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ORIGINAL ARTICLE ***Reproductive genetics***

The clinical utility of PGD with HLA matching: a collaborative multi-centre ESHRE study

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STUDY QUESTION: Has PGD-HLA been successful relative to diagnostic and clinical efficacy?

SUMMARY ANSWER: The diagnostic efficacy of PGD-HLA protocols was found lower in this study in comparison to published PGD-HLA protocols and to that reported for general PGD by ESHRE (78.5 vs 94.1% and vs 92.6%, respectively), while the clinical efficacy has proven very difficult to assess due to inadequate follow-up of both the ART/PGD and HSCT procedure outcomes.

WHAT IS KNOWN ALREADY: The first clinical cases for PGD-HLA were reported in 2001. It is now a well-established procedure, with an increasing number of cycles performed every year. However, PGD-HLA is still offered by relatively few PGD centres, the currently available data is fragmented and most reports on PGD-HLA applications are limited in number and scope. Published systematic details on methodology, diagnostic results, overall ART success and haematopoietic stem cell transplantation (HSCT) outcomes are limited, precluding an evaluation of the true clinical utility of PGD-HLA cycles.

STUDY DESIGN, SIZE, DURATION: This retrospective multi-centre cohort study aimed to investigate the diagnostic and clinical efficacy of the PGD-HLA procedure and the aspects of PGD-HLA cycles influencing positive outcomes: birth of genetically suitable donor-baby (or babies) and HSCT. In April 2014, 32 PGD centres (Consortium members and non-members) with published/known PGD-HLA activity were invited to participate. Between February and September 2015, 14 centres submitted their data, through a custom-designed secure database, with unique login access for each centre. Data parameters covered all aspects of PGD-HLA cycles (ART, embryology and genetic diagnosis), donor-babies born and HSCT.

PARTICIPANTS/MATERIALS, SETTING, METHODS: From 716 cycles submitted by 14 centres (performed between August 2001 and September 2015), the quality evaluation excluded 12 cycles, leaving 704, from 364 couples. The online database, based on REDCap, a free, secure, web-based data-capture application, was customized by Centre for Clinical Epidemiology and Outcomes Research (CLEO),

Athens. Continuous variables are presented using mean, standard deviation, median and interquartile range, and categorical variables are presented as absolute and relative frequencies.

MAIN RESULTS AND THE ROLE OF CHANCE: The data included 704 HLA-PGD cycles. Mean maternal age was 33.5 years. Most couples (81.3%) requested HLA-typing with concurrent exclusion of a single monogenic disease (58.6% for beta-thalassaemia). In 92.5% couples, both partners were fertile, with an average 1.93 HLA-PGD cycles/couple. Overall, 9751 oocytes were retrieved (13.9/cycle) and 5532 embryos were analysed (7.9/cycle). Most cycles involved fresh oocytes (94.9%) and Day 3 embryo biopsy (85.3%). In 97.5% of cycles, the genotyping method involved PCR only. Of 4343 embryos diagnosed (78.5% of analysed embryos), 677 were genetically suitable (15.4% of those analysed for HLA alone, 11.6% of those analysed for HLA with exclusion of monogenic disease). Of the 364 couples, 56.6% achieved an embryo transfer (ET) and 598 embryos were transferred in 382 cycles, leading to 164 HCG-positive pregnancies (pregnancy rate/ET 41.3%, pregnancy rate/initiated cycle 23.3%) and 136 babies born (live birth rate/ET 34.3%, live birth rate/initiated cycle 19.3%) to 113 couples. Data analysis identified the following limitations to the overall success of the HLA-PGD procedure: the age of the mother undergoing the treatment cycle, the number of oocytes collected per cycle and genetic chance. HSCT was reported for 57 cases, of which 64.9% involved combined umbilical cord-blood and bone marrow transplantation from the HLA-identical sibling donor; 77.3% of transplants reported no complications.

LIMITATIONS REASONS FOR CAUTION: The findings of the study may be limited as not all PGD centres with PGD-HLA experience participated. Reporting bias on completion of the online database may be another potential limitation. Furthermore, the study is based on retrospective data collection from centres with variable practices and strategies for ART, embryology and genetic diagnosis.

WIDER IMPLICATIONS OF THE FINDINGS: This is the first multi-centre study evaluating the clinical utility of PGD-HLA, indicating variations in practice and outcomes throughout 15 years and between centres. The study highlights parameters important for positive outcomes and provides important information for both scientists and couples interested in initiating a cycle. Above all, the study underlines the need for better collaboration between all specialists involved in the ART-PGD/HLA procedure, as well as the need for comprehensive and prospective long-term data collection, and encourages all specialists to aim to properly evaluate and follow-up all procedures, with the ultimate aim to promote best practice and encourage patient informed decision making.

STUDY FUNDING/COMPETING INTEREST(S): The study wishes to acknowledge ESHRE for funding the customization of the REDCap database. There are no competing interests.

TRIAL REGISTRATION NUMBER: N/A.

Key words: preimplantation HLA typing / sibling donor / haematopoietic stem cell transplantation / HSCT / HLA matched donor / PGD with HLA

Introduction

Over the last 27 years, PGD has often been the topic of ethical debates. One of its most controversial applications was reported in 2001 and involved HLA typing of preimplantation embryos (Devolder, 2005; Shenfield *et al.*, 2005). The procedure aimed to achieve treatment of an affected child in need of haematopoietic stem cell transplantation (HSCT) by establishing a pregnancy with an HLA-compatible embryo (Verlinsky *et al.*, 2001). PGD-HLA can be performed as a sole indication when the affected child requires transplantation to treat an acquired disease related to the haematopoietic and/or immune system, or, alternatively, in combination with PGD to exclude the familial monogenic disorder and concurrently avoid the risk of producing another affected child. The procedure is regulated and viewed very differently in various countries, and in some countries it is only considered acceptable when it is combined with the exclusion of a monogenic disease. The fundamental clinical value of PGD-HLA is that it enables HSCT with a matched related donor, a procedure which shows superior outcomes to all other procedures using allogeneic HSCT. HSCT with a matched related donor is associated with fewer complications, higher survival rates and better outcomes than when using a related haploidentical or mismatched unrelated donor, but is also superior even in comparison to HSCT with a matched unrelated donor (Gale and Eapen, 2015; Kindwall-Keller and Ballen, 2017). This is important considering that only 30% of patients are able to find a suitable donor within their family (Besse *et al.*, 2016). With the aim of providing the optimum donor, PGD-HLA has become a well-established option. However, it is a complex procedure that requires close collaboration between specialists of many different disciplines (Kakourou *et al.*, 2017).

