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ORIGINAL ARTICLE ***Reproductive genetics***

Principles guiding embryo selection following genome-wide haplotyping of preimplantation embryos

Eftychia Dimitriadou[1], Cindy Melotte[1], Sophie Debrock[2], Masoud Zamani Esteki[1], Kris Dierickx[3], Thierry Voet[1,4], Koen Devriendt[1], Thomy de Ravel[1], Eric Legius[1], Karen Peeraer[2], Christel Meuleman[2], and Joris Robert Vermeesch[1,*]

[1]Department of Human Genetics, Centre for Human Genetics, University Hospitals Leuven, O&N I Herestraat 49 - box 602, KU Leuven, 3000 Leuven, Belgium [2]University Hospitals Leuven, Leuven University Fertility Center, Herestraat 49, 3000 Leuven, Belgium [3]Centre for Biomedical Ethics and Law, KU Leuven, 3000 Leuven, Belgium [4]Single-cell Genomics Centre, Welcome Trust Sanger Institute, Hinxton CB10 1SA, UK

*Correspondence address. Department of Human Genetics, Centre for Human Genetics, University Hospitals Leuven, 3000 Leuven, Belgium. Tel: +32-16-3-45941 or +32-16-3-45904; Fax: +32-16-3-46060; E-mail: joris.vermeesch@uzleuven.be

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STUDY QUESTION: How to select and prioritize embryos during PGD following genome-wide haplotyping?

SUMMARY ANSWER: In addition to genetic disease-specific information, the embryo selected for transfer is based on ranking criteria including the existence of mitotic and/or meiotic aneuploidies, but not carriership of mutations causing recessive disorders.

WHAT IS KNOWN ALREADY: Embryo selection for monogenic diseases has been mainly performed using targeted disease-specific assays. Recently, these targeted approaches are being complemented by generic genome-wide genetic analysis methods such as karyomapping or haplarithmisis, which are based on genomic haplotype reconstruction of cell(s) biopsied from embryos. This provides not only information about the inheritance of Mendelian disease alleles but also about numerical and structural chromosome anomalies and haplotypes genome-wide. Reflections on how to use this information in the diagnostic laboratory are lacking.

STUDY DESIGN, SIZE, DURATION: We present the results of the first 101 PGD cycles (373 embryos) using haplarithmisis, performed in the Centre for Human Genetics, UZ Leuven. The questions raised were addressed by a multidisciplinary team of clinical geneticist, fertility specialists and ethicists.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Sixty-three couples enrolled in the genome-wide haplotyping-based PGD program. Families presented with either inherited genetic variants causing known disorders and/or chromosomal rearrangements that could lead to unbalanced translocations in the offspring.

MAIN RESULTS AND THE ROLE OF CHANCE: Embryos were selected based on the absence or presence of the disease allele, a trisomy or other chromosomal abnormality leading to known developmental disorders. In addition, morphologically normal Day 5 embryos were prioritized for transfer based on the presence of other chromosomal imbalances and/or carrier information.

LIMITATIONS, REASONS FOR CAUTION: Some of the choices made and principles put forward are specific for cleavage-stage-based genetic testing. The proposed guidelines are subject to continuous update based on the accumulating knowledge from the implementation of genome-wide methods for PGD in many different centers world-wide as well as the results of ongoing scientific research.

WIDER IMPLICATIONS OF THE FINDINGS: Our embryo selection principles have a profound impact on the organization of PGD operations and on the information that is transferred among the genetic unit, the fertility clinic and the patients. These principles are also important for the organization of pre- and post-counseling and influence the interpretation and reporting of preimplantation genotyping results. As novel genome-wide approaches for embryo selection are revolutionizing the field of reproductive genetics, national and international discussions to set general guidelines are warranted.

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Key words: haplotyping-based PGD / haplarithmisis / embryo selection / embryo prioritization / guidelines

Introduction

PGD is offered to couples that carry disease causing mutations or chromosomal abnormalities and wish to avoid transmitting the disorder to their offspring (Handyside *et al.*, 1990; Verlinsky *et al.*, 1994). Embryos are produced by IVF and cultured until Day 3 post-fertilization, when one or two cells are biopsied from the cleavage-stage embryo for genetic analysis (Harton *et al.*, 2011). Alternatively, PGD can be performed on polar bodies (Verlinsky *et al.*, 1998) or following trophectoderm biopsy on Day 5, where 5–10 cells are removed from the blastocyst-stage embryo (Kokkali *et al.*, 2005).

Until recently, PGD for monogenic disorders has mainly been performed via amplification of the mutated locus using (multiplex) PCR, combined with the analysis of linked polymorphic single tandem repeats (Renwick and Ogilvie, 2007; Spits and Sermon, 2009). Chromosomal abnormalities, such as translocations, insertions and inversions which are present in the parents in a balanced form, can lead to unbalanced chromosomal constitution. The transmission of fragments in an unbalanced form can lead to miscarriages or genetic disorders in the child. Historically, fluorescence *in situ* hybridization (FISH) was implemented for the simultaneous testing of the copy number (CN) status of multiple loci in the genome (Vanneste *et al.*, 2009a). To tackle the limitation in the number of loci that could be tested in a single FISH experiment, array comparative genomic hybridization (Dimitriadou *et al.*, 2015) and more recently massive parallel sequencing (Deleye *et al.*, 2015) have been introduced, allowing genome-wide aneuploidy screening (PGS) (Harper and Harton, 2010; Vermeesch *et al.*, 2016). The resolution of these techniques ranges between 1 and 4 Mb when performed on single cells (Munné, 2012).

