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ORIGINAL ARTICLE *Topic*

The experience of two European preimplantation genetic diagnosis centres on human leukocyte antigen typing

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BACKGROUND: Two European centres report on human leukocyte antigen (HLA) typing of preimplantation embryos for haematopoietic stem cell (HSC) transplantation: 'UZ Brussel' in Brussels and 'Genoma' in Rome. Both centres have 6 years' experience with technical and clinical aspects of this type of genetic analysis on single blastomeres.

METHODS: Both centres apply a similar technique for preimplantation HLA typing using short tandem repeats linked to the HLA locus in multiplex PCR for haplotyping.

RESULTS: At present, a conclusive HLA diagnosis could be assured in 92.8% and 90.3% of the embryos at UZ Brussel and at Genoma, respectively. The implantation rates were 32.4% and 28.2%, respectively, and the birth rates per cycle were 9.4% and 18.6%, respectively. The HLA programme at UZ Brussel and at Genoma resulted in the birth of 9 babies and 3 successful HSC transplantations, and 42 babies and 7 successful HSC transplantations, respectively, so far.

CONCLUSIONS: Drastic embryo selection for preimplantation HLA typing (in theory 1/4 for HLA, 1/8 for HLA in combination with sexing for X-linked recessive diseases, 3/16 for HLA in combination with autosomal recessive disorders) resulted overall in the birth of 51 babies (15.9% live birth rate per started cycle) in two European centres.

Key words: preimplantation HLA typing / preimplantation genetic diagnosis / HLA matching / short tandem repeats / live birth rate

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Introduction

Human leukocyte antigens (HLAs) are tissue antigens that play a major role in the immune system. They are responsible for rejection following organ/tissue transplantations. HLA identical cord blood transplantation (CBT) is the best therapeutical option for genetic (e.g. β-thalassaemia) diseases affecting the haematopoietic and/or immune system in children (Gaziev *et al.*, 2000; Locatelli *et al.*, 2003). Exceptionally, HLA identical CBT is also an option for acquired diseases (e.g. leukaemia). If there is no HLA identical donor in the family and if there is no matching unrelated donor available in banks, IVF in combination with genetic analysis of the embryos can be used to select (an) embryo(s) for intrauterine transfer with a view to giving birth to an unaffected HLA matching sibling (Verlinsky *et al.*, 2001). This embryo should be HLA identical in case of acquired disease (25% or 1/4 of the embryos), or HLA identical and unaffected (in case of mutation analysis for autosomal recessive diseases, 19% or 3/16 of the embryos; 12.5% or 1/8 of the embryos in case of sex selection for X-linked recessive diseases). At birth, haematopoietic stem cells (HSCs) can be collected from the cord blood and used to transplant the affected sibling (Bielorai *et al.*, 2004; Grewal *et al.*, 2004; Reichenbach *et al.*, 2008).

HLA typing on one cell is complex because the HLA locus is highly polymorphic and large (4 Mb) and recombination within the locus has been observed (Martin *et al.*, 1995; Malfroy *et al.*, 1997). The development of a reliable single-cell PCR for each couple requesting a matched donor for their affected child would be time-consuming, especially when the HLA typing has to be combined with preimplantation genetic diagnosis (PGD) for a monogenic disease.