Based on the annual data collections of the ESHRE PGD Consortium, overall, more than 600 PGD-HLA cycles have been reported that have led to the birth of over 100 babies, however, only 15% of ESHRE member-centres offer PGD-HLA (De Rycke *et al.*, 2015). Considering the entire PGD-HLA HSCT procedure, assuming that a healthy matched embryo has been successfully detected, implanted and a baby born, the ultimate benchmark for the success of the procedure should be defined by having the affected sibling cured by HSCT. This valuable information is thus far missing from the ESHRE data and has only been reported in isolated centre series and several individual cases (Kahraman *et al.*, 2011, 2014; Rechitsky *et al.*, 2015; Kurekci *et al.*, 2017). The true clinical utility of PGD-HLA cycles performed to date has been difficult to evaluate due to the limited availability of published details on PGD-HLA methodologies, diagnostic results, overall success of the ART and finally the HSCT outcomes.

This is the first multi-centre cohort study attempting to define how often PGD-HLA achieves the ultimate clinical utility, which is to cure a

sick child, following selection and birth of an HLA-compatible sibling. The study aimed to provide answers to the following questions:

- Has PGD-HLA been successful in terms of diagnostic and clinical efficacy?
- Which parameters of assisted-reproduction, embryology and genetic diagnosis influence the likelihood of donor-live-born baby/babies and a HSCT treatment for an affected sibling?

Furthermore, the findings of the study highlight variations in practice between different centres and outcomes throughout 15 years of PGD-HLA application, from 2001 to 2015.

Materials and Methods

Study design

The study was initiated in April 2014 when the ESHRE-PGD Consortium established a PGD-HLA working group, which approached, by email, 32 PGD centres, with published or known PGD-HLA activity, to invite them to participate in this retrospective multi-centre study. The centres approached included both members of the PGD Consortium and non-members.

Study data were collected and managed using REDCap electronic data capture tools hosted at the Centre for Clinical Epidemiology and Outcomes Research (CLEO), Athens, Greece (Harris *et al.*, 2009). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies (https://projectredcap.org/, 25 January 2018, date last accessed). A unique login access code was given for each centre. The data covered aspects of ART treatment, embryology, genetic diagnosis, donor-babies born and transplantation procedure. The database fields were agreed by members of the PGD Consortium HLA working group (Table I). Fields relevant to patient demographic details, ART cycle details and embryo diagnosis results were mandatory for submission of data, while all remaining fields remained optional as initial data collection suggested that only partial data was available on pregnancy, baby and HSCT follow-up. All centres, however, endeavoured to obtain as much information as possible for all performed cycles. From February to September 2015, participating centres submitted their data on PGD-HLA cycles performed from 2001 to 2015.

Data analysis

Data management and statistical analysis was conducted in STATA SE v.11 and was performed by the CLEO centre, Athens, Greece. The quality of all submitted data was evaluated for missing and inconsistent data and clarifications were requested through re-contacting the relevant participating centres. Following re-contact, data inconsistencies were corrected and cycles with missing information were excluded from further analysis. Continuous variables were presented using mean, standard deviation, median and interquartile range, and categorical variables were presented as absolute and relative frequencies. Evaluation of associations between variables was performed with both parametric and non-parametric tests, depending on whether normality was held or not, respectively. Steps of the data analysis are presented in Table II.

Results

Study participants

Of the 32 centres that were contacted and offered to participate in this study, only 14 agreed to participate. Reasons for denying participation were not always clear, despite several attempts to communicate with the corresponding centres. Some of the known reasons were inability to complete the required database fields (lack of information, lack of follow-up), lack of time and desire to separately publish the centre's experience.

Participating centres were from Turkey, Spain, Greece, United Arab Emirates, Sweden, Israel, Portugal, Hong Kong, Poland and France, in order of contribution with regards to the number of PGD-HLA cycles submitted. A total of 716 cycles were submitted by the 14 participating centres. Quality evaluation excluded 12 cycles due to significant missing data. The final analysis was performed on 704 cycles from 364 couples undergoing PGD-HLA. The number of cycles per country is as indicated in Fig. 1. The majority of cycles (70%) were contributed by a single centre, the Istanbul Memorial Hospital in Turkey. This centre-to-centre imbalance with regards to the reported PGD-HLA cycles is in proportion to the imbalance of cycles reported to be performed amongst the invited centres.

For the majority of participating centres, the ART unit and genetics laboratory were in the same institution, and PGD was performed in-house. Two of the participating centres performed transport PGD, i.e. ART procedures including embryo biopsy were performed in one location and the biopsied cells were shipped to a genetics laboratory for analysis.

Results from data analysis are indicated below for each of the main database fields from Table I.

Family history and indications

Mean maternal age was 33.5 years (range: 17.6–48.2). The highest and lowest maternal age, representing the extremes of the entire cohort, were from a single centre (Supplementary Table SI).

In the overwhelming majority of patients (94.2%) both or one of the partners was certainly fertile, in 0.5% of the couples both partners were infertile, while in 1.4% of the couples, at least one partner was infertile and the other one had unknown fertility status. For 3.9% of couples, the fertility status for both partners was unknown.

The majority (81.3%) of couples (296 couples, 585 cycles) requested HLA-typing with concurrent exclusion of a single monogenic disease and the remaining 18.7% of couples (68 couples, 119 cycles) requested HLA typing to achieve an HLA matched and related donor for a child with an acquired disorder.