Most recently, generic methods enabling concomitant detection of both disease alleles as well as genome-wide numerical and structural chromosomal anomalies have been developed and clinically implemented. Two different approaches are in use, namely karyomapping (Natesan *et al.*, 2014) and haplarithmisis (Zamani Esteki *et al.*, 2015). The process involves genome-wide single nucleotide polymorphism (SNP) analysis of the parents and additional family members (e.g. grandparents or a sibling), enabling the identification of informative loci for each of the parental haplotypes across all chromosomes. By following the inheritance of the parental haplotypes and localizing the meiotic homologous recombination sites, the haplotype of each cell biopsied from the embryo is reconstructed and the inheritance of Mendelian disease variants can be inferred genome wide. The advantages of such an approach are multifold: (i) the technique is generic and thus no protocol optimization per family or per locus is required, given that the analysis is performed genome-wide at high-resolution using hundreds of thousands of SNPs spread across the genome; (ii) random allelic drop-out events can be solved by using the information of neighboring informative markers present at high density for each locus genome wide; (iii) meiotic homologous recombination sites can be accurately localized; (iv) in cases of parental reciprocal translocation carriers, it is not only possible to detect embryos carrying unbalanced chromosomes but also to distinguish embryos that are carriers of a balanced translocation versus chromosomally normal embryos and (v) genome-wide CN variation can be detected, enabling the identification of monosomies or trisomies and the distinction of meiotic from mitotic origin of the trisomies. As a result, the selection of the embryos to be transferred to the uterus is optimized.

Here, we present the clinical implementation of haplarithmisis and report on 101 PGD cycles, providing insights on the incidence of meiotic and mitotic trisomies, uniparental disomies (UPDs), blastomeres with multiple aneuploidies, polyploid, monosomic and segmental imbalances as well as the frequency of blastomeres where no definitive diagnosis can be set. Whereas most PGD testing methods thus far focused on analyzing a single locus or a localized region of the genome, those new methods provide a genome-wide haplotype of the embryo. As a consequence, not only the genetic variants of interest are interrogated, but also other genomic variation can be analyzed. This genome-wide view offers the potential to select embryos and prioritize embryo transfer not only on the basis of the absence of the mutation, but also based on the overall genetic constitution.

During the clinical implementation of haplarithmisis, several questions arose concerning embryo selection and embryo prioritization. The questions raised were addressed by a multidisciplinary team of clinical geneticists, fertility specialists and ethicists. Here, we present the principles guiding embryo selection. These principles are based not only on technical and biological, but also on ethical criteria. They impact on the interpretation of the genotyping results, the reporting of the results to the fertility center and the patients as well as the pre- and post PGD-counseling.

Materials and Methods

Patient enrollment and clinical protocol

Sixty-three couples presented with either (i) inherited genetic variants and/or (ii) chromosomal translocations (Supplementary Table SI). PGD is not offered for alleles with low penetrance (e.g. checkpoint kinase 2 (*CHEK2*) mutations with only marginal increased risks for breast cancer)

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nor for genetic variants (e.g. sex determining region Y (*SRY*) mutations) or 'healthy' conditions (e.g. sex selection for non-medical reasons) (Shenfield *et al.*, 2003; De Wert *et al.*, 2014).

Prospective parents are counseled by a clinical geneticist, a gynecologist-fertility specialist and/or a counselor and, whenever appropriate, a psychologist.

For 74.6% ($n = 47$) of the families enrolled, haplotypes were deduced via the parents of one or both of the prospective parents. For the remaining 16 families, a sibling was used to perform the haplotype phasing.

PGD preparation

DNA samples from the couple and other family members are analyzed before the couple starts a PGD cycle. Genotyping is performed by SNP arrays (Human CytoSNP12v2.1, Illumina, USA) and analyzed with Genome Studio (Illumina) as described (Zamani Esteki *et al.*, 2015). Genotypes from parents, grandparents and/or siblings are not interpreted.

PGD and embryo selection

Following ICSI, embryo biopsy is performed by the removal of one blastomere from the developing Day 3 embryo (Goossens *et al.*, 2008). The genome of the blastomere is then whole genome amplified (WGA) using isothermal multiple displacement amplification (REPLI-g single cell kit, Qiagen, Germany), quantified (Qubit, Thermo Fisher Scientific, USA) and genotyped by SNP arrays (Human CytoSNP12v2.1, Illumina). Subsequently, genotyping data are fed to single-cell haplotyping and imputation of linked disease variants (siCHILD) (Zamani Esteki *et al.*, 2015) that reconstructs genome-wide haplotype architectures as well as the CN and segregational origin of the haplotypes. This is possible by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions using haplarithmisis. The output of siCHILD includes haplotyping information, via which the presence or absence of the disease allele can be defined.

All embryos with six or more cells on Day 3 after injection are biopsied. All biopsied embryos are further cultured until Day 5/6. Only embryos with normal morphology on Day 5/6 are considered for embryo transfer and/or cryopreservation.

During the first phase of the clinical implementation of the protocol (three cycles of three families), fresh embryo transfer was performed. Test results were obtained within 2 days and unaffected, morphologically normal embryos were transferred on Day 5. During the subsequent cycles, all embryos developing to morulas and/or blastocysts were vitrified at Day 5/6. Test results were obtained within 4 weeks following biopsy. Unaffected embryos were warmed and transferred in a following single frozen thawed embryo transfer cycle.

Embryo transfer management with respect to the disease locus

For autosomal dominant disorders, selection against carriers of the mutation is carried out.

For autosomal recessive disorders both non-carrier and carrier embryos can be transferred.

For X-linked recessive (XLR) disorders both carrier (female) as well as non-carrier (males and females) embryos can be transferred. Information regarding the carrier status is not disclosed, unless the couple has explicitly stated that they do not wish the transfer of carrier female embryos. In this case, carriership information will be used for embryo selection. Carrier status is used for embryo prioritization, as preference will be given to non-carrier(s). For specific X-linked dominant disorders, such as Fragile X syndrome, the selection strategy is as follows. If the mother is a carrier of a pre-mutation, then the risk for expansion is calculated. Given that the presence or absence of the high-risk allele can be determined in the embryo the couple can opt for transfer of non-carriers of the disease allele and cryopreservation of female carriers and make a final decision at a later time point. In such cases, information regarding the sex and the carrier status of the embryos are communicated.

