.4 (0.64-9.07)
010106	4 (5.8)	0 (0)	0.03	0
010107	2 (2.9)	1 (2.9)	1	0.99 (0.09-11.26)
010401	1 (1.44)	0 (0)	1	0
010403	4 (5.8)	6 (11.6)	0.08	0.29 (0.08-1.08)
010404	1 (1.44)	0 (0)	1	0
All alleles	69	34		

[a]CI, confidence interval.

[b]Odds ratios were generated from two-by-two tables, and statistical significance was assessed using the Fisher exact test.

Table 6. Distribution of intronic SNPs frequencies (intron 2) of HLA-G in 100 couples with history of ART failure and 50 healthy couples in Iranian population ($p \leq 0.05$)

SNP position	Nucleotide change	SNP Frequency in cases group	SNP Frequency in control group	*P*- value
+482	T>C	4.5%	0%	0.032
+485	G>T	1%	2%	0.603
+494	A>C	3.5%	3%	1
+505/+506	->CC	0.5%	0%	1
+506	->C	6%	0%	0.01
+615	A>-	12%	2%	0.05
+636	C>T	15.5%	20%	0.332
+644	G>T	1%	4%	0.098
+685	G>A	28%	30%	0.787

Nucleotide Variation in Intron 2

We took into consideration nucleotide variation from exon 2 to exon 3 which included the intron 2 sequence. Table 5 shows the observed nucleotide variation in intron 2 of the *HLA-G* gene in the studied couples. We have suggested that these variations are noteworthy, because some have different frequencies in these groups of patients. Sequence analysis of the *HLA-G* gene indicated that nucleotide variability in the intron 2 of the gene had a different frequency between the two groups. SNP at the +482T/C position was more common among failed ART couples (p=0.032); the frequency of nucleotide variation at the +506 -/C position in ART couples was higher than healthy couples (p=0.01); and we observed an increase in SNP at the +615 A/- position in ART couples (p=0.05). Finally, the results indicated that these SNPs were mostly together (Table 6).

DISCUSSION

According to previous studies sHLA-G has been detected not only in plasma of pregnant women but also in the supernatant culture media from in vitro cultured embryos.[28] Based on expression of HLA-G before, during and after implantation, and the protein's critical role, it is believed that nucleotide polymorphisms in some regions of the gene can lead to implantation failure in unexplained infertile couples.[6] This study has demonstrated a significant increase in the frequencies of G*0106, G*01010106, G*010106, and G*0105N alleles in patient couples compared with healthy couples. The *HLA-G**0106 allele is defined by an amino acid change from threonine (Thr) to methionine (Met) at amino acid 258 in exon 4 of the gene which encodes the α3 extracellular domain of the HLA-G protein. In addition, this domain exists only in the HLA-G1, G2, G5 and G6 isoforms.[6] The importance of expression of these isoforms during implantation is clear; perhaps this amino acid change affects HLA-G protein function. The *HLA-G**0105N allele has a frame shift mutation in exon 3 of this gene that leads to distorted protein production. According to other reports, the G*0105N allele is associated with lower plasma sHLA-G concentrations;[26] correlation of lower sHLA-G concentrations have been demonstrated with pregnancy outcome after IVF. Therefore an increase in soluble HLA-G concentration in plasma from pregnant women is associated with successful pregnancy.[6,19] In our study, we have observed a high frequency of the G*0105N allele in failed ART couples (3%) compared to control couples (0%). The highest concentrations of sHLA-G were found among HLA-G 010102, 010101 and 010108 allele carriers; these concentrations in HLA-G 010101, 010102 and 010108 were significantly lower than HLA-G 10401 carriers (p<0.001). The highest concentration of sHLA-I was noted among women with the *HLA-G* 10401 allele (p<0.0001).[29] Therefore these results could explain the increased frequencies of G*010108 (p=0.04; OR= -1.63) and G*010403 (p=0.01; OR=0.51) alleles in healthy compared to infertile couples. As mentioned above, we observed some SNPs in intron 2 of the gene; this observation was higher in infertile couples compared to healthy couples [+482T/C (p=0.032); +506 -/C (p=0.01); and +615 A/- (p=0.05)]. These