Verlinsky *et al*. (2001) were the first to report on HLA typing combined with PGD in a case of Fanconi anaemia in 2001 (Grewal *et al.*, 2004). At that time, allele-specific primers were used for direct typing in combination with one short tandem repeat (STR). A strategy that is based on the presence/absence of an allele may be susceptible to technical errors including allelic drop out (ADO, one of the two alleles is not amplified) and contamination, resulting in misdiagnosis (Sermon *et al.*, 2004). UZ Brussel was the first to report a novel approach for HLA typing using four evenly distributed informative STRs in multiplex PCR on single cells (Van de Velde *et al.*, 2004). The HLA haplotype was indirectly determined by segregation analysis in order to control for recombination within the locus, but also for aneuploidy of chromosome 6, ADO (or preferential amplification) and contamination at the single-cell level. In Italy, Genoma had initially reported on the use of a minisequencing-based genotyping of HLA regions combined with STRs (Fiorentino *et al.*, 2004; Kahraman *et al.*, 2004), but a technique selecting 9–13 STRs from a panel of 50 STRs is currently also used (Fiorentino *et al.*, 2005; Kahraman *et al.*, 2007). At present, the PGD centre in Chicago also uses STRs for indirect HLA typing (Rechitsky *et al.*, 2004, Verlinsky *et al.*, 2004, Kuliev *et al.*, 2005) as do the PGD centres in Detroit (M. Hughes, personal communication) and Sydney (Marshall *et al.*, 2004). In some European countries such as the UK, Spain and France, HLA typing in combination with PGD is allowed, but no reports are available so far. We present the results of 6 years of preimplantation HLA typing in two European centres: UZ Brussel and Genoma (Rome).

Materials and Methods

Couples treated for HLA typing

Between 1 January 2001 and 31 December 2006, 32 couples were treated for HLA typing at UZ Brussel for distinct indications. The major indication for HLA-only typing was leukaemia and the major indication for HLA typing in combination with PGD was sickle cell anaemia. At Genoma, 107 couples were treated for HLA typing; of these the majority was treated for HLA typing in combination with PGD for β-thalassaemia or for HLA-only typing (leukaemia) (Table I).

Haplotyping

The technical details for HLA haplotyping at UZ Brussel (Van de Velde *et al.*, 2004) and at Genoma (Fiorentino *et al.*, 2004; Fiorentino *et al.*, 2005) are described elsewhere. There are differences in methodology between the two centres: at UZ Brussel, up to 7 informative STRs were selected from a panel of 16 STRs (Table II and Fig. 1) for segregation analysis. The multiplex PCR usually consists of two rounds of amplification: in the first amplification round, all primers are added together in the reaction mix; in the second amplification round, the first mix is split into distinct mixtures containing two or three primer combinations. Before a new test is applied clinically, amplification efficiency, ADO and contamination rates are determined on 30–50 single heterozygous lymphoblasts (Sermon *et al.*, 2004).

At Genoma, a panel of 50 different STRs (Table II and Fig. 1) was studied during the set-up phase to ensure the required informativity in all families. The policy was to select, for the subsequent PGD, at least 13 informative STR markers evenly spaced throughout the HLA complex to obtain an accurate haplotyping of the HLA complex, allowing identification of double recombination events, which if not detected may lead to misdiagnosis in HLA typing. A hemi-nested multiplex PCR assay was used to co-amplify all the selected loci. A multiplex PCR was performed in the first round, followed by separate second round PCRs for each locus. In order to evaluate single-cell amplification efficiency, ADO and contamination rates, all primers used were first tested on single heterozygous lymphocytes of the couple.

The number of embryos with a diagnosis was calculated as {(no. of embryos analysed) − (no. of embryos without diagnosis because of a total PCR amplification failure in the single cell or in both cells biopsied)}. Embryos showing monosomy, trisomy or uniparental disomy of chromosome 6 were considered to be abnormal. The number of embryos with a conclusive HLA diagnosis was calculated as {(no. of embryos analysed) − (no. of embryos without amplification) − (no. of abnormal embryos)}. The embryos with recombination were considered to be HLA non-identical.

Clinical data and definitions

Data presented by Genoma are cumulative data because they are derived from seven different ART centres in five countries (Belgium, Greece, Italy, UK and Turkey).