For translocation carriers, both balanced carriers and non-carrier embryos are transferred. Nevertheless, if both balanced and non-carrier embryos of the same quality are available, preference will be given to the non-carriers.

Carrier information (autosomal and XLR or balanced translocations) is available in the genetic lab but is not communicated with the fertility laboratory. If wanted, this information can be communicated to the prospective parents after birth.

Embryo transfer management guidelines with respect to genetic variation not related to the disease locus

Genetic properties which exclude embryo transfer are: (i) the embryo is a carrier of numerical or structural abnormality in the chromosome or one of the chromosomes carrying the disease locus; (ii) the embryo is a carrier of a trisomy 13, 18 or 21, which might lead to Patau, Edwards and Down syndrome, respectively; (iii) the embryo carries multiple aneuploidies genome-wide with gains and/or losses of several chromosomes; (iv) the embryo is carrier of a trisomy of any chromosome of meiotic origin, which is highly likely to be present in all blastomeres and (v) the presence of UPD of chromosome 6, 7, 11, 14 and/or 15, which are known causes of developmental disorders.

Embryo prioritization guidelines

Embryo prioritization was based on biological (i) and/or ethical (ii and iii) criteria. (i) A numerical chromosomal abnormality, a segmental chromosomal abnormality or UPD not mentioned above. That chromosomally abnormal embryos can be transferred may seem counterintuitive. However, the mitotic error rate in cleavage embryos is higher than the meiotic error rate (Vanneste *et al.*, 2009c; Zamani Esteki *et al.*, 2015) and mosaic embryos may develop normally, implant and lead to the birth of healthy individuals (Greco *et al.*, 2015); (ii) an XLR mutation in a female carrier embryo. Preference will be given to non-carrier embryos. This ranking criterion is secondary to (i); (iii) a balanced translocation in the embryo. If the embryo selection takes place in the context of balanced translocations present in (one of) the parents, a selected embryo can either be chromosomally normal, or carrier of the balanced translocation transmitted from one of the parents. Preference will be given to the chromosomally normal embryos. This choice is secondary to the criteria (i) and (ii).

The ranking will be communicated to the embryologist, but the rationale underlying the prioritization not. The embryologists are responsible to choose the embryo that will be transferred, taking into account (i) the quality of the embryo, i.e. a score based on morphological criteria, (ii) the developmental stage of the embryo, which needs to be compatible with the stage of the endometrial development and (iii) the genetic ranking, based on the aforementioned criteria.

Results

First year experience

Sixty-three couples underwent 101 IVF cycles. Out of 868 metaphase II oocytes, 668 led to the formation of a Day 1 embryo (76.9 % fertilization rate), of which 85% ($n = 568$ embryos) were of adequate quality on Day 3 to be biopsied and 48.5% ($n = 324$ embryos) developed into a morphologically normal blastocyst at Day 5/6 (Fig. 1; Table I; Supplementary Table SII).

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Embryos that underwent analysis were classified in five categories: unaffected, affected, inconclusive, abnormal and no result (Fig. 2). Unaffected embryos either lack the disease allele (recessive disorders, dominant disorders, unbalanced translocation) or are heterozygous carriers of one (recessive disorders) or two disease alleles (balanced translocations). Affected embryos carry one (dominant) or two (recessive) copies of the disease allele or even two different disease alleles (combined heterozygotes for recessive disorders). Abnormal embryos carry chromosomal aneuploidies (Fig. 3). Inconclusiveness is indicated when a homologous recombination site is present closer than 150 SNPs to the locus of interest (Zamani Esteki *et al.*, 2015). No result can be reported in cases of whole genome amplification failure or low SNP call rate. In such cases, following thawing of the embryo, a trophectoderm rebiopsy was performed in two cases, leading to an ongoing clinical pregnancy in one of them.

In total 372 out of 568 biopsied embryos have been tested, namely 324 blastocysts, 36 morulas and 12 embryos that were of adequate quality for biopsy on Day 3, but failed to develop further (fresh cycles) (Supplementary Table SII). Informative and conclusive results were obtained for 92.7% ($n = 345$): 143 embryos (38.4%) were unaffected, 148 (39.8%) were affected and 54 (14.5%) abnormal.

Among the abnormal embryos (Supplementary Table SIII), the most common anomaly was a haploid or polyploid profile ($n = 16$) followed by aneuploidy of the chromosome of interest ($n = 13$), gain and/or loss of multiple chromosomes ($n = 11$), aneuploidies of chromosomes 13, 18 and 21 ($n = 10$) as well as of other chromosomes ($n = 4$). Following genetic analysis of trophectoderm samples from embryo rebiopsy

