# Combination of uterine natural killer cell immunoglobulin receptor haplotype and trophoblastic HLA-C ligand influences the risk of pregnancy loss: a retrospective cohort analysis of direct embryo genotyping data from euploid transfers

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**Objective:** To compare maternal uterine natural killer cell immunoglobulin receptor (KIR) genotype and haplotype frequencies between patients whose euploid single-embryo transfer resulted in pregnancy loss and those that resulted in delivery and to determine if the risk of pregnancy loss was affected by the HLA-C genotype content in the embryo.
**Design:** Retrospective cohort.
**Setting:** Academic research center.
**Patient(s):** Autologous fresh IVF cycles resulting in positive serum $\beta$-hCG during 2009–2014.
**Intervention(s):** None.
**Main outcome measure(s):** 1) Relative risk of pregnancy loss according to maternal KIR genotypes and haplotypes. 2) Comparison of pregnancy loss rates within each KIR haplotype according to HLA-C ligand present in trophectoderm biopsy samples.
**Result(s):** A total of 668 euploid single-embryo transfers with stored maternal DNA and available preamplification DNA from prior trophectoderm biopsy samples were studied. KIR2DS1, KIR3DS1, and KIR2DS5 were more common in patients who experienced pregnancy loss. Carriers of KIR A haplotype exhibited a decreased risk of pregnancy loss compared with KIR B haplotype carriers. However, among KIR A haplotype carriers, the risk of loss was significantly influenced by whether the transferred embryo carried a C1 allele versus no C1 alleles.
**Conclusion(s):** KIR A haplotype carriers experienced fewer pregnancy losses than KIR B haplotype carriers after euploid single-embryo transfer. However, this risk was modified by HLA-C alleles present in the embryo. High-risk combinations (KIR A/homozygous C2 and KIR B/homozygous C1) resulted in a 51% increased risk of loss over all other combinations. (Fertil Steril® 2017;107:677–83. ©2016 by American Society for Reproductive Medicine.)
**Key Words:** Uterine natural killer cells, human leukocyte antigen, pregnancy loss

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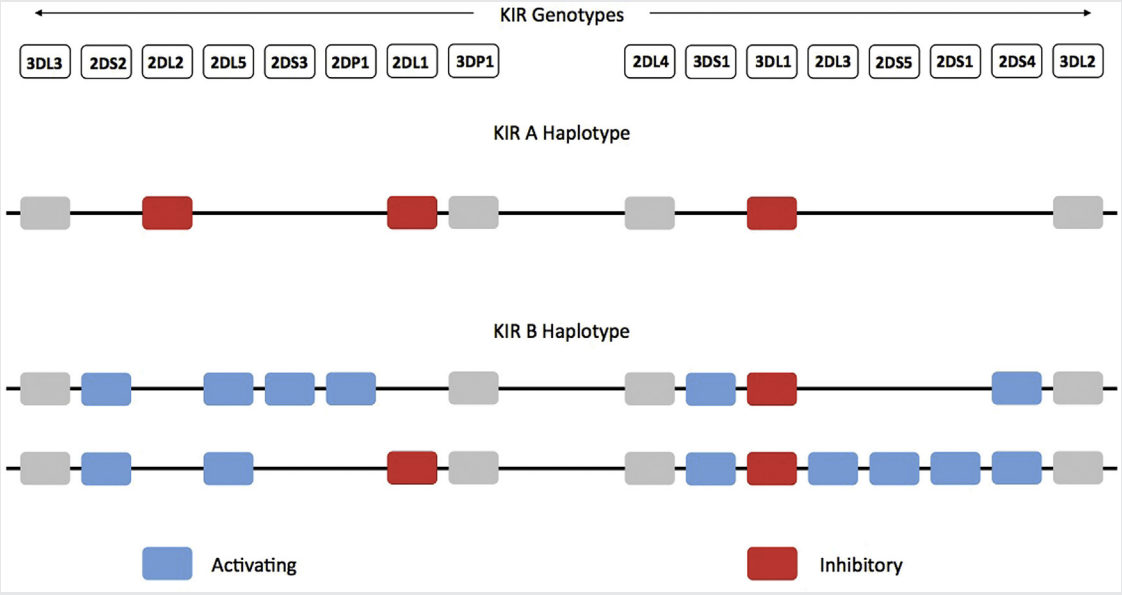
Uterine natural killer cells (UNKs) are the dominant immune cells in the uterine mucosa, accounting for >70% of leukocytes in the secretory endometrium (1). These cells differ radically from peripheral-blood natural killer cells in their cell surface markers and functionality (2, 3). There is strong evidence that UNKs' primary role involves regulating early placentation, because they differentiate and proliferate in the window of implantation (4) and populate the decidua immediately adjacent to the infiltrating extravillous trophoblast (EVT) and around the spiral arterioles during early pregnancy (5). They also secrete a range of factors involved in vascular remodeling and angiogenesis on encountering invading EVT soon after implantation (6). Thus, UNKs appear to play a significant role in establishing normal early placentation through vascular remodeling late in the implantation process.