The number of fertilized (2 pronuclei) oocytes and the number of biopsied embryos were calculated on the basis of the total number of mature-injected oocytes. For the outcome of the pregnancies (Zegers-Hochschild *et al.*, 2006), according to ICMART, a clinical pregnancy is 'evidence of pregnancy by clinical or ultrasound parameters (ultrasound visualization of a gestational sac—thus with and without fetal heart beat. It includes ectopic pregnancy. Multiple gestational sacs in one patient are counted as one clinical pregnancy'; miscarriages include preclinical (biochemical) abortions ('an abortion that takes place before clinical or ultrasound evidence of pregnancy'), clinical abortions ('abortion of a clinical pregnancy

Table I Number of families treated at UZ Brussel and at Genoma with distinct indications for HLA typing, clinical pregnancies, babies born alive and CBTs

	UZ Brussel				Genoma				Overall
	Indications	Clinical pregnancies	Babies born alive	CBT	Indications	Clinical pregnancies	Babies born alive	CBT	Babies born alive
HLA typing combined with PGD									
Sickle cell disease	9	2	3		2	0			3
β-Thalassaemia	6	2	1		77	36	31	5	32
Fanconi anaemia	3	1	1	1	1	0			1
Wiskott Aldrich' syndrome	1*	1	1	0[a]	1*	1	1		2
Chronic granulomatous disease	1°	1	1	1[b]	1*	1	2	1	3
Duncan syndrome	1°	0			1*	0			
Hyper IgM syndrome	1	0							
Mannosidosis Alpha					1	0			
Hurler syndrome					1	0			
Gaucher disease					1	0			
Bruton agammaglobulinaemia					1*	1	2		2
Glanzmann thrombasthenia					1	0			
Adrenoleukodystrophy					2*	1	1		1
HLA-only typing									
Acute lymphoblastic leukaemia	7	2	2	1	13	4	3	1	5
Severe aplastic anaemia	3	0							
Diamond Blackfan anaemia					3	3	1		1
Histiocytosis					1	1	1		1
Total	32	9	9	3	107	48	42	7	51

[a]Affected child died.
[b]Bone marrow transplantation.
*PCR.
°Sex selection by FISH.

Table II Panel STR markers used at UZ Brussel and at Genoma

UZ Brussel	Mb	Genoma	Mb
		D6S299	24.0
		D6S276	24.3
D6S1571	25.1		
D6S1260	28.0		
		D6S105	27.9
		D6S306	28.0
		D6S248	28.8
		D6S1624	28.9
		D6S1615	29.0
		D6S258	29.1
		D6S1683	29.3
MOG	29.7	MOG-CA	29.7
		MOG-TAAA	29.7
		RF	29.8
		HLA-F	29.8
HLA-A			
D6S510	30.0	D6S510	30.0
D6S265	30.1	D6S265	30.1
		D6S388	30.4
		HLAC-CA	31.2
		HLABC-CA	31.4
HLA-B			
		MIB	31.5
		MICA	31.6
		62	31.7
		TNFa	31.7
TNFB	31.7	TNFb	31.7
		82-1	31.8
		9N-2	31.8
		D6S273	31.8
D6S2670	32.1		
		D3A	32.2
		LH-1	32.3
HLA-DR			
		DRA-CA	32.5
D6S1666	32.7	D6S1666	32.7
HLA-DQ			
		DQCARII	32.8
		DQCAR	32.8
D6S2443	32.8	D6S2443	32.8
D6S2444	32.8	D6S2444	32.8
		D6S2447	32.8
		G51152	32.9
		TAP1CA	32.9
		D6S2414	33.0
		D6S2445	33.0
		Ring3CA	33.0
		D6S497	33.5
D6S1560	33.7	D6S1560	33.6
D6S1583	33.8	D6S1583	33.8
		D6S1629	33.9
		D6S1568	34.1
D6S1618	34.2		
		D6S439	35.2
		D6S1611	35.4
		D6S1645	35.7
		D6S291	36.4
D6S1680	39.3		
D6S1610	39.4		
		D6S426	40.7

that takes place between the diagnosis of pregnancy and 20 completed weeks' gestational age') and ectopic pregnancies. The implantation rate was calculated as the number of embryos (with and without fetal heart beat) implanted on the total number of embryos transferred. Live birth rate was calculated as 'the number of babies born alive per started cycle; multiple live births are registered as one live birth'.