SNPs were mostly together. In order to justify the results, we checked causes such as: occurrence of a new open reading frame (ORF) in intron 2; the existence of CpG islands following the change of DNA methylation and gene expression as an epigenetic regulation; and the possibility of an effect on the alternative splicing processes for HLA-G isoforms within intron 2 (HLA-G1, G4 and G5). However none were suitable reasons for the different detected frequencies. Until now, 29 SNPs have been identified for the *HLA-G* promoter[6] which might influence regulation of HLA-G expression. The -725 G/C/T variant is very close to ISRE, a target site for interferon regulatory factor-1 (IRF-1), in the gene promoter.[30] An association between the -725G variant with sporadic miscarriage has been reported.[23] On the other hand, *HLA-G* alleles that presented the 14-bp (5'-ATTTGTTCATGCCT-3') sequence have been associated with lower mRNA production for most membrane-bound and soluble isoforms in trophoblast samples.[7,31] In addition, both G*01:01:03 and G*01:01:02 alleles are associated with different 3'UTR haplotypes that differ at the +3035, +3187 and +3196 positions - all preserved after alternative splicing.[32,33] The 14-bp insertion is always accompanied by the +3142G and +3187A alleles which are associated with low mRNA production.[7] Based on the above mentioned studies, we have suggested that possibly some SNPs in the noncoding regions of the *HLA-G* gene, such as intron 2 have an association with polymorphisms in other regions of the gene, such as 3'UTR or 5'UTR. However it is apparent that these SNPs can significantly exist in the failed ART group (+482 T>C: p=0.32; +506 ->C: p=0.01; +615 A>-: p=0.05) and may have a relation with implantation failure.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to the Genetics Department staff at Royan Institute (Tehran, Iran) for technical support.

REFERENCES

1. Tan BK, Vandekerckhove P, Kennedy R, Keay SD. Investigation and current management of recurrent IVF treatment failure in the UK. BJOG 2005; 112(6):773-80.
2. Scott L. Embryological strategies for overcoming recurrent assisted reproductive technology treatment failure. Hum Fertil (Camb) 2002; 5(14):206-14.
3. Margalioth EJ1, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod 2006; 21(12):3036-43.
4. Simon A, Laufer N. Repeated implantation failure: clinical approach. Fertil Steril 2012; 97(5):1039-43.
5. Choudhury SR, Knapp LA. Human reproductive failure I: immunological factors. Hum Reprod Update 2001; 7(2):113-34.
6. Donadi EA1, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. Cell Mol Life Sci 2011; 68(3):369-95.
7. Hviid TV. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. Hum Reprod Update 2006; 12(3):209-32.
8. Carosella ED1, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. Adv Immunol 2003; 81:199-252.
9. Rizzo R1, Vercammen M, van de Velde H, Horn PA, Rebmann V. The importance of HLA-G expression in embryos, trophoblast cells, and embryonic stem cells. Cell Mol Life Sci 2011; 68(3): 341-52.
10. Shaikly VR1, Morrison IE, Taranissi M, Noble CV, Withey AD, Cherry RJ, et al. Analysis of HLA-G in maternal plasma, follicular fluid, and preimplantation embryos reveal an asymmetric pattern of expression. J Immunol 2008; 180(6):4330-7.
11. Yao YQ, Barlow DH, Sargent IL. Differential expression of alternatively spliced transcripts of HLA-G in human preimplantation embryos and inner cell masses. J Immunol 2005; 175(12): 8379-85.
12. Carosella ED, Moreau P, Lemaoult J, et al. HLA-G: from biology to clinical benefits. Trends Immunol 2008; 29(3):125-32.
13. Carosella ED1, Favier B, Rouas-Freiss N, Moreau P, Lemaoult J. Beyond the increasing complexity of the immunomodulatory HLA-G molecule. Blood 2008; 111(10):4862-70.
14. Bahri R, Hirsch F, Josse A, Rouas-Freiss N, Bidere N, Vasquez A, et al. Soluble HLA-G inhibits cell