This hypothesis is further bolstered by evidence of a direct ligand-receptor interaction between EVT and UNKs. The UNKs feature specialized cell surface receptors called killer cell immunoglobulin receptors (KIRs), which bind to human leukocyte antigen (HLA) C ligands present on the EVT. KIRs are encoded by an array of highly polymorphic genes which produce wide variations in KIR structure and differentially influence downstream UNK function. Although many polymorphisms exist, they are classically divided into two categories, activating and inhibitory, based on the extent to which they up-regulate or down-regulate downstream UNK cytotoxic and angiogenic behavior. A given patient carries alleles for multiple KIR genotypes, often from both inhibitory and activating categories. However, the genotypes exist in linkage disequilibrium, such that specific genotypes tend to migrate together (7). Therefore, the KIR genotype profile of a given patient is traditionally categorized into two haplotypes: KIR A (only inhibitory genotypes present) and KIR B (activating haplotypes also present) (8) (Fig. 1).

The ligand for KIRs is the highly polymorphic HLA-C molecule that exists on the EVT. Although hundreds of variants have been described worldwide, HLA-C genotypes are typically organized into two epitopes based on the amino acid present at position 80 (HLA-C1: asparagine; HLA-C2: lysine) (9). Although both HLA-C1 and HLA-C2 bind to KIR receptors, the strength of the downstream UNK response is strongly influenced by the C1 and C2 zygosity of the new pregnancy (Fig. 2). The extreme variability in both the maternal KIR and fetal HLA-C ligands means that each pregnancy will feature a range of different combinations. Therefore, the balance of KIR genotypes in a given patient and the HLA-C exposure in a given pregnancy may influence vascular remodeling and the initiation of normal placentation.

Multiple investigators have examined the impact of the HLA-KIR system on pregnancy outcomes. In a series of studies, Hiby et al. reported that specific KIR genotypes are more commonly seen in patients with morbidities typically attributed to abnormal placentation—recurrent pregnancy loss (RPL), fetal growth restriction, and preeclampsia (10–12)—whereas other KIR genotypes have been suggested to reduce the risk of these disorders (3, 13). Interestingly, in patients with exclusively inhibitory genotypes (KIR A haplotype carriers), the risk of these morbidities may be