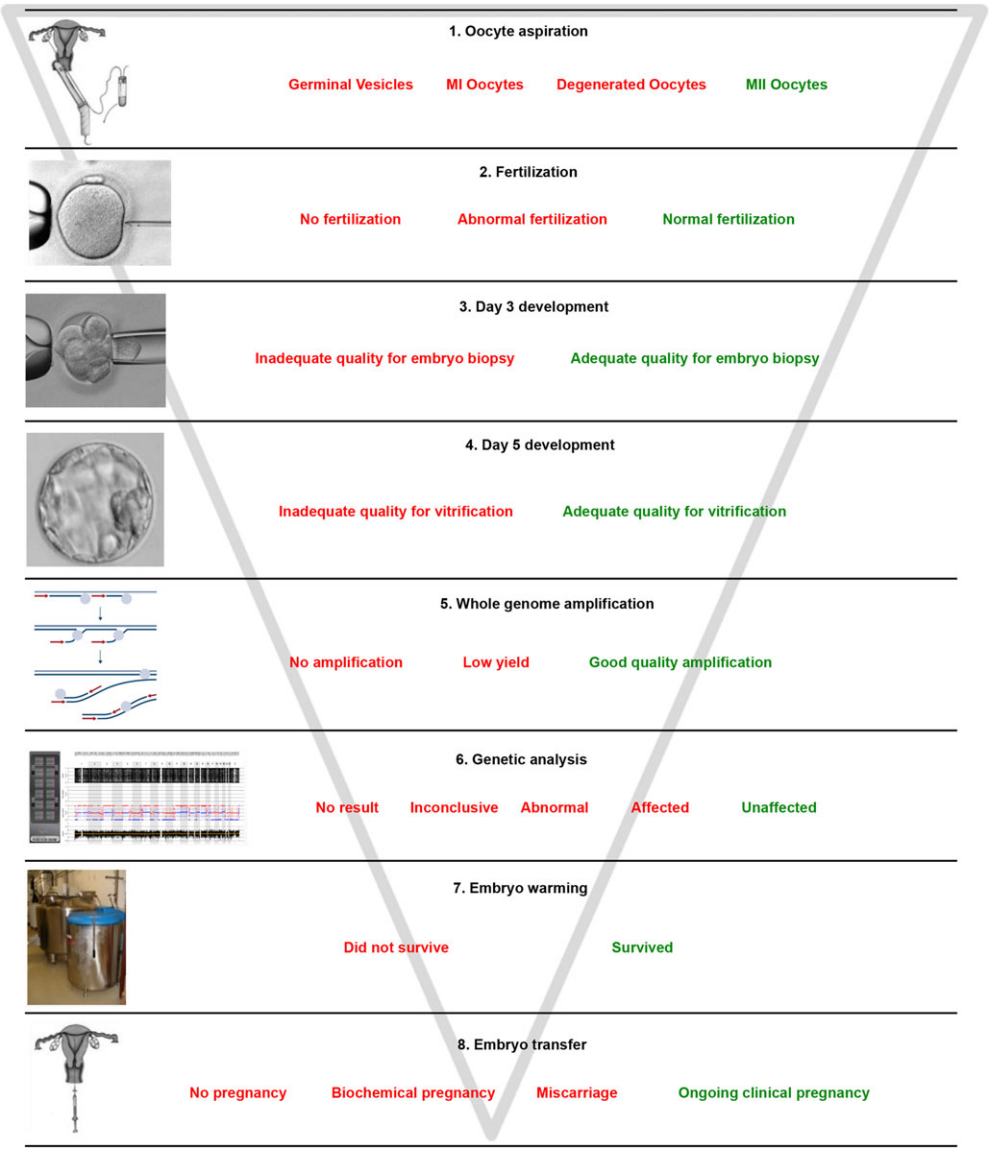

Figure 1 Selection procedure from oocyte aspiration until embryo transfer. Flow chart showing the eight consecutive selection steps that are followed during a PGD cycle.

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Table I Summary of the cycles per category of indication.

	AR	AD	XLR	XLD	Chromosomal	Combined indications	Total
Cycles	14	62	11	4	2	8	101
Oocytes	107	580	105	12	14	50	868
Fertilized	81	449	72	10	13	43	668
Biopsied	77	377	62	8	12	32	568
Blastocysts	55	213	29	6	6	15	324
Unaffected	26	86	19	2	4	6	143

AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive; XLD, X-linked dominant.

($n = 14$), two embryos were excluded from transfer due to the presence of segmental aneuploidies likely present in all cells. Ranking of embryos occurred in 22 cycles (26 embryos) (Supplementary Table SIV). Two embryos (1 cycle) were given Ranking II because of the presence of the balanced form of a reciprocal translocation, while diploid non-carrier embryos (ranking I) were also available and priority was given to them. The remaining 24 embryos were carriers of aneuploidies involving whole chromosomes ($n = 12$), chromosomal segments ($n = 6$) or a combination ($n = 6$). Interestingly, two blastomeres of different embryos carried each one UPD, namely a maternal UPD of Chromosome 1 and a maternal UPD of Chromosome X. Meiotic trisomies were detected in 11 embryos (Supplementary Tables SIII–SV).

Inconclusive results were indicated in 14 (3.7%) embryos and no result for 13 (3.5%) embryos. The latter is inflated due to one cycle, where six out of eight tested embryos failed amplification. (Fig. 4; Supplementary Table SII).

Embryo transfer was possible in 70 out of 101 cycles (69.3%) (Fig. 4). In three cycles the only available unaffected embryo did not survive the warming procedure. From the remaining 67 cycles, embryos have already been transferred in 60 cycles. In total, 27 embryo transfers led to a clinical pregnancy, 12 of which are ongoing, while another 15 resulted in the birth of 17 healthy babies. This gives a pregnancy rate of 45% per finalized cycle with embryo transfer.

Thirteen embryos with aneuploidies at Day 3 (ranking II) were transferred (Supplementary Table SIV), four of which implanted. One embryo in which the blastomere contained a partial trisomy of chromosome four led to the birth of a healthy baby. For two embryos with respectively a segmental deletion of the long arm of Chromosome 22 and a monosomy of Chromosome 8 pregnancies are ongoing. Non-invasive prenatal testing of the latter showed a normal diploid profile. In one case (PGD105_C1; E03_BI001), analysis of a blastomere on Day 3 showed the presence of the non-risk (normal) allele, but the presence of multiple monosomies genome-wide. Nevertheless, because of the normal embryo development and morphology at Day 5, it was decided to use this embryo for transfer. Notably, embryo transfer led to a clinical pregnancy, which is still ongoing.