cycle progression in human alloreactive T lymphocytes. J Immunol 2006; 176(3):1331-9.
15. Lila N, Rouas-Freiss N, Dausset J, Carpentier A, Carosella ED. Soluble HLA-G protein secreted by allo-specific CD4+ T cells suppresses the allo-proliferative response: a CD4+ T cell regulatory mechanism. Proc Natl Acad Sci U S A 2001; 98(21):12150-5.
16. Shiroishi M, Kuroki K, Rasubala L, Tsumoto K, Kumagai I, Kurimoto E, et al. Structural basis for recognition of the nonclassical MHC molecule HLA-G by the leukocyte Ig-like receptor B2 (LILRB2/LIR2/ILT4/CD85d). Proc Natl Acad Sci U S A 2006; 103(44):16412-7.
17. Gao GF, Willcox BE, Wyer JR, Boulter JM, O'Callaghan CA, Maenaka K, et al. Classical and nonclassical class I major histocompatibility complex molecules exhibit subtle conformational differences that affect binding to CD8alphaalpha. J Biol Chem 2000; 275(20):15232-8.
18. Wold AS, Arici A. Natural killer cells and reproductive failure. Curr Opin Obstet Gynecol 2005; 17(3):237-41.
19. Moffett-King A. Natural killer cells and pregnancy. Nat Rev Immunol 2002; 2:656-63.
20. Rebmann V, Switala M, Eue I, Grosse-Wilde H. Soluble HLA-G is an independent factor for the prediction of pregnancy outcome after ART: a German multi-centre study. Hum Reprod 2010; 25(7):1691-8.
21. Hviid TV, Hylenius S, Lindhard A, Christiansen OB. Association between human leukocyte antigen-G genotype and success of in vitro fertilization and pregnancy outcome. Tissue antigens 2004; 64(1):66-9.
22. Pfeiffer KA, Fimmers R, Engels G, van der Ven H, van der Ven K. The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. Mol Hum Reprod 2001; 7:373-8.
23. Ober C, Aldrich CL, Chervoneva I, Billstrand C, Rahimov F, Gray HL, et al. Variation in the HLA-G promoter region influences miscarriage rates. Am J Hum Genet 2003; 72(6):1425-35.
24. Kuroshli Z, Gourabi H, Bazrgar M, Sanati MH, Bahraminejad E, Anisi K. HLA-G allele and Haplotype Frequencies in a Healthy Population of Iran. Iran J Allergy Asthma Immunol 2014; 13(3):207-13.
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.
26. Rebmann V, van der Ven K, Pässler M, Pfeiffer K, Krebs D, Grosse-Wilde H. Association of soluble HLA-G plasma levels with HLA-G alleles. Tissue antigens 2001; 57(1):15-21.
27. Coulam CB, Moore SB, O'Fallon W. Investigating unexplained infertility. Am J Obstet Gynecol 1988; 158(6 Pt 1):1374-81.
28. Hunt JS, Langat DK, McIntire RH, et al. The role of HLA-G in human pregnancy. Reprod Biol Endocrinol 2006; (4 Suppl 1):S10.
29. Sipak-Szmigiel O, Ronin-Walknowska E, Cybulski C, Plonka T, Lubiński J. Antigens HLA-G, sHLA-G and sHLA- class I in reproductive failure. Folia Histochem Cytobiol 2007; (45 Suppl 1):S137-41.
30. Gobin SJ, van Zutphen M, Woltman AM, van den Elsen PJ. Transactivation of classical and nonclassical HLA class I genes through the IFN-stimulated response element. J Immunol 1999; 163(3):1428-34.
31. Hviid TV1, Hylenius S, Rørbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 2003; 55(2):63-79.
32. Castelli EC, Mendes-Junior CT, Deghaide NH, de Albuquerque RS, Muniz YC, Simões RT, et al. The genetic structure of 3'untranslated region of the HLA-G gene: polymorphisms and haplotypes. Genes Immun 2010; 11(2):134-41.
33. Castelli EC, Moreau P, Oya e Chiromatzo A, Mendes-Junior CT, Veiga-Castelli LC, Yaghi L, et al. In silico analysis of microRNAS targeting the HLA-G 3' untranslated region alleles and haplotypes. Hum Immunol 2009; 70(12):1020-5.