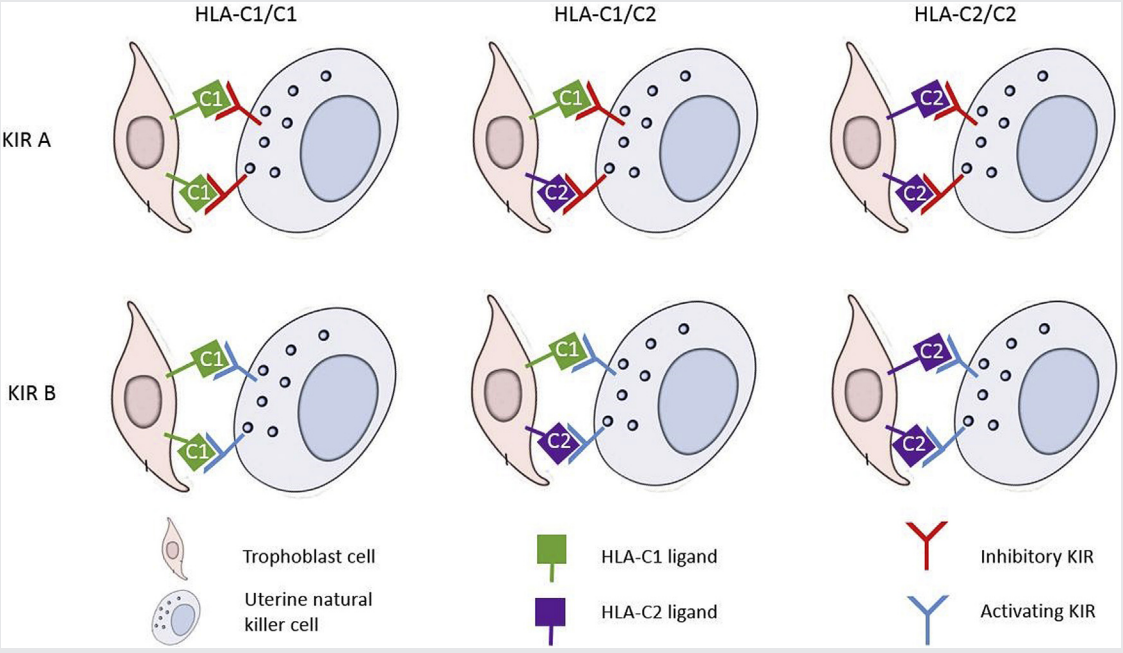
## FIGURE 1




Uterine natural killer cell immunoglobulin receptor (KIR) haplotype determination. Each patient carries multiple KIR genotypes. If all genotypes in a given patient are inhibiting, she is a carrier of the KIR A haplotype. If she carries a combination of inhibitory and activating genotypes, she is a carrier of the KIR B haplotype.

*Morin. KIR haplotype, HLA ligands, and loss risk. Fertil Steril 2016.*

FIGURE 2




Possible combinations of trophoblastic HLA-C ligands and maternal uterine natural killer cell immunoglobulin receptors (KIRs). There are six possible HLA-KIR combinations at the fetomaternal interface, based on trophoblastic HLA zygosity and maternal KIR haplotype.

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exacerbated in the presence of a paternal HLA-C2 ligand in the conceptus (14). Only one study has been performed in the IVF setting. Alecsandru et al. examined a series of patients with recurrent implantation failure or RPL and also demonstrated that carriers of the KIR A haplotype were at increased risk of pregnancy loss compared to KIR B. However, no information on the trophoblastic HLA-C content was reported in that study (15).

Although the above-noted studies provide compelling evidence of a contribution of the HLA-KIR system to abnormal placentation and early pregnancy loss, there are significant limitations to the current literature. First, despite the fact that chromosomal abnormalities account for at least one-half of all miscarriages (16), none of the above studies provides information on the contribution of aneuploidy to the pregnancy losses reported. Second, none of the studies had direct evidence of the HLA-C ligand present on EVT. Instead, all of the studies relied on supposition of HLA-C status based on paternal genotypes. In vitro fertilization with trophectoderm (TE) biopsy offers the unique opportunity to control for both of these issues. Comprehensive chromosomal screening and direct genotyping of the HLA locus can be performed from the same sample.

With this in mind, this study attempted to more robustly characterize the contribution of the HLA-KIR system to early pregnancy loss by controlling for aneuploidy and directly evaluating the HLA-C alleles in TE biopsy samples and comparing pregnancy loss risks within the different KIR haplotype categories.

## MATERIALS AND METHODS

### Population

This was a retrospective cohort study of patients who underwent their first IVF cycles with the use of comprehensive chromosomal screening (CCS) and euploid single-embryo transfer at a single academic-affiliated private practice from March 2009 through July 2015. All patients with stored maternal DNA in an on-site DNA repository (for KIR genotyping) and with residual preamplification genomic DNA from TE biopsy samples (for HLA-C genotyping) were evaluated. These samples were obtained as part of an Institutional Review Board–approved longitudinal study aimed at studying the association of genetic variation with infertility (Copernicus IRB, protocol RMA-00-10). All patients entering treatment were offered the opportunity to participate during the study period and thus this population is representative of the infertile population seen at the clinic during this period.

Only the first embryo transfer for each patient was included in the analysis to eliminate bias introduced by patients with multiple failures. Because the suspected mechanism for pregnancy failure in patients with a deleterious HLA-C/KIR combination is suboptimal remodeling of the spiral arterioles, culminating in pregnancy loss after implantation, only pregnancies that resulted in a positive serum test for $\beta$-hCG were included in the analysis. Any positive pregnancy test that did not proceed to live birth was considered to be a pregnancy loss. Pregnancy losses were further categorized as biochemical losses (defined as pregnancy failure

following positive serum β-hCG but no ultrasound evidence of pregnancy) and clinical losses (defined as pregnancy loss after the sonographic visualization of a gestational sac). Ultrasounds were performed for each patient with a rising β-hCG before 6 weeks' gestation in all patients.

All RPL patients (defined as at least two previous pregnancy losses) were excluded from the analysis owing to the potential for a confounding cause of pregnancy loss. Patients with endometrial thickness <7 mm also were excluded from the study.

## Treatment

All patients underwent IVF care with CCS per usual routine. Of note, CCS is routinely offered to all patients as part of their routine infertility care at this center as a means to increase pregnancy rates per transfer, decrease miscarriage rates, and decrease transfer order. Standard regimens for controlled ovarian hyperstimulation with the use of purified urinary FSH or recombinant FSH and LH activity in the form of low-dose hCG or hMG. Protocols included a GnRH agonist or antagonist for prevention of premature LH surge. Oocyte maturation was induced with the use of recombinant hCG or purified urinary hCG alone or in combination with GnRH agonist when the follicular cohort was deemed to be mature.