Indications for PGD-HLA were recorded for 700 cycles. For the remaining four cycles PGD was performed for HLA-typing only, although the specific indication was not recorded. Out of the 700 cycles, the most common indication for requesting HLA-typing was to treat beta-thalassaemia (58.6% of couples, 61.6% of cycles), followed by acute lymphoid leukaemia (8.5% of couples, 7.1% of cycles) and alpha-thalassaemia (4.6% of couples, 4% of cycles). There was one case with HLA-matching along with PGD to exclude two familial single gene disorders (beta-thalassaemia and sideroblastic anaemia), the details of which have been previously reported in the literature (Kakourou *et al.*, 2016). Overall, the study included referrals for 42 different indications as summarized in Table III.

Details of cycles

Each couple performed on average 1.93 HLA-PGD cycles (range: 1–14). Overall, 9751 oocytes were retrieved, 5532 embryos were

Table I List of fields included in the database.

Family history and indication	
1	Record ID
2	Patient ID
3	Centre ID
4	HLA alone (yes/no)
5	HLA indication (HLA plus any other indication, e.g. two SGDs or PGS) (other indication should be specified)
6	Monogenic disease (autosomal dominant, autosomal recessive or X-linked) (specify OMIM)
7	Mother's age (dob)
8	Mother's fertility status (fertile, infertile or unknown)
9	Father's fertility status (fertile, infertile or unknown)
10	Number of affected children
11	Number of unaffected children
12	Age of candidate BMT recipient at start of PGD-HLA request
Details of cycle	
13	Cycle ID
14	Year of genetic analysis
15	Number of fresh oocytes
16	Number of oocytes (specify whether fresh, frozen, mixed or unknown)
17	Number of embryos analysed
18	Number of thawed embryos analysed
19	Biopsy stage (specify whether polar body, Day 3, blastocyst or mixed)
20	Number of HLA matched embryos (for HLA only protocols)
21	Number of HLA unmatched embryos (for HLA only protocols)
22	Number of HLA matched and unaffected embryos
23	Number of HLA matched and affected embryos
24	Number of HLA unmatched and unaffected embryos
25	Number of HLA unmatched and affected embryos
26	Number of embryos with inconclusive diagnosis
27	Number of embryos inconclusive or undiagnosed
28	Number of embryos inconclusive or undiagnosed: specify reason: technical failure, amplification failure, embryo aneuploidy or other (specify 'other')
29	Embryo transfer (yes/no)
30	Number of HLA matched embryos frozen (for HLA only protocols)
31	Number of HLA unmatched embryos frozen (for HLA only protocols)
32	Number of HLA matched and unaffected embryos frozen
33	Number of HLA unmatched and unaffected embryos frozen
34	Date of transfer
35	Genetic status of embryo 1 transferred (specify whether HLA matched, HLA matched and unaffected, HLA unmatched, HLA unmatched and unaffected)
36	Genetic status of embryo 2 transferred (specify whether HLA matched, HLA matched and unaffected, HLA unmatched, HLA unmatched and unaffected)
37	Genetic status of embryo 3 transferred (specify whether HLA matched, HLA matched and unaffected, HLA unmatched, HLA unmatched and unaffected)
38	HCG positive (specify yes, no, unknown)
39	Foetal heart beat positive (yes, no, unknown)
40	Number of foetal heartbeats
41	Pregnancy lost (yes, no)
42	Number of babies born
43	Confirmation of PGD diagnosis (specify prenatal, postnatal)
44	Genetic diagnosis confirmed for monogenic disease for embryo 1 (yes/no)
45	Genetic diagnosis confirmed for monogenic disease for embryo 2 (yes/no)
46	Genetic diagnosis confirmed for monogenic disease for embryo 3 (yes/no)
47	Genetic diagnosis confirmed as concordant for HLA typing for embryo 1 (yes/no)
48	Genetic diagnosis confirmed as concordant for HLA typing for embryo 2 (yes/no)
49	Genetic diagnosis confirmed as concordant for HLA typing for embryo 3 (yes/no)
	Fields 34–49 should be completed for any additional embryo transfers performed from a single cycle
Genotyping analysis for cycle	
50	Genotyping method (specify whether PCR only, WGA + PCR, karyomapping or NGS)
51	Was PGS also performed (yes/no)
52	PGS method (if performed) (specify whether aCGH, karyomapping, NGS)
53	Number of informative or semi-informative HLA markers included in PGD protocol
54	Number of markers upstream to HLA-A
55	Number of markers between HLA-A and HLA-B
56	Number of markers between HLA-B and HLA-DRA
57	Number of markers between HLA-DRA and HLA-DQB1
58	Number of markers downstream to DQB1
59	Number of embryos with recombination in the HLA region (out of all embryos analysed)
60	Position of recombination (specify whether maternal, paternal and between which markers recombination occurred, fill in the name of the marker and coordinates, include gene build)
Transplantation procedure	
61	Transplantation centre (specify name)
62	Disease category for cure (leukaemia, monogenic or other)
63	Specify disease for cure
64	Type of transplant (cord blood, bone marrow or both)
65	Age of donor at transplantation
66	Age of recipient at transplantation
67	Transplantation complications (none, graft-versus-host disease, rejection, other)
68	'Other' complications of the transplantation procedure (specify)
69	Timing of transplantation complications
70	Length of follow up post the transplantation procedure

Table II Parameters of data analysis.

	Parameters investigated
Basic data analysis	PGD-HLA indications, number of couples and cycles, average maternal age, number of attempts per couple, results of ART stimulation, embryo diagnosis, embryo transfer, pregnancy and live birth rate
Sub-analysis 1	Comparative analysis of PGD-HLA cycles performed before and after 2012 (2011 being the year when guidelines for PGD-HLA typing were published and newer PGD methodologies were introduced)
Sub-analysis 2	Comparative analysis of cycles between the centre that contributed the largest number of cycles (495 cycles) vs all remaining centres (209).
Sub-analysis 3	Identification of factors that impact on the overall success of the procedure
	Details of genetic analysis, detection of recombination

