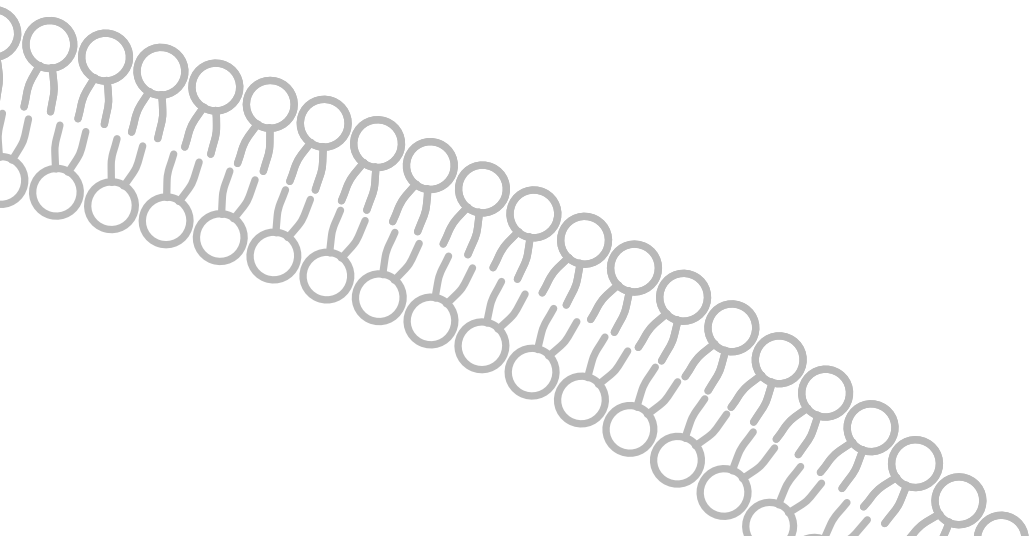
Chapter 6

Selective advantage of HLA matching in successful uncomplicated oocyte donation pregnancies

Lisa Lashley, Geert Haasnoot, Marijke Spruyt, Frans Claas

Submitted for publication



Abstract

Oocyte donation (OD) enables women with various causes of reproductive failure to conceive, but is accompanied with a high risk for certain pregnancy disorders. Possibly, the allogeneic nature of the fetus in OD pregnancies plays a role in the development of these disorders. In this study, we investigated whether there is a selection for some degree of HLA matching in successful and uncomplicated OD pregnancies.

Mothers and children from OD pregnancies that made use of unrelated donors (n=75) were typed for HLA-A,-B,-C,-DR,-DQ and the observed number of HLA matches of the child was compared with the expected number of HLA matches. Moreover, we studied the possibility of a preferential selection for maternal KIR and fetal C combinations.

We observed a significantly higher level of HLA matching between mother and child than expected by chance. Especially the incidence of children with 5 or more HLA matches, which is the situation in normal pregnancy, was higher than expected. A higher level of matching was shown especially for HLA class I, while no significant differences were observed for the individual HLA loci. With respect to maternal KIR and fetal HLA-C no selection for a favourable combination was found.

Larger observational studies including uncomplicated, preeclamptic and aborted pregnancies are essential to determine to what extent HLA matching affects the outcome of OD pregnancies.

Introduction

Oocyte donation (OD) was first introduced in 1984 [1] and enables women with various causes of reproductive failure to conceive. In their latest annual report on assisted reproductive therapies, the European Society of Human Reproduction and Embryology (ESHRE) published data of 20 countries performing oocyte donation [2]. A total of 15825 fresh transfers after OD were performed resulting in 6568 pregnancies, giving a pregnancy rate of 47.4%. This pregnancy rate number is not associated with indication for the oocyte donation [3]. In comparison, the pregnancy rate after IVF transfer is only 33.2% [2]. At a first glance this lower pregnancy rate is surprising, as the techniques are to a large extent similar. However, contrary to the known cause of reproductive failure and reason for OD, for IVF a large portion of patients has unexplained infertility and failure of implantation. Furthermore, although female age is a large predictor for success in IVF [4] and age-related infertility is nowadays the most common indication to perform OD, the pregnancy rate seems to depend on quality and age of the donor [5], rather than age of the uterus [6,7].