Transvaginal oocyte aspiration was performed 36 hours later. Cumulus stripping was performed and all mature oocytes underwent intracytoplasmic sperm injection to avoid contamination when performing CCS. Trophectoderm biopsy was performed per standard protocol on all expanded blastocysts on day 5 or 6. Embryo transfers took place fresh either on day 6 or as part of a subsequent synthetic frozen embryo transfer cycle per standard clinic protocol. Decisions on the use of frozen transfer were made to reduce risk of ovarian hyperstimulation syndrome or to optimize embryo-endometrial synchrony.

## KIR Genotyping

Genomic DNA was extracted from maternal whole blood, stored, and never thawed before analysis. KIR genotyping was performed with the use of the Powerup Sybr Green–based quantitative polymerase chain reaction (PCR). Real-time PCR was performed in a 5-μL reaction mixture containing 2.5 μL 2× Powerup Sybr Green Master Mix (Thermo Fischer Scientific), 0.5 μL forward and reverse primers, and 2 μL genomic DNA at 5 ng/μL. PCR cycling was performed on the Applied Biosystems 7900 Real-Time PCR System. Conditions were set to 2 minutes at 50°C, 10 minutes at 95°C, 40 15-second cycles at 95°C, and 1 minute at 60°C. The cycle threshold for detection of Sybr Green fluorescent signal was 30 cycles.

The following KIR genotypes were analyzed: *2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DP1, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3,* and *3DS1*.

## KIR Haplotype Assignment

Haplotypes were assigned according to the World Health Organization (WHO) Human Genome Organization Subcommittee on KIR Nomenclature (17). More specifically, KIR B haplotype was assigned if any of the following activating genes were present: *KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5,* or *KIR3DS1*. KIR A haplotype was assigned if none of these genes were present. Although previous authors have further categorized KIR haplotypes according to the centromeric and telomeric gene motifs (KIR AA, KIR AB, KIR BB), these subclassifications were not assigned for simplicity's sake and to remain in keeping with the WHO KIR Nomenclature Subcommittee recommendations.

## HLA-C Genotyping

HLA-C genotyping was performed with excess preamplification DNA obtained from TE biopsy samples. The remainder of the DNA obtained from TE biopsy samples had previously been processed for CCS per usual clinic protocol. Preamplification reaction mixture included 25 μL excess DNA, 50 μL Taqman Preamp Master Mix, and 25 μL 0.1× HLA-C SNP Genotyping Assay. PCR cycling conditions were set to 10 minutes at 95°C, 18 cycles of 15 seconds at 95°C, and 4 minutes at 60°C on the Applied Biosystems 2720 Thermal Cycler. PCR was performed in a 5-μL reaction mixture volume containing 2.5 μL of 2× Taqman Universal PCR Master Mix, 0.25 μL 20× HLA-C SNP Genotyping Assay, 1 μL amplified DNA, and 1.25 μL molecular biology–grade water. PCR cycling conditions were set to 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C on the Applied Biosystems PCR System 9700, followed by allelic discrimination on the Applied Biosystems 7900 Real-Time PCR System.

HLA-C calls were made based on zygosity for each embryo. The possible options included C1/C1, C1/C2, and C2/C2. In evaluating cell line control samples containing six cells or more (to recapitulate TE biopsy samples) during assay development, no allelic dropout was observed. Therefore, we anticipated an allelic dropout rate of <1%, which is consistent with previous literature that used a similar methodology (18).

## Data Analysis

Each patient was then assigned a KIR haplotype based on their individual complement of genotypes according to the above criteria. The percentage of pregnancies ending in loss was compared between KIR haplotypes A and B by means of $\chi^2$ analysis. Relative risk (RR) and 95% confidence interval (CI) for pregnancy loss were then calculated by assigning KIR A haplotype as the exposure. The proportions of pregnancies ending in loss within each KIR haplotype were then compared according to the HLA-C alleles present in the TE biopsy specimens by means of $\chi^2$ analysis with Bonferroni correction. A subgroup analysis was also performed by dividing pregnancy losses into biochemical versus clinical losses and comparing among the groups with the use of $\chi^2$ analysis with Bonferroni correction. Owing to evidence of differences in HLA and KIR genotype frequencies in different populations, these same relationships were evaluated according to reported ethnicity (19). Continuous data, such as patient age and number of previous losses, were compared with the use of Student *t* test. A *P* value of $<.05$ was considered to be significant.