Results

HLA haplotyping

Both methodologies for HLA typing on preimplantation embryos turned out to be efficient, as shown in Table III representing the overall data as well as the data classified into a category of cells analysed for HLA-only typing and a category for HLA typing in combination with PGD. In cases for HLA typing in combination with PGD, at both centres usually two cells were taken from each embryo for single-cell multiplex PCR. This stringent rule was also followed for HLA typing at the start of the HLA programme, but both centres switched to one cell when they experienced that a conclusive and reliable HLA diagnosis for the embryos could be obtained on the basis of one cell. The percentage of embryos with no amplification was lower at UZ Brussel (0.9%) than at Genoma (5.8%), most likely because less stringent criteria for embryo biopsy were applied at the IVF centres collaborating with Genoma resulting in more embryos without diagnosis. Therefore, in order to compare the efficiency of the HLA typing techniques at the two centres, data were calculated on the basis of the number of embryos that could be diagnosed based on the result of at least one cell with PCR amplification. Fewer couples were treated at UZ Brussel (32 versus 107 at Genoma), and consequently, fewer embryos were tested at UZ Brussel (321 versus 1772 at Genoma), but the percentage of embryos with a conclusive HLA diagnosis was similar (92.8% and 90.3%, respectively). A similar amount of abnormal embryos with monosomies (5.3% and 7.4%, respectively) or trisomies (1.5% and 2.0%, respectively) of chromosome 6 were found. One and five

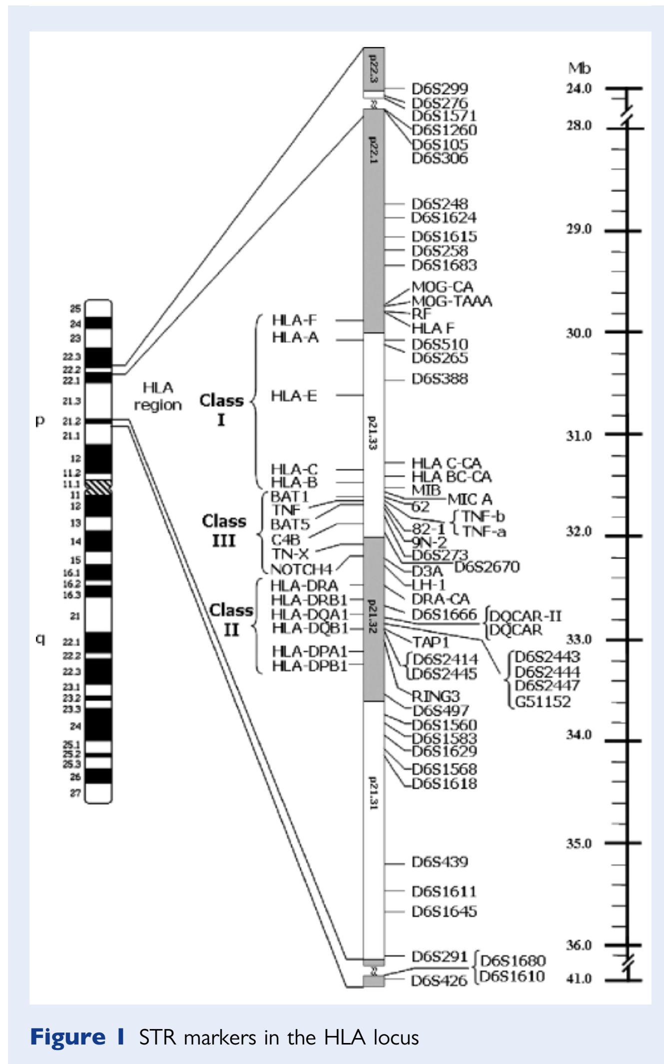

Figure 1 STR markers in the HLA locus

embryos showed uniparental disomy of chromosome 6 at UZ Brussel and at Genoma, respectively. Looking into detail at the embryos with a conclusive diagnosis for HLA, the percentage of HLA identical embryos was 23.5% and 20.7%, respectively, and the percentage of HLA identical unaffected embryos in case of PGD was 16.7% and 15.0%, respectively. A number of embryos showed recombination within the HLA locus (2.3% and 2.1%, respectively). ADO and contamination rates were lower than 0.5% at both centres (data not shown).