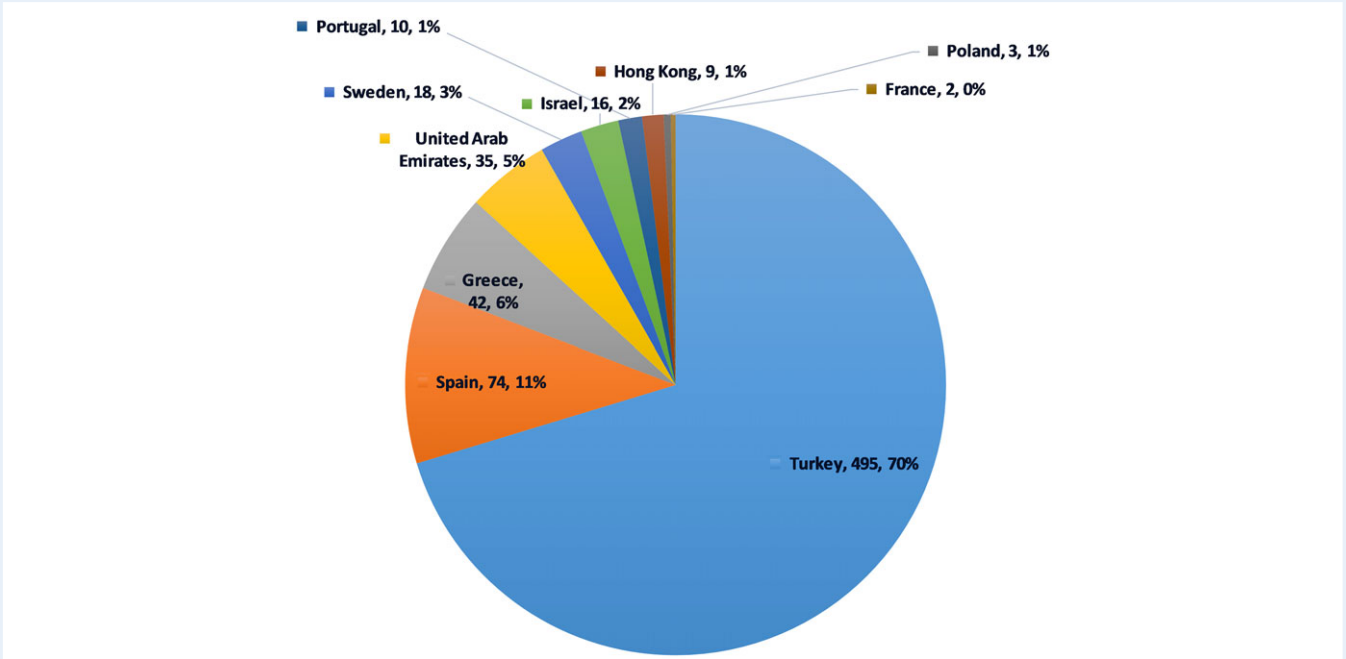

Figure 1 Number of contributed cycles per country.

analysed and 4343 embryos had characterized genotypes (78.5% of embryos analysed). The average number of oocytes retrieved per cycle was 13.9 ± 8.2 SD (range: 0–46), the average number of embryos analysed per cycle was 7.9 ± 4.9 SD (range: 0–25) and the average number of embryos with characterized genotypes per cycle was 6.26 ± 4.02 SD. All but one of the participating centres reported that they do not have a policy to obtain a minimum number of oocytes before initiating the PGD cycle. One centre reported a policy of obtaining at least 10 follicles for all PGD cycles; this centre only contributed two cycles to the complete data set.

The majority of reported cycles involved collection and fertilization of fresh oocytes (94.9%), although use of frozen oocytes (1.1% of cycles), use of a mixture of fresh and frozen oocytes (3% of cycles) or use of oocytes of unknown status (1%) was also reported.

Information on the embryo biopsy stage was available for 702 of the cycles performed. The majority of cycles involved blastomere biopsy on Day 3 of development (599 cycles, representing 85.3% of cycles). Trophectoderm biopsies were performed in 40 cycles (5.7%), polar body biopsy was performed in one cycle (0.1%) and a combination of mixed stages for biopsy was reported in 62 cycles (8.9%).

A total of 677 embryos were genetically suitable for transfer. Of the embryos analysed, 3.6% had gone through cryopreservation and thawing prior to diagnosis.

Information on the achievement of embryo transfer (ET) was available for 341 couples of whom, 56.6% had an ET and 43.4% did not.

The number of transferred embryos per couple, ranged from 1 to 12 embryos. Of the couples with a transfer, 38% of couples had one embryo transferred, 32.9% of couples had two embryos transferred, 12.7% of couples had three embryos transferred and 6% of couples had four embryos transferred. The remaining 10.3% of couples had between 5 and 12 embryos transferred. The 12 embryos were transferred in a couple through a total of five PGD-HLA cycles.

Of the cycles where at least one genetically suitable embryo was identified, 96% had an ET. Overall, 598 embryos were transferred, of

Table III Indications for preimplantation HLA matching in this study.

Indication	Number of cycles	Number of couples
Acute lymphoid leukaemia	50	31
Acute myeloid leukaemia	19	11
Adrenoleukodystrophy	7	5
Alpha mannosidosis	4	1
Alpha thalassaemia	28	17
Anaplastic large cell lymphoma	3	1
Aplastic anaemia	3	3
Beta thalassaemia	431	214
Beta-thalassaemia and sideroblastic anaemia	4	1
Burkitt's lymphoma	2	1
CD3 deficiency	1	1
Chronic myeloid leukaemia	7	4
Congenital dyserythropoietic anaemia	1	1
Diamond Blackfan anaemia	22	10
Fanconi anaemia (FANCA, FANCD2-8)	25	14
Gaucher disease	5	1
Glanzmann thrombasthenia	4	3
Hemophagocytic lymphohistiocytosis	1	1
Haemophilia A (F8)	3	2
Histiocytosis	3	1
Hurler syndrome	2	2
Hyper-IgD syndrome	1	1
Hyper-IgM type 1, X-linked	5	3
Juvenile myelomonocytic leukaemia	2	2
Kostmann syndrome	2	2
Leukaemia	2	2
Leucocyte adhesion deficiency, type III FERMT3	2	1
Lymphoproliferative syndrome, X-linked,	3	1
MHC type II deficiency	2	1
Mucopolysaccharidosis type I	3	2
Mucopolysaccharidosis type VII	3	1
Myelodysplastic syndrome	2	1
Neuroblastoma	4	2
Neutropenia, severe congenital	4	1
Non-hodgkin lymphoma	1	1
Porphyria, congenital erythropoietic	2	1
Pyruvate kinase deficiency	3	2
Schwachman Diamond	3	2
SCID due to adenosine deaminase deficiency	2	1
sickle cell anaemia	10	8
Wiskott–Aldrich syndrome	4	3
X-linked adrenoleukodystrophy	15	2
Total	700	365

which 92.8% were genetically suitable (matched embryos) and 7.3% were unmatched embryos. Unmatched embryos were transferred in 27 cycles alone or along with matched embryos. This led to the birth of seven babies of which five were unmatched (see data on live births below).