## TABLE 1

**Patient demographics.**

| Variable | KIR A | KIR B | *P* value |
|---|---|---|---|
| No. of patients | 176 | 492 | |
| Age (y) | 36.5 (21–46) | 36.2 (22–45) | .46 |
| No. of previous clinical losses | 0.4 (0–1) | 0.5 (0–1) | .66 |
| Frozen embryo transfers | 71.0 (125/176) | 70.1 (345/492) | .61 |
| Fresh embryo transfers | 29.0 (51/176) | 29.9 (147/492) | .61 |
| White | 54.5 (96/176) | 58.3 (286/492) | .88 |
| Asian | 17.0 (30/176) | 20.5 (101/492) | .37 |
| Hispanic | 9.1 (16/176) | 9.3 (46/492) | .99 |
| African American | 5.1 (9/176) | 5.9 (29/492) | .84 |
| Other | 14.2 (25/176) | 6.1 (30/492) | .01 |

*Note:* Values are presented as mean (range) or % (n/n). Between KIR A and B haplotype carriers, there was no significant difference in age, number of previous clinical losses, and proportion of fresh versus frozen embryo transfers. KIR = uterine natural killer cell immunoglobulin receptor.

*Morin. KIR haplotype, HLA ligands, and loss risk. Fertil Steril 2016.*

# RESULTS

## Population Demographics

A total of 668 euploid single embryo transfers resulting in positive serum $\beta$-hCG met the criteria for inclusion in the analysis. The proportions of fresh and frozen embryo transfers were 29.7% (198/668) and 70.3% (470/668), respectively. A live birth resulted from 75.3% (503/668) of positive $\beta$-hCG results, and pregnancy loss resulted from 24.7% (165/668). In total, 176 women (26.3%) were carriers of KIR A haplotype and 492 (73.7%) were carriers of KIR B haplotype. The allele frequencies of C1 and C2 were 61.1% and 38.9%, respectively. The remainder of demographic information is presented in Table 1.

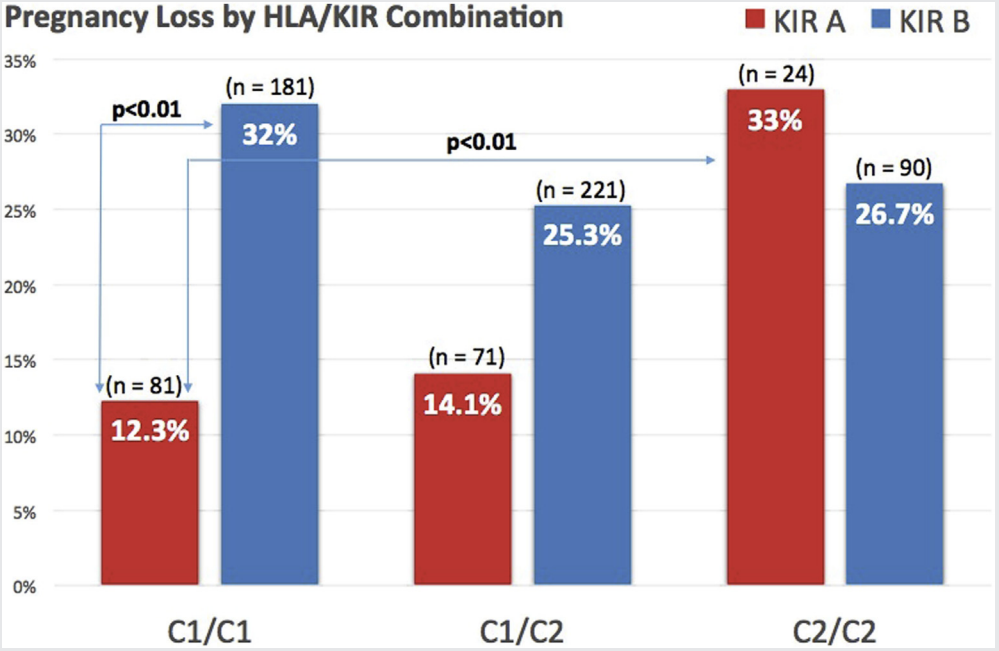
## Pregnancy Outcomes by KIR Haplotype and Embryonic HLA-C Status

Pregnancy outcomes were analyzed from three perspectives: 1) maternal KIR haplotype (activating/inhibiting) status; 2) maternal KIR haplotype status in relation to embryonic HLA status; and 3) embryonic HLA status in relation to maternal KIR haplotype status. The maternal KIR genotype status in relation to pregnancy outcomes was evaluated.