Clinical results

Table IV represents the clinical results at UZ Brussel and at Genoma. A subdivision was made between cycles for HLA-only typing on the one hand, and for HLA typing in combination with PGD on the other. Overall, 32 and 107 couples were treated at UZ Brussel and at Genoma, respectively, and 85 and 199 cycles were performed at UZ Brussel and at Genoma, respectively. At Genoma, 77 of 107 couples came for HLA typing in combination with PGD for β-thalassaemia, which is a prevalent disease in Italy and in the Mediterranean countries (Lucarelli *et al*. 2002). The major indication at UZ Brussel was sickle cell disease. The maternal age was 35.9 ± 4.1 and 32.5 ± 5.1 at UZ Brussel and at Genoma, respectively.

The fertilization rate was 68.0% and 88.5% at UZ Brussel and at Genoma, respectively. At UZ Brussel, fewer embryos were analysed (40.3% versus 76.5% of the mature oocytes at the distinct IVF centres collaborating with Genoma). The overall number of embryos analysed per cycle was lower at UZ Brussel (3.8 ± 3.7) when compared with Genoma (9.5 ± 5.9).

At UZ Brussel, overall 34 embryos were transferred in 27 transfer cycles (31.8% of the couples had a transfer). There were 11 HLA identical embryos transferred in the couples for HLA-only typing. In this group, four pregnancies (leukaemia) were obtained: two biochemical and two clinical pregnancies. The clinical pregnancy rate was 6.1% per cycle and 20.0% per transfer, the implantation rate was 18.2%

Table III **Results HLA haplotyping at UZ Brussel and at Genoma**

	UZ Brussel			Genoma			Overall
	HLA + PGD	HLA-only	Total	HLA + PGD	HLA-only	Total	
No. of embryos analysed	218	106	324	1572	309	1881	2205
No. of embryos diagnosed (%)*	216 (99.1)	105 (99.1)	321 (99.1)	1490 (94.8)	282 (91.3)	1772 (94.2)	2093 (94.9)
No. of embryos with conclusive HLA diagnosis (%)$	203 (94.0)	95 (90.5)	298 (92.8)	1356 (91.0)	244 (86.5)	1600 (90.3)	1898 (90.7)
HLA identical embryos (%)§	53 (26.1)	17 (17.9)	70 (23.5)	281 (20.7)	50 (20.5)	331 (20.7)	401 (21.1)
HLA identical healthy embryos (%)§	34 (16.7)			204 (15.0)			
HLA non-identical embryos (%)§	150 (73.9)	78 (82.1)	228 (76.5)	1075 (79.3)	194 (79.5)	1269 (79.3)	1497 (78.9)
Embryos with recombination (%)§	4 (1.9)	3 (3.2)	7 (2.3)	29 (2.1)	5 (2.0)	34 (2.1)	41 (2.2)
Abnormal embryos (%)$	13 (6.0)	10 (9.5)	23 (7.2)	134 (9.0)	38 (13.5)	172 (9.7)	195 (9.3)
Monosomy chromosome 6 (%)$	8 (3.7)	9 (8.6)	17 (5.3)	102 (6.8)	29 (10.3)	131 (7.4)	148 (7.0)
Trisomy chromosome 6 (%)$	4 (1.8)	1 (0.9)	5 (1.5)	28 (1.9)	8 (2.8)	36 (2.0)	41 (2.0)
Uniparental disomy (%)$	1 (0.5)	0	1 (0.3)	4 (0.3)	1 (0.3)	5 (0.3)	6 (0.3)

*Calculated on no. of embryos analysed.
$Calculated on no. of embryos diagnosed.
§Calculated on no. of embryos with conclusive HLA diagnosis.