There were no records of cryopreservation of unmatched unaffected or inconclusive embryos.

As the majority of biopsy procedures were performed on Day 3, the majority of ETs were performed on Day 4 (51.7%) or Day 5 (39.4%), with a very limited number of transfers (3.2%) performed on Day 6. In 5.7% of ETs, the day of transfer was not reported.

The 397 ETs led to 164 HCG-positive pregnancies (pregnancy rate/ET 41.3%, pregnancy rate/initiated cycle 23.3%), 160 foetal heartbeats (FBH/embryo transferred: 26.7%, FHB/ET: 40.3%) and 136 babies born, representing a live birth rate (LBR) per cycle initiated of 19.3% and LBR per ET of 34.3% (Fig. 2). All submitted cycles have been completed with regards to the potential of delivering a donor child, with no pending pregnancies remaining. Overall, 31% of couples (113 couples) were reported to have achieved a baby following the ART/PGD-HLA procedure, 58 of them (51%) following their first ART/PGD-HLA cycle.

The number of babies born corresponds to 2.45% of all embryos analysed. Of the couples who achieved at least one live birth, maternal age was on average 31.5 years (range: 18–48), while for the couples with ET and no live birth, maternal age was 33 years (range: 22–48). There were 40 reported cases of a lost pregnancy where the age of the mothers ranged between 23 and 41 years (average 34 years).

Genotyping analysis of cycles

Information on the genotyping methodology applied was available for 486 cycles. In 97.5% of cycles, the genotyping method involved PCR only and 2.5% involved whole genome amplification (WGA) with subsequent PCR analysis. No cycles of karyomapping or next-generation sequencing (NGS) were reported in this data set. All diagnostic protocols applied fulfilled the ESHRE PGD Consortium best-practice guidelines for amplification-based PGD, with regards to amplification efficiency of at least 90% and allele dropout rates of <10% (Harton *et al.*, 2011).

Of 4343 embryos diagnosed (78.5% of embryos analysed), 677 were genetically suitable (HLA-matched), representing 15.4% of those analysed for HLA alone and 11.6% of those analysed for HLA along with exclusion of monogenic disease (Table IV).

Of the cycles, 51% had up to three embryos with inconclusive diagnosis, the main reasons being amplification failure or indication of chromosome aneuploidy. Other reported reasons for inconclusive results were high allele dropout rate, biopsy of anucleate cells or cell-lysis during biopsy, contamination with extraneous DNA, discordant results (in cases where two cells from a single embryo were tested), detection of recombination in the HLA region, uninformative STRs in the family and low DNA quality leading to very low signal of STR peaks.

There were six cycles with concurrent embryo aneuploidy screening (PGS) for women of 33–46 years old (average 38 years). One of these PGS cycles involved FISH for chromosomes 13, 15, 16, 17, 18, 21, 22, X and Y and the remaining PGS cycles involved aCGH analysis,

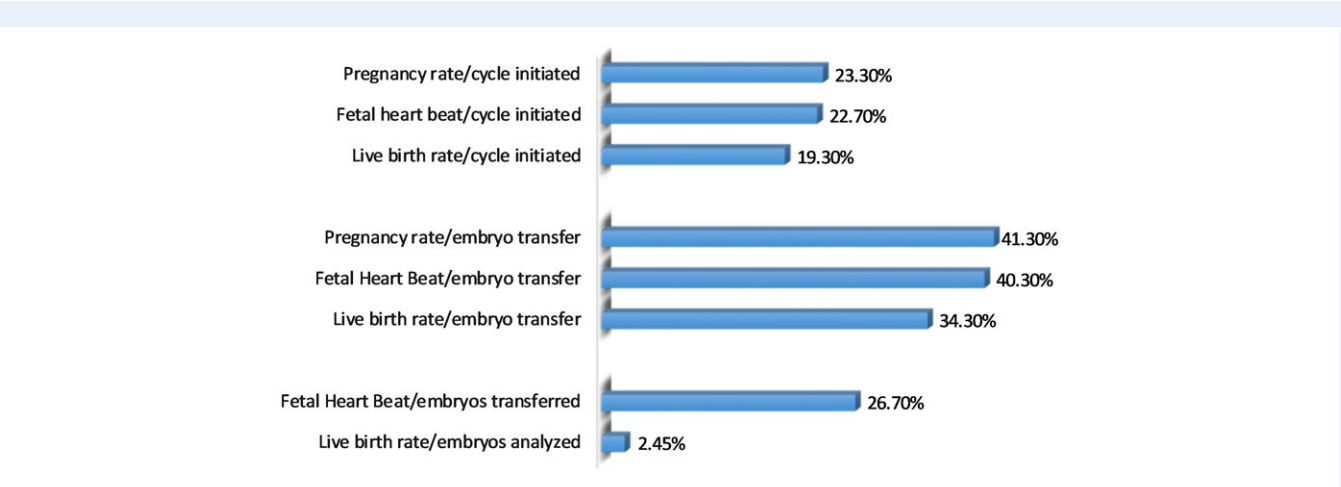

Figure 2 Results from all (HLA-only and PGD-HLA) cycles included in the analysis.

Table IV Number of transferable embryos per indication for preimplantation HLA typing.