Remarkably, the actual delivery rates after OD and IVF are quite comparable which is 29.4% after OD (fresh and thawed) and 25.5% after IVF [2]. This means that a large number of the clinical OD pregnancies do not lead to the birth of a child. While in normal pregnancy the fetus is semi-allogeneic to the mother, oocyte donation may be associated with a complete HLA incompatible pregnancy. The high number of unsuccessful pregnancies after OD may be related to this aberrant HLA-match between the combination of oocyte donor and sperm and the gestational carrier.

In addition, reproductive failure has been associated with the interaction between maternal KIR AA genotype and fetal HLA-C2 [8-10]. This combination is proposed to result in too much inhibition of uterine NK cells leading to inadequate placentation. As a result, the disadvantageous combination is selected against in evolution: populations with high frequency of KIR AA genotype have low frequencies of HLA-C2 alleles and vice veRSM [10]. With the foreign background of the donated oocyte, this protective effect however may not be present, possibly causing a high frequency of spontaneous abortions.

Nevertheless, 27% of all OD embryo transfers result in a successful pregnancy. In this study we tested the hypothesis that these successful pregnancies show less HLA mismatches and less KIR AA/C2 combinations as expected by chance.

Method

Subjects

This study is based on 75 successful pregnancies conceived by oocyte donation, of which the delivery took place between February 2004 and 2014. Exclusion criteria were pregnancies with a gestational age <24 weeks, pregnancies complicated by preeclampsia (gestational hypertension and proteinuria), maternal autoimmune diseases (e.g. SLE or APS) and use of immunosuppressive medication. Medical records were reviewed and clinical data were summarized. Clinical data of the donor and indication for the oocyte donation was unknown to our laboratory. All donors were unrelated to the recipients. The study protocol was approved by the ethics committee of the Leiden University Medical Center (LUMC) and informed consent of every patient was obtained.

HLA typing

Maternal blood samples and, either umbilical cord blood (UCB) after delivery or saliva samples (Oragene Discover, DNA Genotek, USA) from the children were obtained. DNA was extracted using a ChemaGen DNA isolation robot system (PerkinElmer); the loci HLA-A, -B, -C, -DR and -DQB were typed using the Reverse Sequence Specific Oligonucleotides PCR technique. For class I, a commercially available assay was applied (LIFECODES HLA-A,B and C SSO Typing kits from Immucor), HLA-DRB and HLA-DQB typing was performed with a locally developed SSO technique [11].

The maternal and fetal HLA allele frequencies were tested for Hardy-Weinberg equilibrium [12,13]. The Hardy-Weinberg principle states that in absence of admixture or migration, the genotype frequency in a certain population will remain constant from generation to generation.

The number of maternal-fetal HLA matches was calculated at the national reference laboratory for histocompatibility testing (LUMC). On basis of the HLA-A, -B, -C, -DR and -DQ antigens, the maximal number of (mis)matches between mother and child is 10.

Genotyping of maternal KIR was performed for 60 women using the RT PCR SSP (Sequence Specific Primers) technique as described by Gomez-Lozano et al [15]. The KIR genes typed are 2DL1, 2DL2, 2DL3, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1 and 3DS1. We assigned the KIR group B as the presence of 2DL2, 2DL5, 2DS1, 2DS2, 2DS3, 2DS5 or 3DS1; group A was assigned when these genes were absent. The HLA-C1 and HLA-C2 groups were established on basis of the presence of SER77ASN80 (C1) and ASN77LYS80 (C2).

Statistical analysis

The presence of Hardy-Weinberg equilibrium was assessed for both the genotypes of the mother and their children using Pypop Software 0.7.0. All other statistical analyses were performed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) and SPSS Statistics 20 (IBM SPSS Software).

The Woolf-Haldane analysis [16,17] was performed with a control population consisting of 1305 healthy Dutch blood donors. This group is considered to be a proper representation of the HLA gene distribution in the Dutch population [18]. We tested all women with their first pregnancy in our dataset and, in case of twin pregnancies, all firstborn children. Genotypic and phenotypic frequencies were determined by direct counting.