In total, KIR A patients were significantly less likely to experience pregnancy loss than KIR B patients (16% vs. 27.8%; RR 0.57, 95% CI 0.39–0.83; $P<.01$). However, the risk of pregnancy loss within each KIR haplotype was significantly affected by the transferred embryo's HLA-C genotype (Fig. 3).

In KIR A carriers, the proportion of pregnancies that ended in loss was significantly higher when a C2/C2 embryo was transferred than when C1/C1 or C1/C2 embryos were transferred (33% vs. 12.3% vs. 14.1%, respectively; $P<.01$; Fig. 3). In KIR B carriers, the proportion of pregnancies that ended in loss was no different whether the embryo was C2/C2, C1/C1, or C1/C2 (26.7% vs. 32% vs. 25.3%, respectively; $P=.56$).

## FIGURE 3




Pregnancy loss according to HLA-KIR combination. Among KIR A haplotype carriers, the risk of pregnancy loss was significantly higher if the embryo transferred was C2/C2 ($P<.01$). Among C1/C1 embryos, the chance or pregnancy loss was significantly higher if the recipient was a KIR B haplotype carrier ($P<.01$). KIR = uterine natural killer cell immunoglobulin receptor.

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Conversely, the risk of pregnancy loss from an embryonic HLA-C perspective was significantly affected by the KIR haplotype status of the recipient. If an embryo carried any C1 alleles (C1/C1 or C1/C2), the rate of pregnancy loss was significantly lower if the embryo was transferred to a KIR A haplotype carrier (13.2% [20/152] vs. 25.9% [104/402]; $P=.002$).

There were three KIR genotypes that were seen more frequently in patients who experienced a pregnancy loss than in those who delivered (*KIR2DS1*, *KIR2DS5*, and *KIR3DS1*; Supplemental Table 1, available online at www.fertstert.org). All three of these are activating genotypes, which would have yielded a KIR B haplotype. None of the genotypes was found more frequently in patients who delivered. The prevalence of each inhibitory genotype was >89% in both groups.

## Subgroup Analyses

Pregnancy losses were then categorized as biochemical or clinical losses. In total, 48.4% of losses (80/165) were clinical and 51.7% (85/165) were biochemical. There was no difference in the proportion of losses that were clinical in nature between KIR A and B haplotype carriers (50% vs. 47.6%, respectively; $P=.98$). However, the timing of pregnancy loss was influenced by the HLA-C content in the embryos. Pregnancy losses of C1/C1 embryos were more likely to be biochemical losses than clinical losses (66.1% vs. 33.9%, respectively; $P<.001$). In contrast, pregnancy losses of C2/C2 embryos were less likely to be biochemical losses than clinical losses (35.7% vs. 65.4%, respectively; $P<.01$). Pregnancy losses among heterozygous C1/C2 embryos were more evenly distributed (44.3% vs. 55.7%, respectively; $P=.8$).

Given that KIR haplotypes vary by ethnicity, a subgroup analysis of outcomes according to self-reported ethnicity was also performed. KIR A haplotype was associated with a greater risk of loss in white and Asian patients ($P<.05$). There were trends toward increased risks of loss among KIR A haplotype carriers in other self-reported ethnicities, but these differences failed to reach statistical significance. There were insufficient numbers of HLA-KIR combinations in every group to make meaningful comparisons in each ethnic group. These data are presented in Supplemental Table 2 (available online at www.fertstert.org).

# DISCUSSION

This study demonstrates that maternal KIR haplotype influences the risk of pregnancy loss after euploid single-embryo transfer. More importantly, these data suggest strongly that the HLA-C ligand genotype present in the transferred embryo significantly influences this risk. Although earlier studies have hypothesized that this relationship exists, this is the first investigation to demonstrate this phenomenon concretely with direct embryo HLA genotyping data. Furthermore, this is the first study to use aneuploidy screening to better isolate the impact of the HLA-KIR interaction on early pregnancy loss.

These findings represent the largest patient database examining the influence of the HLA-KIR system on early pregnancy loss. These data build upon the findings of earlier studies that suggest that pregnancy loss is significantly influenced by the combination of HLA ligands present on the extravillous trophoblast and the KIR composition on UNKs. Indeed, KIR A haplotype carriers overall experienced a lower rate of pregnancy loss than KIR B haplotype carriers. However, within the KIR A haplotype group, the risk of loss varied significantly according to the embryonic HLA-C ligand complement: Pregnancies with C1/C1 embryos resulted in a 2.5-fold decrease in pregnancy loss rate compared with C2/C2 pregnancies (12.3% vs. 33%, respectively; $P<.01$) in this group. However, the improved outcomes of C1/C1 embryos disappeared if transfer occurred to a KIR B haplotype carrier. The loss rate in those pregnancies was 32%.