	Indication				
	HLA only	**HLA with single cell disorder[a]**	**Autosomal dominant**	**Autosomal recessive**	**X-linked disorder**
Number of embryos analysed	920	4612	60	4243	253
Number of HLA-matched	142				
Unaffected		535	8	492	28
Affected		224	1	205	11
Number of non-HLA matched embryos	603				
Unaffected		2036	27	1881	111
Affected		803	12	719	58
Incomplete/inconclusive diagnosis	175	1014	12	946	45
Number of transferable embryos/total number of embryos analysed	142/920 (15.4%)	535/4612 (11.6%)	8/60 (13.3%)	492/4243 (11.6%)	28/253 (11.1%)
Number of transferable embryos/total number of embryos with complete diagnosis	142/745 (19.1%)	535/3598 (14.9%)	8/48 (16.6%)	492/3297 (14.9%)	28/208 (13.5%)

[a]Includes 56 additional embryos that were tested for HLA matching with two single gene disorders that have been previously reported.

employing the Illumina SurePlex DNA amplification System and 24sure V3 arrays. Overall, these six cycles included 42 embryos, of which only four were genetically suitable for transfer (9.5%), 28 were genetically unsuitable for transfer (66.7%), four were not considered for transfer due to detection of aneuploidy (9.5%) and six embryos had failure of amplification (inconclusive) (14.3%).

For haplotyping of the HLA region, 11 polymorphic STRs were utilized on average spanning the HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ regions. Some cycles had no markers in some key HLA regions (range per region was 0–7 STRs). Only 57% of cycles had at least one marker in each of the HLA regions as recommended on the published guidelines for preimplantation HLA haplotyping (Harton *et al.*, 2011).

There were five cycles where polymorphic HLA STRs were not used as HLA typing was performed directly on single blastomeres using sequence specific primers following WGA.

Where confirmation of PGD-HLA diagnosis was performed, it was mostly during pregnancy (83%) but also postnatal (17%). One misdiagnosis was reported at prenatal diagnosis testing, which involved the HLA haplotyping in a β-thalassaemia-HLA case.

Information on HLA haplotypes was provided for 2832 embryos and recombination within the HLA region was detected in 99 embryos (3.5%). Further information was provided for 85 of these 99 embryos with recombination, of which 51 indicated recombination on the maternal allele and 30 on the paternal allele, while four embryos indicated recombination on both maternal and paternal alleles. A double recombination event was detected in four embryos, two with maternal and two with paternal recombination. A complex case with nine recombination events detected in three embryos from the same PGD cycle was noted but no further details were provided. The distance between the two reported markers within which recombination was detected

ranged from 25 kb to 5.8 Mb for both maternal and paternal recorded events.

Most recombination events were noted in the region downstream to HLA-DQB1 (21 embryos) and upstream of HLA-A (12 embryos), but overall, recombination events were detected throughout the whole HLA region. There were 11 couples (average maternal age 32 years) whose embryos (32 embryos overall) indicated recombination in more than one PGD cycle. For four of these couples, recombination events were solely maternal, for three other patients recombination events were paternal, while three patients had both maternal and paternal recombination events in their embryos; further information on recombination was unavailable for one case.

Transplantation procedure

HSCT was performed on average within approximately two years of PGD request. HSCT was reported for 57 out of the 364 children in need of transplantation (50% of cases with at least one baby born) for the following indications (number of cases): acute lymphoid leukaemia (1), acute myeloid leukaemia (1), adrenoleukodystrophy (2), beta thalassaemia (39), Diamond Blackfan anaemia, (3) Fanconi anaemia (3), Glanzmann thrombasthenia (1), Kostmann syndrome (1), mucopolysaccharidosis type I (1), sickle cell anaemia (1), Wiskott–Aldrich syndrome (3), X-linked lymphoproliferative syndrome (1). The majority of HSCTs (52/57, 91.2%) were reported by Istanbul Memorial Hospital, Turkey.

Of the 57 cases, stem cells were sourced from umbilical cord blood (UCB) in three cases, bone marrow (BM) was used in 17 cases, while 37 cases (64.9%) involved a combined UCB and BM transplantation from the HLA-identical sibling donor. Follow-up was available for 44 out of the 57 HSCT. Of these, no complications were reported in 34 (77.3%), graft-versus-host disease (GvHD) was reported in one (2.27%), rejection was reported in one (2.27%) and additional complications were reported in eight (18.2%) of the cases. The mean BMT recipient age was 8 years and the mean BMT donor age was 23.5 months. Details on the transplantation centres where HSCT was performed were not provided for any of the procedures reported.

Additional analyses

Factors affecting procedure outcome

Important factors influencing the outcome of the procedure were found to be maternal age, number of oocytes and number of STRs used for diagnosis. Each of these parameters was separately investigated with regards to procedure outcome. There was a significant negative correlation between maternal age and number of embryos analysed (rho = −0.283, $P < 0.001$) and transferred (rho = −0.146, $P = 0.001$). Maternal age and number of oocytes collected per cycle were significantly associated with the number of babies born and the chance of an ET ($P < 0.05$, Supplementary Table SII). PGD-HLA cycles that led to a live birth following fresh ET had on average 17 fresh oocytes collected per cycle, in comparison to the remaining cycles, where 13 oocytes were retrieved on average.

The number of STRs included in the diagnostic protocol was significantly positively correlated with the number of embryos definitively characterized/diagnosed (rho = 0.157, $P = 0.001$).

PGD-HLA cycles performed prior to and post PGD-HLA guidelines publication

When comparing the results from cycles performed before and after 2012 (the year after the ESHRE PGD guidelines were published), there were significantly more cycles and more oocytes collected per couple, more embryos analysed per cycle and more cycles with an ET achieved in cycles prior to 2012 ($P < 0.05$). However, there was no difference with regards to the pregnancy rate and number of babies born (Supplementary Table SIII).

Before 2012, 477 cycles were performed and 227 cycles were performed after 2012. With respect to the number of informative STRs used for HLA haplotyping, the details submitted were incomplete for 235 cycles performed before 2012 but for the remaining 242 cycles, an average of 10 STRs were included in the PGD protocol (range: 4–19). For cycles performed after 2012, 11 STRs were used on average in the PGD protocol (range: 4–18) in 187 cycles, five cycles did not proceed to HLA haplotyping (the embryos were first characterized for the monogenic disease and were genetically unsuitable or undiagnosed) and for 35 cycles information was not provided.