The Chi-Square test was used to examine the observed and expected number of HLA and triplet (mis)matches between mother and child and to examine the observed and expected maternal KIR or HLA-C with fetal HLA-C combination. For all tests a value of $p<0.05$ was defined as significant.

Results

Subjects

The clinical characteristics of the included pregnancies (n=75) are depicted in Table 1. We selected 66 women with their first pregnancy and 9 additional pregnancies derived from the same women. The 75 pregnancies resulted in 89 children, 18.7% of the pregnancies consisted of twin pregnancies. There were no triplet (or higher) pregnancies noted.

Pregnancy (n=75)		
	Gestational age (days)*	273 [178-297]
	Multiple pregnancy (%)	
	- Singleton	81.3
	- Twin	18.7
	Mode of delivery (%)	
	- Vaginally	50.7
	- Caesarean	49.3
Mother (n=66)		
	Age (years)*	40 [27-51]
	Gravidity (number)*	2 [1-7]
	Parity (number)*	0 [0-2]
	Spontaneous Abortions (number)*	0 [0-6]
Child (n=89)		
	Gender (%)	
	- Male	51.4
	- Female	48.6
	Birth weight (gr)*	3440 [754-4775]

Table 1 Clinical data of included subjects *Values are medians with minimum and maximum

Analysis of genotype frequencies

We tested whether the genotype frequencies of the HLA alleles, including the HLA-C1 and –C2 group, from the mothers and their children were in Hardy-Weinberg equilibrium. All homozygote and heterozygote alleles showed a normal distribution.

According to the Woolf-Haldane analysis, the distribution of the HLA antigens in our study group (both women and children) was not significantly different from a control population of Dutch blood donors (n=1305) (data not shown).

Selection for HLA and epitope (mis)matches

We investigated the possibility that a preferential selection for HLA (mis)matching is essential for a successful oocyte donation pregnancy by comparing the observed HLA (mis)matches with expected HLA (mis)matches. The expected number of HLA (mis) matches was calculated by assigning the HLA phenotype of the children randomly to the mothers. In case of twin pregnancy, we calculated the expected and observed number of HLA (mis)matches on basis of the HLA type of the first child.

A significant difference between observed and expected number of HLA matches was observed: mother-child combinations showed a higher level of matching and especially

the incidence of ≥5 HLA matches was higher than expected by chance (Table 2). Additional analyses showed that the level of matching was only significantly higher for HLA class I and not for the number of HLA class II matches (Table 2). Next we studied the level of matching at the individual HLA loci but, despite a trend towards more matching, no significant differences were observed (Table 3). Also for the HLA-C1 and HLA-C2 group, we did not observe more (mis)matching than expected by chance (Table 3).

	Number of HLA matches	Observed*	Expected*	P value
Total	0	7 9.3%	7 9.3%	0.01
	1-2	29 38.7%	28 37.3%	
	3-4	19 25.3%	29 38.7%	
	≥5	20 26.7%	11 14.7%	
Class I	0	13 17.3%	18 24.0%	0.005
	1-2	40 53.4%	45 60.0%	
	≥3	22 29.3%	12 16.0%	
Class II	0	29 38.7%	26 34.7%	0.65
	1	15 20.0%	18 24.0%	
	≥2	31 41.3%	31 41.3%	

Table 2 Observed and expected number of fetal-maternal HLA matches between mother and child (n=75) *Values are numbers of patients and percentages. χ^2 test

	Match	Observed*	Expected*	P value
A-locus	0	39 52.0%	44 58.6%	0.24
	>0	36 48.0%	31 41.3%	
B-locus	0	48 64.0%	54 72.0%	0.12
	>0	27 36.0%	21 28.0%	
C-locus	0	24 32.0%	30 40.0%	0.16
	>0	51 68.0%	45 60.0%	
DR-locus	0	49 65.3%	53 70.6%	0.31
	>0	26 34.6%	22 29.3%	
DQ-locus	0	30 40.0%	28 37.3%	0.63
	>0	45 60.0%	47 62.6%	
C1	0	13 17.3%	16 21.3%	0.39
	>0	62 82.6%	59 78.7%	
C2	0	46 61.3%	47 62.6%	0.81
	>0	29 39.7%	28 37.3%	