The wide variation in loss risk among the various HLA-KIR combinations has significant clinical implications. Deleterious HLA-KIR combinations may contribute to a substantial percentage of euploid pregnancy losses. Pregnancies featuring either a KIR A/homozygous C2 or KIR B/homozygous C1 combination exhibited a 51% increased risk of pregnancy loss compared with all other combinations (32.2% [66/205] vs. 21.3% [99/463]; $P<.01$). These two high-risk combinations were present in 30% of couples.

The observation that KIR A haplotype alone was associated with a decreased risk of pregnancy loss also is notable. These results are consistent with data from Faridi et al. (20). In that study, KIR genotype frequencies were compared between 205 RPL patients and 224 control subjects. Activating genotypes were more common in RPL patients, whereas inhibitory genotypes were more common in control subjects. The activating genotype with the greatest association with recurrent loss in that study was *KIR3DS1*, which was overrepresented in the pregnancy loss group in our study as well. In fact, only activating genotypes were overrepresented in the pregnancy loss group in our study population. These findings are in contrast with those of Alecsandru et al., who reported that pregnancy loss after IVF was more common in KIR A haplotype carriers than in KIR B haplotype carriers (22.8% vs. 11.1%). No data regarding HLA-C status was available in either of the above studies, however.

There are two explanations for these discrepant results. First, no previous studies have accounted for the contribution of aneuploidy on pregnancy loss. Given that aneuploidy is responsible for a substantial proportion of clinical pregnancy losses, it is difficult to isolate the contribution of the HLA-KIR system to pregnancy loss without controlling for aneuploidy. Not only is the present study larger, but considering only euploid embryo transfers improves the resolution of our results. Second, the geographic distribution of earlier studies varies widely. Faridi et al. examined an exclusively North Indian population. Alecsandru et al. included only white Europeans. It is well established that KIR genotype frequencies vary substantially by geographic region and that these variations are linked to HLA-C genotype frequency (19). For example, in Japan, there is an inverse correlation between the KIR A haplotype frequency and the HLA-C2 frequency (7). Using trophectoderm biopsy to directly evaluate embryo HLA-C zygosity and comparing loss risk within KIR haplotype groups results in a more comprehensive evaluation.

It is important to note that the population studied here was different from earlier studies that have investigated the role of the HLA-KIR interaction on miscarriage. All previous studies focused on RPL patients. We chose to focus on patients seeking their first IVF cycle in our center and excluded RPL patients. We sought to study this population as part of an ongoing effort to identify reasons why the transfer of some euploid embryos fails to result in delivery. RPL patients represent a different population of patients than the standard infertile population and may have confounding reasons for RPL. As these relationships become clearer, we intend to focus on RPL patients because they may be at increased risk of deleterious HLA-KIR pairings.

The differential timing of pregnancy loss according to embryonic HLA-C content also raises important questions regarding the temporal relationship of the HLA-KIR interaction during the implantation process. Embryos carrying two C1 alleles experienced a greater number of biochemical pregnancy losses than clinical losses. It is possible that HLA-C1 and HLA-C2 are expressed at different time points during implantation. If so, a suboptimal HLA/KIR interaction involving C1 alleles may fail earlier than those involving C2 alleles. This may also explain why previous studies that examined only clinical losses identified only KIR A/HLA-C2 as a deleterious combination and did not observe any decrement in outcomes in KIR B/HLA-C1 combinations. Further study is required to better delineate whether some very early failures can be explained by the HLA-KIR interaction.

Many patients pursuing traditional IVF care with their own gametes may not have any option but to transfer embryos that result in high-risk HLA-KIR combinations. However, if confirmed in prospective studies, these data may represent an opportunity for embryo prioritization in the case of paternal HLA-C heterozygosity. Avoiding high-risk combinations may reduce the risk not only of pregnancy loss, but also of obstetrical morbidity associated with abnormal placentation. Furthermore, HLA-KIR genotyping may be of particular benefit to patients pursing third-party reproduction. KIR A haplotype carriers with partners carrying a C2 allele may be able to reduce their risk of abnormal placentation by selecting an egg donor carrying a C1 allele. Other patients may be able to select a gestational carrier with a more favorable KIR haplotype based on their own HLA status.

In conclusion, KIR haplotype status appears to influence the risk of pregnancy loss. Furthermore, HLA-C ligands modify the risk and result in wide variations in the rates of pregnancy loss, even after the transfer of a euploid embryo. Although these data require further exploration in prospective studies, this relationship may represent an embryo selection tool to decrease the risk of pregnancy loss.