Comparison between the centre with the highest number of cycles and the remaining centres

The majority of data on PGD-HLA cycles (495/704 cycles) was contributed from a single centre (Istanbul Memorial Hospital, Turkey). Comparison of the data from this centre with pooled data from the remaining centres demonstrates that this centre had significantly younger mothers, more oocytes and more embryos analysed and diagnosed and a lower number of cycles per couple ($P < 0.05$ for all comparisons). Furthermore they had a higher percentage of cycles with transfer, a higher pregnancy rate, and an overall higher number of cycles with at least one baby born, although these latter parameters were not significantly different. This centre also had a higher percentage of cycles requesting HLA alone ($P < 0.05$, Supplementary Table SIV).

Comparison of HLA-PGD with monogenic disease exclusion and HLA-only cases

When comparing the HLA alone and PGD-HLA cycles, there were no statistically significant differences detected (Supplementary Table SV).

Discussion

The present study is the first multi-centre collaboration to evaluate the diagnostic and clinical utility of the PGD-HLA procedure and attempt to identify parameters that may potentially influence the procedure outcome by investigating data from 15 years of PGD-HLA practice from 14 different centres. The data represents a valuable information resource for both health practitioners and couples interested in initiating a cycle.

The diagnostic and clinical efficacy of the PGD-HLA procedure was found to be lower in this study than that reported for general PGD by ESHRE (diagnosis: 78.5 vs 92.6% and pregnancy rate/cycle initiated 23.3 vs 25%) but once ET is achieved, the LBR/ET was comparable with the general ESHRE PGD data (34.3 vs 34%). In addition, in comparison to other published PGD-HLA protocols (rather than general PGD), diagnostic efficacy in this study was again found to be lower

(78.5 vs 89.5–94.1%) (De Rycke et al., 2015; Calhaz-Jorge et al., 2016).

Evaluation of true clinical efficacy was limited as the participating centres had incomplete data on patient follow-up following PGD, such that the overall success of the procedure may not be accurately evaluated. Partial data involves the ET outcome (fresh transfer or subsequent transfer from cryopreserved embryos), the pregnancy outcome and eventually the HSCT treatment. Consequently the 136 children (for 113 couples) reported in this study, may be an underrepresentation. In addition, information on HSCT was only provided for 57 of these 113 couples. Out of the remaining 56 cases, the family is still waiting for HSCT in 17 cases, most commonly because cord blood was not sufficient for treatment so the donor child needs to come to a certain age for BMT, in six cases the affected child passed away before HSCT was attempted, in 11 cases HSCT was not required as the affected child was in remission, in seven cases the patient could not be reached, in two cases the affected child received a transplant from an outsource donor. In three cases, the children born were unmatched (following intentional transfer of knowingly unmatched embryos). For the remaining 10 cases, the reasons remained unspecified.

Data analysis identified the following factors that may influence cycle success: (i) the age of the mother undergoing the treatment cycle, (ii) the number of oocytes collected per cycle and (iii) genetic chance. Each of these parameters was separately investigated with regards to procedure outcome, however, the potential that maternal age and number of oocytes collected may not be independent limitations cannot be excluded, and is indeed quite likely.

With respect to maternal age there was a significant negative correlation between maternal age and number of embryos analysed (rho = −0.283, $P < 0.001$) and transferred (rho = −0.146, $P = 0.001$). Maternal age and number of oocytes collected per cycle were also significantly correlated with the number of babies born and the chance of an ET. With respect to genetic chance, the probability that embryos will be HLA-matched with the sibling awaiting HSCT is 25%. If a familial monogenic disease needs to be excluded, then the genetic chance drops to 18.8% for an autosomal recessive or X-linked recessive disease and 12.5% for an autosomal dominant disease. Despite the decreased chance of identifying a transferable embryo in a PGD-HLA case, in comparison to general PGD, the majority of participating centres (with the exception of one centre that contributed only two cycles to the data cohort) confirmed that they do not have a policy for obtaining a minimum number of oocytes before a PGD-HLA cycle. Having such a policy would imply that in cases of poor ovarian response the patient would potentially need to undergo additional stimulation cycles, which may not always be possible for medical or financial reasons.

All participating centres have underlined the importance of adequate evidence-based patient counselling and the patient's informed consent prior to initiating the PGD-HLA procedure, to ensure that the couple appreciates their chances of success. Counselling should provide information on the potential ART, PGD, HLA and HSCT risks and complications and the procedural limitations, based on response to stimulation, biopsy technique and genetic chance, and the time required to initiate a cycle, achieve a pregnancy and reach the stage of HSCT, underlining the fact that a low percentage of patients will eventually achieve transplantation (Kakourou et al., 2017).

With respect to the actual diagnostic approach, the number of STRs used for HLA-haplotyping seems to significantly affect whether a diagnostic result is achieved. The robustness of the STR linkage approach is strongly correlated with the number of STR markers used for HLA haplotyping, so for optimal clinical utility protocols have to be highly multiplexed to test many loci simultaneously. The HLA-haplotyping result can also be compromised by recombination with the HLA locus on chromosome 6p. A recombination rate of 3.5% within the HLA region was observed in this study. However, it should be emphasized that the methodologies applied may not always detect all cases of recombination and that in the literature over 4% recombination has been reported within the HLA region (Rechitsky et al., 2004; Little et al., 2016). It would be interesting to assess data following other methodologies, such as karyomapping (Handyside et al., 2010). The latter, as previously mentioned, was not reflected in our data set and on a more recent contact with participating centres, none of them reported to have switched to a different methodology for HLA haplotyping.

There was one case of misdiagnosis in this data set, which involved a cycle performed in 2008 that has previously been reported in the literature, where the baby born was unaffected for β-thalassaemia but not HLA matched (Kahraman et al., 2014). Based on data from the ESHRE PGD Consortium, the misdiagnosis rate in PGD has generally been very low (<0.1%), and there are no misdiagnoses reported in the latest ESHRE data set (unpublished data). However, the chance of misdiagnosis should always be discussed and clearly communicated to the couples as part of the informed consent process for the PGD procedure. As the risk cannot be eliminated, it is advisable to perform prenatal diagnosis for every ART/PGD-HLA pregnancy.