Discussion

Since the introduction of PGD in 1990, internationally accepted guidelines have been developed and implemented (De Wert *et al.*, 2014; Harper *et al.*, 2014). Nevertheless, with the development and introduction of novel genome-wide haplotyping technologies, new ethical questions arise (Hens *et al.*, 2013). The available genetic information not only pertains the disease allele, but provides three types of extra genetic information: (i) information about the mutation which remained previously hidden (e.g. balanced translocation), (ii) information about the quality of the embryonic genome, such as the presence or absence of aneuploidies, haploidy, triploidy or UPDs in the biopsied cell and (iii) information about the future child itself, such as carrier status of genetic variants, based on the haplotyping data. Importantly, the genome of the embryos is not being analyzed for other loci, consequently, information regarding genetic variants and mutations on the DNA sequence level is not available and will not be communicated. Thus, informing the couple in advance regarding the nature of the test is essential. Given that the genomes of other family members (parents, grandparents and/or siblings) are only used for haplotype phasing and are not being further analyzed, the discussion of the consequences of potential incidental findings in these individuals is not relevant.

Carrier identification and selection

Occasionally, the balanced translocation causes an abnormal phenotype and selection against it is required. Most often, both categories of embryos (balanced carriers and non-carriers) are expected to develop normally and knowledge of carriership is not required for embryo selection. Nevertheless, chromosomally normal embryos were prioritized above embryos carrying the translocation, thus reducing the future reproductive risks of the future child. Likewise, carriers of recessive mutations can be distinguished from non-carrier embryos. In contrast to translocations, carrier information for recessive disorders was not used for genetic selection nor for prioritization. Most mutations causing recessive disorders are very rare, thus the chance that a carrier child will in the future find a partner carrying a mutation in the same gene is rather unlikely. Moreover, variants can provide advantages under certain environment conditions in a heterozygous state (O'Donald, 1967). Nevertheless, carriership information can be communicated following the embryo transfer. For XLR diseases, on the other hand, it was decided that carrier status information can be disclosed and/or used for embryo selection, given the 50% chance of the future individual (female) carrier to have an affected (male) child.

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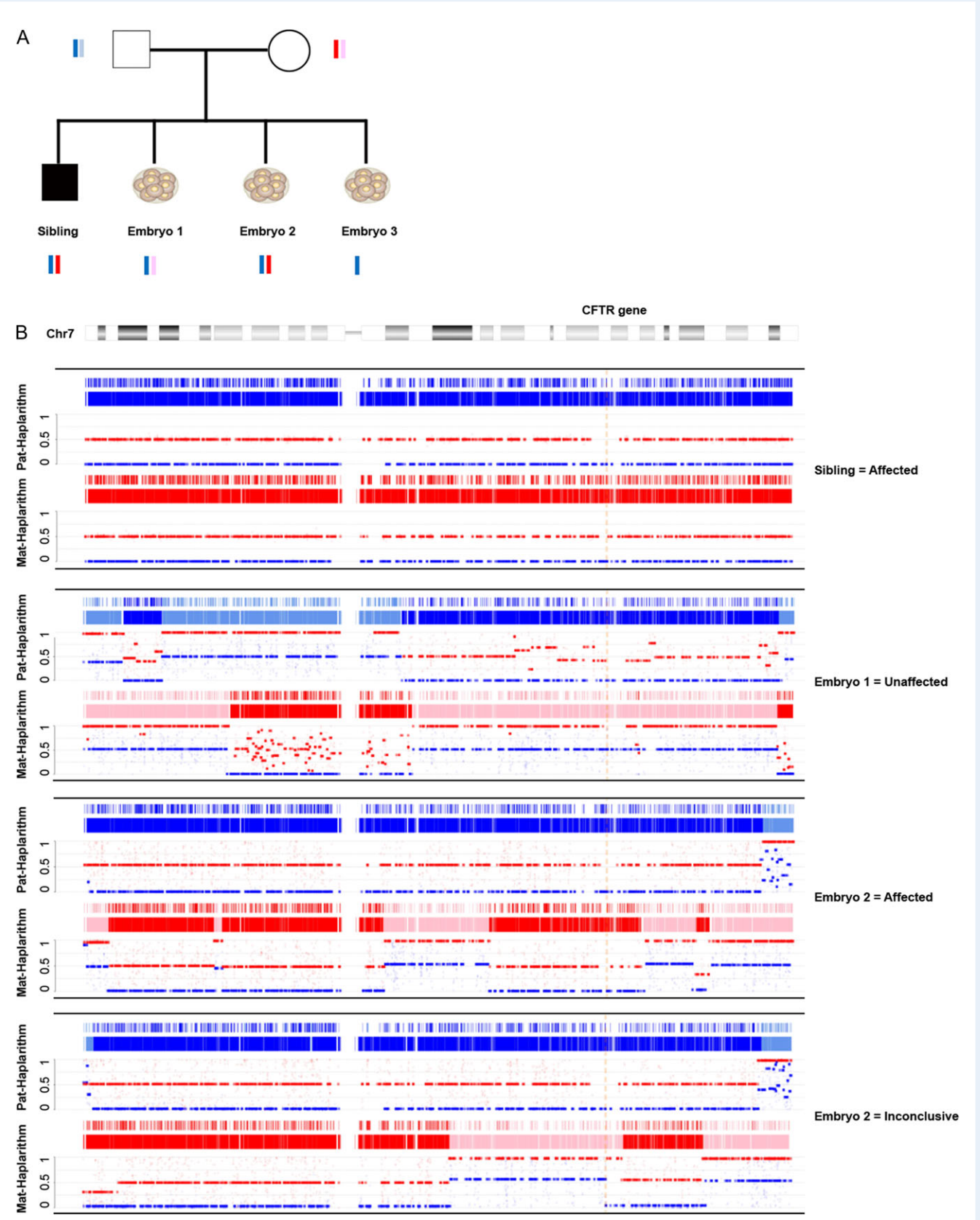