Table 3 Observed and expected number of fetal-maternal HLA matches per HLA locus (n=75) *Values are numbers of patients and percentages. χ^2 test

KIR analysis

We analysed the frequencies of maternal KIR haplotype and allele frequency of HLA-C1 and C2 groups in 66 mothers and their children. The frequency of KIR A was 32.8% in the women. The allele frequency of HLA-C1 was 63.6% and 68.2% and of HLA-C2 36.4% and 31.8% in the mothers and children respectively.

We investigated the possibility of a preferential selection for maternal KIR and fetal C combinations resulting in a successful oocyte donation pregnancy by comparing the observed- with expected KIR-C combinations (Table 4). The C group of the fetus was randomly assigned to the mothers to compute the expected KIR-C combinations. We observed no significant difference between observed and expected combinations. Furthermore, we randomly assigned the fetal HLA-C group to the mothers to compute the expected maternal C- fetal C combinations (Table 4). Again, we observed no significant difference between observed and expected combinations.

	Observed*		Expected*	
KIR A/ HLA-C1	14	23.3%	16	26.7%
KIR A/ HLA-C2	12	20.0%	9	15.0%
KIR B/ HLA-C1	39	65.0%	37	61.7%
KIR B/ HLA-C2	20	33.3%	23	38.3%
Maternal C1/ Fetal C1	62	82.9%	59	81.8%
Maternal C1/ Fetal C2	37	47.7%	39	48.9%
Maternal C2/ Fetal C1	41	56.8%	42	56.8%
Maternal C2/ Fetal C2	29	35.2%	28	35.2%

Table 4 Observed and expected KIR/ HLA-C allele combinations (n=60) and observed and expected maternal/ fetal HLA-C allele combinations (n=75) *Values are numbers of patients and percentages. Overall comparison KIR/HLA-C combination χ^2 test p= 0.63 Overall comparison maternal C/ fetal C combination χ^2 test p= 0.95

Discussion

Oocyte donation (OD) is a specific method of artificial reproduction that is accompanied with a higher incidence of early and late obstetrical problems compared to spontaneous and IVF pregnancies [19]. Even after correction for maternal age and multiple gestation there is a higher risk for spontaneous miscarriages, hypertensive disorders during pregnancy, caesarean section deliveries and bleeding complications [20-24]. In this study we examined whether a certain degree of HLA matching is beneficial for the development

of an uncomplicated oocyte donation pregnancy. In addition to HLA matching, we investigated the interaction between maternal KIR and the fetal HLA-C group in uncomplicated OD pregnancies. We observed a significant higher level of HLA matching between mother and child than expected by chance. With respect to maternal KIR and fetal HLA-C no evidence for a favorable combination was found.

Though the indication for oocyte donation was unknown to our laboratory, it is likely that a substantial portion of the subjects underwent OD because of the autoimmune disease premature ovarian failure. Most important genetic factors influencing susceptibility to autoimmune disease are located in the HLA complex and we therefore compared the prevalence of HLA class I (A, B and C) and class II (DR, DQ) alleles of our oocyte donation patients with a control population of healthy Dutch blood donors. We showed no significant differences and in addition, all HLA alleles heterozygotes and homozygotes were found to be in Hardy- Weinberg equilibrium.