Several potential limitations of the study must be clarified. First of all, low participation rate (fewer than 50% of the invited centres agreed to participate in the study), potentially introducing non-response bias, the potential of reporting bias through individual centre completion of the online database and the partial nature of the data, as is often observed when data collection is performed retrospectively. A strong point of the trial, however, that merits highlighting is that we encouraged participation of centres with variable practices and strategies for ART, embryology and genetic diagnosis. The combined results can be compared with those from single centres performing a large number of PGD-HLA cycles that were published after this study was initiated, although such comparison may not be accurate as the precise details on many aspects (e.g. indication) are not similarly presented in all studies (Table V) (Van de Velde et al., 2009; Kahraman et al., 2011, 2014; De Rycke et al., 2015; Rechitsky et al., 2015; Kakourou et al., 2017).

Another major limitation of the study was that the majority of PGD-HLA cycles were contributed by a single centre (Istanbul Memorial Hospital, Turkey). A comparison of data from these 495 cycles and the remaining 209 cycles contributed by the 13 different centres indicates that the former had a statistically significant number of younger mothers, statistically significant more oocytes, more embryos analysed and diagnosed, a higher percentage of cycles with transfer, a higher pregnancy rate, and overall higher number of cycles with at least one baby born. It may be that the experience of this centre's both clinical and laboratory staff, plays a major role in the overall outcome (potentially, maintaining optimal culture conditions, biopsy techniques and quality controls for both the embryology and the genetics laboratory). This centre also potentially has a strategy to initiate the procedure in a

Table V Data from the main studies to date on clinical experience of preimplantation HLA-typing with or without concurrent exclusion of a single gene disorder (PGD-HLA and HLA-only). Data referring to the HLA-only cases is underlined in the table. Table adapted from Kakourou *et al.* (2017).

PGD-HLA or HLA-only cases	Van de Velde *et al.* (2009) (data from two centres)		ESHRE PGD Consortium data collection De Rycke *et al.* (2015)	Kahraman *et al.* (2011)	Kahraman *et al.* (2014)	Rechitsky *et al.* (2015)	This study
Period of study	2001–2006		2010	2003–2010	2003–2013	Not specified	2001–2015
Number of couples	22	90	n/a	136	192	79	296
	10	17		35	50	19	68
Number of cycles	52	164	72	262	371	180	585
	33	35	36	65	90	44	119
Maternal age	35.2 ± 4.2	31.6 ± 4.8	35	32 ± 4.83	31.9 ± 4.7	Not specified	33.1
	35.8 ± 3.3	37.3 ± 3.6	33	34.2 ± 5.65	34.1 ± 5.7		34.5
Cycles to embryo transfer (%)	32.7	68.3	33	63.4	57.1	61.7	54.9
	30	74.3	21	70.8	70	68.2	63.9
Number of clinical pregnancies/ oocyte retrieval (%)	13.5	24.4	21	22.9	21.6	18.3	22.2
	6.1	22.9	28	21.5	25.6	15.9	28.6
Clinical pregnancy rate/embryo transfer (%)	41.2	35.7	45	36.1	37.7	29.7	40.5
	20	30.8	48	30.4	36.5	23.3	44.7
Live birth rate/cycle initiated (%)	11.5	19.5	19	16.4	16.2	12.8	15
	6.1	14.3	22	13.8	22.2	13.6	21

timely and efficient way (by counselling and referral of parents soon after diagnosis in the affected child) or optimize the ART treatment to achieve the maximum number of oocytes employing individualized ovarian stimulation. These are potential approaches that should be considered by centres offering ART/PGD to support further optimization of treatment outcome.

The future in both ART and PGD looks promising, through the continuous advances with regards to the hormonal stimulation protocols, gamete/embryo manipulation and culture conditions and the methodologies used for genetic analysis as well as the improved criteria for embryo selection. The application of next generation sequencing techniques will change the capacity, reliability and timing of analyses for PGD diagnosis (Treff *et al.*, 2013). In addition, the latest studies support a move towards a combination of PGD with aneuploidy screening along with HLA typing on blastocyst biopsies (Rechitsky *et al.*, 2015; Goldman *et al.*, 2016; Sermon, 2017). These new methodologies were not reflected in this study's data. At the same time, with respect to the HSCT treatment that remains a difficult procedure with regards to finding a donor and facing any potential immediate and long-term HSCT complications, it is positive that patient/donor selection, transplant treatment protocols and supportive care treatment are constantly being optimized with the expectation of improved outcomes even without the availability of sibling HLA-matched donors (unrelated transplants). As an alternative, new approaches are promising and may also set a path for cure in the future, such as the use of gene therapy to introduce healthy copies of the defective genes or targeted genome editing approaches (using zinc-finger endonucleases, transcription activator-like effector nucleases, or the clustered regularly interspaced short palindromic repeat-Cas 9 system) to replace the mutated sites, or the use of induced pluripotent stem cells. Some initial results have been encouraging, however, further clinical trials are needed to establish the safety and efficacy of these methodologies (Raya *et al.*, 2009; Tolar *et al.*, 2012; Arnold and Heimall, 2017; Srivastava and Shaji, 2017; Zhang, 2017).

Until the above future prospects are achieved, the requirement for a better collaboration between the different experts (ART, genetic and transplant unit) is highlighted, as well as comprehensive and prospective long-term data collection. It is a matter of urgency to work on the prospect of adequately following and evaluating all PGD-HLA procedures with the ultimate aim to promote best practice and encourage informed patient decision-making.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

J.T.S., G.K.: contribution to study conception and design, acquisition of data, analysis and interpretation of data, drafting and revising the article the article, and final approval of the version to be published. G.Kou.,

E.K.: contribution to design, acquisition, analysis and interpretation of data, revising the article and final approval. S.K., G.C.E., H.A.T., A.C.S., J.M., H.M., C.G., V.G., F.C., C.B., J.F.C.C., X.V., G.Kok., J.L., J.S.: acquisition of data, revising the article and final approval.

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Conflict of interest

None to declare.

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