Figure 2 Embryo diagnosis in relation to the mutation of interest. The disease-locus-specific analysis of two embryos from a family carrying DF408 mutation. (**A**) The family tree showing that both partners carry a mutated allele that has been inherited by their affected child (dark blue and blue red haplotype). (**B**) single-cell haplotyping and imputation of linked disease variants (siCHILD) plots for Chromosome 7, showing that at the locus of

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Impact of genomic information on assessing embryo quality

Selecting against embryos carrying any kind of aneuploidy will reduce the number of embryos for transfer. In our cohort, we detected at least one whole chromosome or segmental aneuploidy in 34.6% of the analyzed embryos (n = 129). Nevertheless, the number of meiotic events was limited (n = 11), which is indicative of the fact that our group consists of young, good prognosis patients (age range = 22–41; mean = 29.4; median = 28). Given that the aim is to help couples to obtain offspring that do not suffer from the specific disease, it was decided to select only against viable trisomies and trisomies of meiotic origin. For 148 embryos, a chromosomal imbalance was detected and in 12 embryos a transfer has been performed leading to at least four pregnancies and one birth. A total of 30 embryos showed an abnormal profile with multiple gains and/or losses of chromosomes upon genetic analysis on Day 3, which nevertheless further developed until Day 5/6, giving rise to morphologically normal blastocysts. Importantly, one of these embryos has been used for transfer, leading to an ongoing clinical pregnancy. Therefore, the identification of chromosomal abnormalities that occurred after fertilization, cannot be used as an uncontested contraindication for embryo selection at the cleavage stage. There is, however, accumulating evidence that the presence of aneuploidy may reduce the overall chance for a pregnancy (Yang *et al.*, 2012; Forman *et al.*, 2013; Scott *et al.*, 2013b). This observation prompts us to prioritize chromosomally normal embryos above embryos with (segmental) aneuploidies.

Any embryo carrying an aneuploidy of the chromosome where the family-specific mutation is located is currently not used for transfer, as it could possibly interfere with the accurate interpretation of the haplotypes regarding the locus of interest. One could argue, however, that in case of monosomy, where the allele from the parental side carrying the mutation is present, a safe and clear conclusion can be made. Moreover, in case of a mitotic trisomy, the same allele will be present twice, which would mean that the aneuploidy would not interfere with the identification of the haplotype. Despite the aforementioned considerations, we decided to currently exclude these embryos from transfer. No embryos carrying a meiotic or a mitotic trisomy for either chromosome 13, 18 or 21, which may lead to a viable trisomy are used for transfer. On the other hand, it could be argued that the trisomic cells may not survive further during embryo development or may not even reside in the inner cell mass that will give rise to the fetus. Moreover, the removal of one trisomic cell from a 6 to 8-cell embryo would reduce the percentage of trisomic cells in the embryo or even vanish them completely, leading to a trisomy-free normal blastocyst. The question also arises how a monosomy of chromosome 13, 18 or 21 should be managed. For monosomies, it cannot be determined whether the detected aneuploidy arose from a mitotic or meiotic event. Given that there is a possibility that it is a mitotic event, trisomic cells would also be present in the embryo. Moreover, by removing one monosomic cell, the relative percentage of trisomic cells in the embryo would have been increased enhancing the overall trisomy risk of the future embryo. Currently, embryos showing monosomy of chromosomes leading to viable trisomies have a low ranking, but are not excluded from transfer.

Impact of detection of genome-wide haplotypes, mutations and variants

More than 40% of individuals who are considered to be healthy have been estimated to carry mutations in genes associated with severe Mendelian disorders that are predicted to be damaging or are annotated as disease causing (Friend and Schadt, 2014; Winand *et al.*, 2014; Chen *et al.*, 2016). Consequently, at present there are still many limitations in terms of analytical and clinical validity and utility of a generalized embryo screening. On top, high-resolution testing will also reveal either genetic variation that is of unknown significance at present (Kingsmore and Saunders, 2011), but which may gain significance in the future or relevant to the general health of the future child, or even information about their non-health related traits. In this case, this information not only affects the decision of the parents and the specialists, but may also have an impact on the quality of life of the future child. Lastly, the introduction of comprehensive embryo testing allows testing and selection for serious health conditions, but could prospectively be used for the embryo selection for non-health-related traits. The debate regarding sex selection for non-medical reasons, for example, has been ongoing for several years already, but the range of characteristics that can be tested for has broadened.

The basic ethical principle for embryo selection and transfer is that the embryos may not be selected based on features that the society considers as normal nor on characteristics that the couple may indicate as desired for their future child (De Wert *et al.*, 2014). Only clinically relevant information leading to known severe diseases can be used for embryo selection and embryo transfer prioritization (Shenfield *et al.*, 2003). Hence, genetic risk factors or genetic lesions at the DNA sequence level other than the reason of referral are currently not considered during embryo selection and prioritization.

Concluding remarks

We envision that the principles implemented in a single center could serve as a guide for other centers adopting those novel technologies. It is likely that some of the views presented are not shared by everyone. Certain aspects of the choices made are likely to be culturally motivated. Other choices may change with protruding insights. We envision the current process to be subject to constant evaluation and refinement. The results of ongoing randomized control trials regarding the clinical outcome of the implementation of genome-wide aneuploidy screening will influence future selection and prioritization criteria. Nevertheless, some of the principles put forward here could potentially serve to search for a broader consensus in the community.

interest (cystic fibrosis transmembrane conductance regulator (CFTR) gene) the affected child carries two mutant alleles (dark blue and dark red); Embryo 1 is unaffected, as it carries a normal allele inherited from the mother (light red) and a mutant allele inherited from the father (dark blue); Embryo 2 is affected, as it carries two mutant allele (dark blue inherited from the father and dark red inherited from the mother); Embryo 3 is inconclusive, as it carries one mutant allele inherited from the father (dark blue) and an inconclusive allele from the mother (light red with distance of 68 single nucleotide polymorphisms (SNPs) from the closest recombination site).