Table IV Clinical data for HLA typing at UZ Brussel and at Genoma

	UZ Brussel			Genoma			Overall
	HLA + PGD	HLA-only	Total	HLA + PGD	HLA-only	Total	
No. of couples treated	22	10	32	90	17	107	139
Maternal age	35.2 ± 4.2	35.8 ± 3.3	35.9 ± 4.1	31.6 ± 4.8	37.3 ± 3.6	32.6 ± 5.1	33.5 ± 4.9
No. of cycles performed	52	33	85	164	35	199	284
Per couple	2.4 ± 1.3	3.3 ± 2.2	2.6 ± 1.6	1.8 ± 1.2	2.1 ± 1.7	1.9 ± 1.3	2.0 ± 1.4
No. of oocytes retrieved	631	341	972	2655	548	3203	4175
Per cycle	12.1 ± 7.7	10.3 ± 5.4	11.4 ± 6.9	16.2 ± 9.5	15.7 ± 7.9	16.1 ± 9.3	14.7 ± 8.9
No. of mature oocytes injected (%)[$]	525 (83.2)	279 (81.8)	804 (82.7)	2036 (76.7)	424 (77.4)	2460 (76.8)	3264 (78.2)
Per cycle	10.1 ± 6.3	8.4 ± 5.4	9.5 ± 6.0	12.4 ± 7.3	12.1 ± 6.2	12.4 ± 7.1	11.5 ± 6.9
No. of oocytes fertilized (%)[§]	350 (66.6)	197 (70.6)	547 (68.0)	1812 (89.0)	366 (86.3)	2178 (88.5)	2725 (83.5)
Per cycle	6.7 + 5.0	6.0 ± 4.2	6.4 ± 4.7	11.0 ± 6.6	10.5 ± 6.1	10.9 ± 6.5	9.5 ± 6.4
No. of embryos analysed (%)[§]	218 (41.5)	106 (38.0)	324 (40.3)	1572 (77.2)	309 (72.9)	1881 (76.5)	2205 (67.6)
Per cycle	4.2 ± 4.3	3.2 ± 2.5	3.8 ± 3.7	9.6 ± 5.8	8.8 ± 5.5	9.5 ± 5.8	7.8 ± 5.8
No. of transfers (%)	17 (32.7)	10 (30.0)	27 (31.8)	112 (68.3)	26 (74.3)	138 (69.3)	165 (58.0)
No. of embryos transferred	23	11	34	172	44	216	250
No. of pregnancies	7	4	11	47	9	56	67
Clinical	7	2	9	40	8	48	57
Clinical per cycle	13.5%	6.1%	10.6%	24.4%	22.9%	24.1%	20.1%
Clinical per transfer	41.2%	20.0%	33.3%	35.7%	30.8%	34.8%	34.5%
Miscarriages	1	0	1	8*	3	11*	12*
No. of embryos implanted	9	2	11	53	9	62	73
Implantation rate	39.1%	18.2%	32.4%	30.8%	20.5%	28.7%	29.2%
No. of pregnancies went to term	6	2	8	32	5	37	45
No. of babies born	7	2	9	37	5	42	51
Live birth rate per cycle	11.5%	6.1%	9.4%	19.5%	14.3%	18.6%	15.9%

*One ectopic pregnancy.
[$]Calculated on no. of oocytes retrieved.
[§]Calculated on no. of mature oocytes injected.

and two babies were born (6.1% live birth rate) (Tables I and IV). There were 23 HLA identical healthy embryos transferred in couples for HLA typing in combination with PGD. Two HLA non-identical unaffected embryos were transferred into two families with sickle cell disease upon their request; one pregnancy occurred and one healthy baby was born. These two cycles were considered as cycles without transfer. Seven pregnancies were obtained (Tables I and IV): one clinical pregnancy (monozygotic twin and singleton) for β-thalassaemia miscarried at 8 weeks, six pregnancies went to term (clinical pregnancy rate was 13.5% per cycle and 41.2% per transfer, implantation rate was 39.1%). Seven healthy HLA-matched babies were born: singletons for β-thalassaemia, Fanconi anaemia, chronic granulomatous disease (CGD), Wiskott Aldrich's syndrome (WAS) and sickle cell disease and one twin for sickle cell disease. The live birth rate per cycle was 11.5%.

Of the 32 couples included in the treatment at UZ Brussel, nine underwent only one PGD cycle, eight had two, seven had three, five had four, one had five, one had six and one couple had eight attempts. Of the eight couples with a baby born, four had just one cycle, two had two attempts, one had three attempts and one couple had four attempts.