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## SUPPLEMENTAL TABLE 1

**Gene frequency of individual KIR genotypes in ongoing pregnancies and pregnancies resulting in loss.**

| Gene | Category | Gene frequency in ongoing pregnancies | Gene frequency in pregnancy losses | OR (95% CI) |
|---|---|---|---|---|
| KIR2DS1 | Activating | 41.1 (208/506) | 52.5 (85/162) | 1.58 (1.1–2.3)[a] |
| KIR3DS1 | Activating | 37.7 (191/506) | 48.1 (78/162) | 1.53 (1.07–2.2)[b] |
| KIR2DS5 | Activating | 35.0 (177/506) | 43.8 (71/162) | 1.45 (1.01–2.1)[b] |
| KIR2DS4 | Activating | 93.7 (474/506) | 89.5 (145/162) | 0.58 (0.31–1.07) |
| KIR2DL5 | Activating | 57.1 (289/506) | 60.5 (98/162) | 1.15 (0.8–1.65) |
| KIR2DS2 | Activating | 53.2 (269/506) | 58.6 (95/162) | 1.25 (0.87–1.78) |
| KIR2DS3 | Activating | 34.6 (175/506) | 34.0 (55/162) | 0.97 (0.67–1.41) |
| KIR2DL3 | Activating | 87.5 (443/506) | 90.1 (146/162) | 1.3 (0.73–2.32) |
| KIR2DP1 | Activating | 96.4 (488/506) | 97.5 (157/162) | 1.15 (0.42–3.17) |
| KIR2DL2 | Inhibitory | 53.6 (271/506) | 57.4 (93/162) | 1.17 (0.82–1.67) |
| KIR3DL1 | Inhibitory | 93.9 (475/506) | 90.7 (147/162) | 1.36 (0.34–1.22) |
| KIR2DL1 | Inhibitory | 96.4 (488/506) | 96.9 (157/162) | 1.15 (0.42–3.17) |
| KIR3DL3 | Framework | 100 (506/506) | 100 (162/162) | – |
| KIR3DP1 | Framework | 100 (506/506) | 100 (162/162) | – |
| KIR2DL4 | Framework | 100 (506/506) | 100 (162/162) | – |
| KIR3DL2 | Framework | 100 (506/506) | 100 (162/162) | – |

*Note:* Values are presented as % (n/n), unless noted otherwise. Three activating genotypes (*KIR2DS1*, *KIR2DS5*, and *KIR3DS1*) were more likely to be present in patients experiencing pregnancy loss after euploid single-embryo transfer. CI = confidence interval; KIR = uterine natural killer cell immunoglobulin receptor; OR = odds ratio.

[a] $P < .01$.

[b] $P < .05$.

*Morin. KIR haplotype, HLA ligands, and loss risk. Fertil Steril 2016.*

## SUPPLEMENTAL TABLE 2

**Pregnancy loss risk according to KIR haplotype and HLA-C status within ethnic categories.**

| | KIR A | | | KIR B | | |
|---|---|---|---|---|---|---|
| **Ethnicity** | **C1/C1** | **C1/C2** | **C2/C2** | **C1/C1** | **C1/C2** | **C2/C2** |
| White (n = 382) | 16.7 (16/96) | | | 28.0 (80/286) | | |
| | 16.7 (16/96) | 28.0 (80/286) | 16.7 (16/96) | 28.0 (80/286) | 28.1 (38/135) | 23 (14/61) |
| Asian (n = 131) | 16.7 (16/96) | | | 28.0 (80/286) | | |
| | 16.7 (16/96) | 28.0 (80/286) | 16.7 (16/96) | 28.0 (80/286) | 25 (4/20) | 21.4 (3/14) |
| Hispanic (n = 62) | 12.5 (2/16) | | | 26.1 (12/46) | | |
| | 12.5 (1/8) | 0 (0/6) | 50 (1/2) | 33 (4/12) | 25 (5/25) | 33 (3/9) |
| African American (n = 38) | 22.2 (2/9) | | | 34.5 (10/29) | | |
| | 33 (1/3) | 33 (1/33) | 0 (0/3) | 20 (2/10) | 66.7 (6/9) | 20 (2/10) |
| Other (n = 55) | 16 (4/25) | | | 30 (9/30) | | |
| | 0 (0/6) | 20 (2/10) | 22.2 (2/9) | 38.5 (5/13) | 75 (3/4) | 7.7 (1/13) |

*Note:* Values are presented as % (n/n), unless noted otherwise. White and Asian KIR A haplotype carriers exhibited an increased risk of pregnancy KIR B haplotype carriers. No other associations were identified. KIR = uterine natural killer cell immunoglobulin receptor.

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