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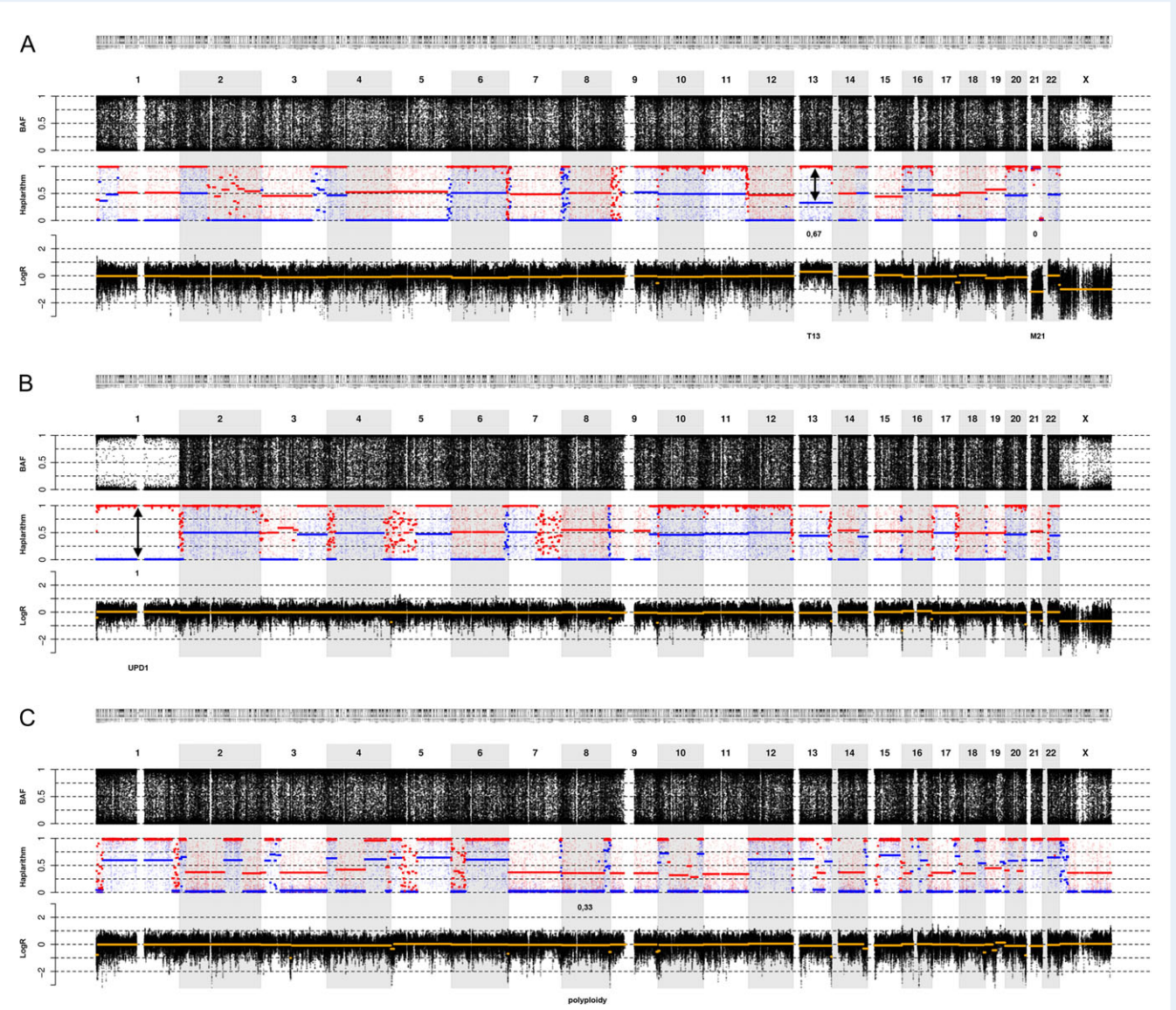

Figure 3 Embryo selection based on genetic variation not related to the disease mutation. Combined information provided by haplarithm profiles and relative copy number (CN) (logR) values show (**A**) a maternal trisomy 13 (T13): the logR value is elevated and the distance between the two lines (blue and red) of the paternal haplarithm show a distance of 0.67, indicating an overrepresentation of maternal copies and a maternal monosomy 21 (M21): the logR value is diminished and the two lines of the paternal haplarithm profile fall on top of each other (distance = 0), indicating the presence of the paternal and absence of the maternal allele, (**B**) a maternal uniparental disomy (UPD) of Chromosome 1 (UPD1): the logR value is neutral and the two lines of the paternal haplarithm profile have a distance of 1, which shows the presence of the maternal and absence of the paternal alleles and (**C**) a polyploidy with extra maternal copies of all chromosomes: neutral CN due to normalization genome-wide accompanied by a distortion of the distance of the two lines of the maternal haplarithm profile for all chromosomes (distance = 0.33). For more details regarding the interpretation of haplarithm signatures see also Zamani Esteki *et al.* (2015).