Three HSC transplantations have so far been done successfully (CGD, Fanconi anaemia and leukaemia) (Table I); in some cases, the HSC transplantation has been postponed, and other families are lost for follow-up. In the CGD case also, few mononuclear cells were recovered in the cord blood; therefore, the transplantation was postponed for 1 year in order to aspirate bone marrow (Reichenbach *et al*., 2008). In another family, the affected child (WAS) died a few weeks before the HLA-matched sibling was born.

Concerning Genoma, overall 216 embryos were transferred in 138 transfer cycles (Table IV). A high number of the couples received a transfer (69.3%). In couples for HLA-only typing, nine pregnancies were obtained, one of which was biochemical and three miscarried spontaneously. The clinical pregnancy rate was 22.9% per cycle and 30.8% per transfer, and the implantation rate was 20.5%. Five singleton pregnancies (three for leukaemia, one for Diamond Blackfan anaemia and one for histiocytosis) delivered five HLA-matched children (Tables I and IV); the live birth rate per cycle was 14.3%.

In couples for HLA typing in combination with PGD for monogenic diseases, 47 pregnancies were achieved, seven of which were biochemical and seven miscarried spontaneously before 12 weeks of pregnancy (Tables I and IV). The clinical pregnancy rate was 24.4% per cycle and 35.7% per transfer, respectively; the implantation rate was 30.8%. One pregnancy was ectopic. There were 32 pregnancies which went to term (19.5% live birth rate per cycle). Five twin and 27 singleton pregnancies (28 for β-thalassaemia, 1 for X-linked adrenoleukodystrophy, 1 for Bruton disease, 1 for WAS and 1 for CGD) resulted in the birth of 37 healthy children, which were confirmed to be HLA identical to their affected sibling by blood samples HLA testing.

Of the 107 couples included in the treatment, 58 underwent only one PGD cycle, 25 had two, 10 had three, 8 had four, 3 had five and 2 couples had seven attempts. Of the 37 couples with positive outcome, 22 had just one cycle, 7 had two, 3 had three, 2 had four, 2 had five and 1 couple had seven attempts.

Haematopoietic stem cells collected after delivery from the umbilical cord blood were transplanted to the affected siblings of seven couples, resulting in a successful haematopoietic reconstruction for all patients (Table I); information on HSC transplantation in the other families is not available at the moment.

Overall, in the two European centres, 139 couples were treated in 284 cycles and 51 healthy HLA-matched babies were born (15.9% live birth rate) (Tables I and IV).

Discussion

HLA haplotyping

We present the results of 6 years of preimplantation HLA typing at UZ Brussel and at Genoma (Rome). Overall, the clinical success rate is acceptable, since 90% of the embryos get a correct HLA diagnosis and the live birth rate per cycle is 15.9%.

Although the methodologies for HLA typing slightly differ between the two European centres, the efficiency in obtaining a conclusive HLA diagnosis is comparable to the results at the Chicago centre (Kuliev *et al*., 2005). Around 2% of the embryos with a conclusive diagnosis show recombination within the HLA locus, which is higher than the 0.3–1% described within the locus (Martin *et al*., 1995; Malfroy *et al*., 1997), but comparable with data from Chicago (Rechitsky *et al*., 2004; Verlinsky *et al*., 2004; Kuliev *et al*., 2005). Monosomies and trisomies as well as maternal heterodisomy of chromosome 6 could be observed. All this implies that using informative STRs that are evenly distributed over the locus for segregation analysis is a good strategy for performing a correct HLA typing on preimplantation embryos. For practical and cost-effective reasons as well as for clinical and psychological reasons, it is important that the time to develop a family-specific PCR protocol should be as short as possible. The use of STR markers enables this goal to be reached. Worldwide, HLA testing on preimplantation embryos is now routinely performed using STRs. The centres in Rome and Chicago combine PGD/HLA with aneuploidy screening for reproductive age (Rechitsky *et al*., 2004; Verlinsky *et al*., 2004; Fiorentino *et al*., 2005; Kuliev *et al*., 2005), but the Brussels centre does not offer this routinely to their patients because the benefits of this technique to improve the success rate have not been unequivocally established (Staessen *et al*., 2004). So far, no misdiagnosis for HLA typing has been officially reported. Based on the results from UZ Brussel and Genoma, we estimate the risk to be <0.5%.