In OD pregnancies, the oocyte is donated by a third party and therefore the entire fetal genome could be allogeneic to the mother, resembling the situation of solid organ transplantation [25]. In transplantation settings the degree of human leukocyte antigen (HLA) compatibility between donor and recipient is relevant for graft survival [26] and both donor and recipient are subjected to extensive screening and comprehensive medical follow up. In contrast, an OD pregnancy occurs mostly via an unknown donor, without prior HLA typing and matching, the pregnant women do not receive extra medical care and do not use any immunosuppression [25]. As immunological acceptance of the fetus is often compared with the state of tolerance to an engrafted organ, the recognition of fetal antigens in pregnancy disorders such as miscarriages and hypertensive complications could be viewed as a kind of graft rejection [27]. This would imply that the high incidence of pregnancy complications in OD pregnancies is a consequence of the high level of HLA mismatching. Indeed, a study that analyzed the relationship between donor and recipient by questionnaire showed an increased incidence of hypertensive disorders during pregnancy when the donor was not related to the recipient, compared to a familiar related donor [28]. HLA compatibility between donor and recipient could therefore also be relevant for survival of the fetal allograft in OD pregnancy. In the present study we observed a significantly higher degree of HLA matching between mother and child in uncomplicated OD pregnancies than expected, suggesting that HLA compatibility is beneficial for the development of successful and uncomplicated OD pregnancies. This is in line with observations showing that in spontaneously conceived uncomplicated pregnancies pre-conceptional partner choice is not random, but aims at an optimal number of HLA matches and mismatches [29]. The mechanism of selection most probably works via the olfactory system, whereby women have preference for the odour of HLA dissimilar males [29]. HLA dissimilar mate preferences may suppress inbreeding and increases

offspring heterozygosity at the HLA, potentially advantageous under immune challenges [30]. However, in oocyte donation the artificial situation of complete HLA incompatibility is introduced and the present study suggests a certain degree of HLA matching is beneficial for a successful OD pregnancy. Especially the increased number of cases with 5 or more matches for total HLA suggests that pregnancies with similar immunogenetic differences as normal pregnancies have a selective advantage. We hypothesize that this selective advantage is absent in complicated OD pregnancies and plan to set up an observational study to test whether the level of antigeneic dissimilarity in complicated pregnancies such as preeclampsia and miscarriage is increased in comparison with uncomplicated OD pregnancies.

Nowadays much research is focusing on the interaction between maternal NK cells and fetal HLA-C on reproductive outcome. Hiby et al showed that the lack of maternal activating KIR, which is the case if the mother is homozygous for KIR haplotype A (KIR AA), is associated with an increased risk for preeclampsia and recurrent spontaneous miscarriages (RSM) and that this risk further increases when the fetus is HLA-C2 [8,10]. The frequency of maternal KIR A haplotype in our uncomplicated OD group was 32.8%, which is comparable to frequencies in uncomplicated control populations [8-10]. Also the frequencies of fetal C2 (31.8%) and maternal C2 (36.4%) are comparable to control groups in earlier studies. We compared the observed maternal KIR-fetal C combinations in uncomplicated OD pregnancies with the expected combinations. No significant difference was observed, indicating that the unfavorable KIR AA/C2 is not avoided in uncomplicated OD pregnancies. Intriguingly, Schonkeren et al showed a significantly higher incidence of the fetal HLA-C2 genotype in pregnancies with a distinct lesion in the chorionic plate of uncomplicated OD pregnancies. This lesion was not observed in placentas from preeclamptic OD pregnancies and an immunological protective mechanism against the allogeneic fetus was suggested [31]. Indeed, in the group that showed this lesion the fetal C2 was more often combined with the protective maternal haplotype KIR B [31]. However, the number of patients was small and the frequency of KIR A/ fetal C2 combination should be tested in preeclamptic OD pregnancies, regardless of the chorionic plate lesion, and in spontaneously aborted OD pregnancies.

Moreover, Hiby et al reported an increased risk for women that are confronted with an additional C2 allele that is inherited from the father [9]. For OD pregnancies, there is a possibility of two extra C2 alleles, paternally and donor inherited, increasing the risk for reproductive failure even more. We therefore compared the observed maternal C- fetal C combination with the combination expected by randomly assigning the fetal HLA-C group to the mothers. In uncomplicated OD pregnancies, we showed no significant differences and avoidance of the situation where the fetus has two more C2 genes than the mother (mother C1/C1 fetus C2/C2) seems not required for an uncomplicated OD pregnancies.

In this study we showed a significant higher level of HLA matching between mother and child in successful and uncomplicated OD pregnancies than expected by chance. Whether this degree of HLA compatibility is indeed essential for survival of the pregnancy and whether HLA typing and matching could be of importance to select the optimal donor however remains to elucidated.