The cumulative live birth rate was twice as high at the centres collaborating with Genoma when compared with UZ Brussel (18.6% versus 9.4%). Comparing results from different IVF and PGD centres is difficult, and the number of couples treated at UZ Brussel was smaller, which may bias the results. Since haplotyping efficiency (90.3% versus 92.8%), availability of HLA identical embryos (20.7% versus 23.5%) or HLA identical healthy embryos in case of PGD (15.0% versus 16.7%) were similar, this could not explain the difference in live birth rate between the centres. Since the implantation capacities were also similar (28.2% versus 32.4%), we suggest that the higher live birth rate at the centres collaborating with Genoma is due to the higher number of mature oocytes available for injection. The number of mature oocytes mainly depends on the hormonal stimulation protocols used, but this is difficult to compare between the centres. Additionally, the fertilization rate and live birth rate were low at UZ Brussel and lower than in the general UZ Brussel

PGD population (Goossens *et al.*, 2008; a study performed during a similar period of time); we suggest that the impaired results for HLA are due to a number of couples (11/32) with repetitive cycles with poor fertilization and/or cleavage. Consequently, there were more embryos available for testing at Genoma than at UZ Brussel (9.5 ± 5.9 versus 3.8 ± 3.7 per cycle). Finally, respectively, 10.3% and 6.3% of the mature oocytes for Genoma and UZ Brussel could be used for transfer, resulting in a higher transfer rate and a higher live birth rate for Genoma.

Ethical and legal considerations

The application of PGD for HLA typing has generated a heated ethical discussion (Pennings *et al.*, 2002; Robertson, 2004; de Wert, 2005; de Wert *et al.* 2007). The first main ethical objection is the selection (and possibly destruction) of embryos on the basis of a feature that does not affect the health of the future child. The idea is that embryo selection is only permitted to prevent harm to the future child. Any selection on the basis of non-pathological characteristics, whether or not combined with testing for genetic diseases, is condemned. The argument in favour of HLA typing is that it is an exception, which can be justified by the fact that it might save the life of another child. The second ethical objection is that the future child would become an instrument to cure another child. By planning to conceive a child for this purpose, we would not fully respect the child as an autonomous person. However, if there were an existing HLA-matched sibling, we would find it acceptable to use that child as a donor of HSCs. Unless using a child as a donor were the sole reason for creating another child, there would be no violation of the child's autonomy.

Because of legal, ethical and most likely financial/technical restrictions, there are worldwide only a limited number of PGD centres offering HLA typing on human preimplantation embryos. Generally speaking, the legal position on HLA typing is determined by the attitude towards PGD. The new Belgian law on medically assisted reproduction includes a special section regulating the use of PGD for HLA typing. The law stipulates two conditions: the selection must have a therapeutic benefit for an existing child of the parents and the fertility centre must evaluate the request to make sure that the desire to have a child is not solely motivated by the therapeutic goal (Pennings, 2007). In Italy, since March 2004, the law regulating IVF techniques imposes very strict conditions (Benagiano and Gianaroli, 2004). According to the text of the new regulation, performing genetic testing on embryos is not illegal. However, the obligation to transfer all embryos, including those shown to be affected by the genetic disease, makes PGD useless. The Roman PGD centre has therefore to collaborate with seven distinct European IVF centres and limits its activity to genetic diagnosis on single cells only, which is not forbidden by the law.

Conclusion

Overall, a 15.9% live birth rate per cycle after drastic genetic selection for HLA in the two European centres is acceptable, but efforts could still be made to improve and individualize hormonal stimulation protocols in order to obtain more and/or better quality oocytes that may result in higher fertilization and biopsy rates.

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