Some of the choices made and principles put forward are specific for cleavage-stage-based genetic testing. At this stage, the embryo is notoriously chromosomally unstable (Vanneste *et al.*, 2009b). Overall, the PGD/PGS field is gradually implementing blastocyst biopsies (Scott *et al.*, 2013a; Deleye *et al.*, 2015; Goldman *et al.*, 2016; Gui *et al.*, 2016), a stage when the overall genome constitution resembles more closely the fetal fate. Nevertheless, increasing data have been showing that mosaicism is not an exclusive characteristic of cleavage-stage embryos, but is also prominent at the blastocyst stage (Fragouli and Wells, 2011; Smith *et al.*, 2015; Vera-Rodríguez *et al.*, 2016). However, recently the birth of healthy babies have been reported following the transfer of mosaic aneuploid embryos, indicating that the presence of mosaic aneuploidies in the trophectoderm are not detrimental for normal embryonic and fetal development (Greco *et al.*, 2015). Understanding the behavior of aneuploid cells following the blastocyst stage and understanding the potential consequences of the

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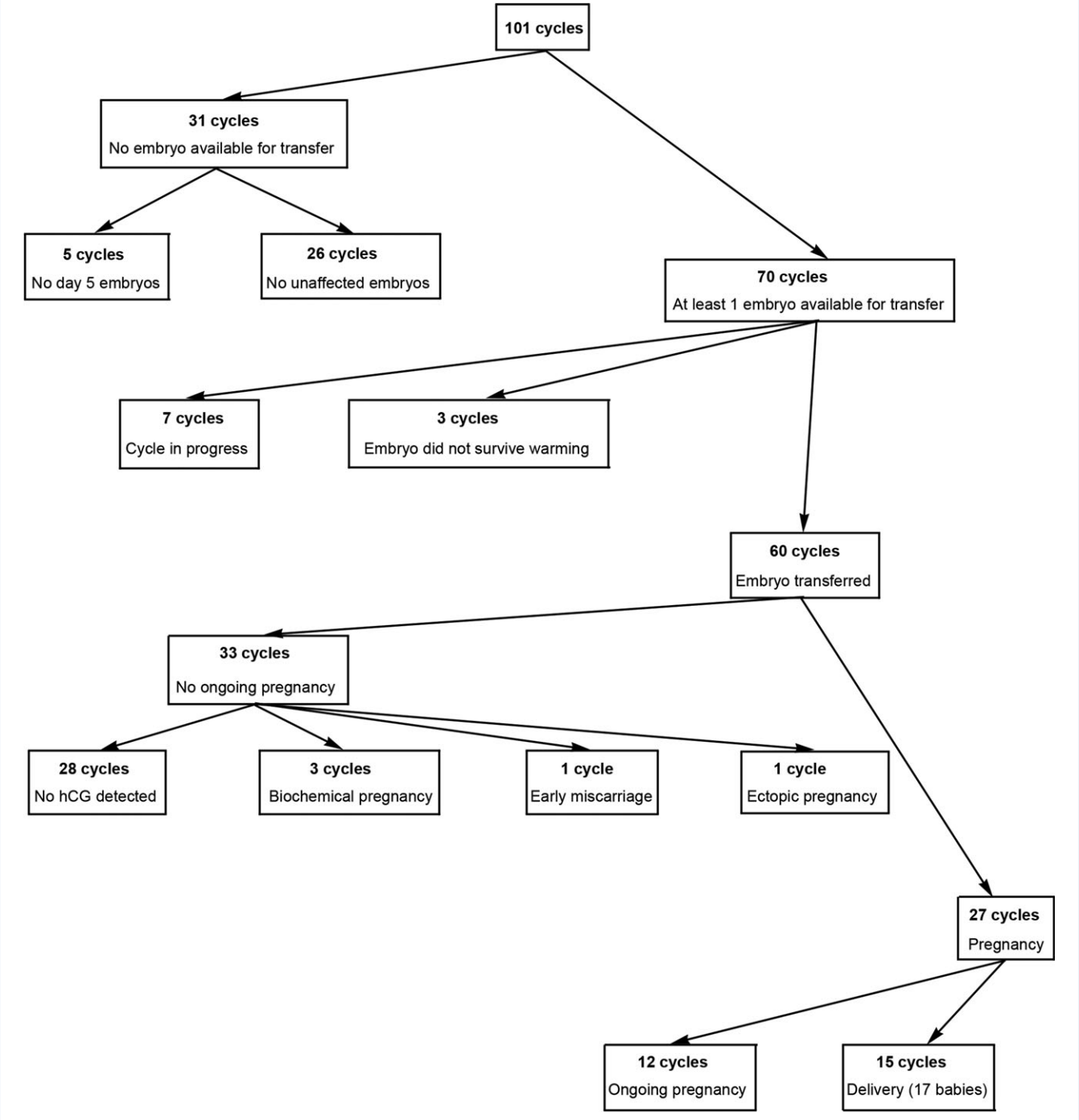

Figure 4 Cycle outcome. Flow chart showing the number of cycles performed and the outcome of the cycles.

presence of (segmental) aneuploidies in the Day 5/6 embryos at the trophectoderm will be essential to shape the decisions on embryo selection and ranking. This warrants more research.

Finally, rapid reduction of sequencing cost over the last decade has rendered it an attractive alternative for single-cell analysis. After having been successfully used by several groups for CN profiling of single cells (Zong *et al.*, 2012; Voet *et al.*, 2013; Binder *et al.*, 2014; Cai *et al.*, 2014), efforts are lately focused on transfer of this technology to the clinic for PGS and PGD purposes (Treff *et al.*, 2013; Yin *et al.*, 2013; Wang *et al.*, 2014; Wells *et al.*, 2014; Deleye *et al.*, 2015; Łukaszuk *et al.*, 2015; Peters *et al.*, 2015). The guidelines introduced here, will not only serve to address the ethical challenges due to genome-wide haplotyping, but will remain valuable in the years to come when full genome sequencing embryos might become a clinical reality.

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Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

E.D., Ci.M. and J.V. conceived and designed the study. S.D., Ko.D, T.d.R, E.L., K.P., Ch.M. consulted the patients and acquired patient data. E.D., Ci.M. and M.Z.E. participated in its execution and analysis. E. D., Ci.M, M.Z.E., T.V. and J.R.V. interpreted the results. J.R.V. supervised the study. E.D. wrote the initial version of the manuscript. Kr.D. took part in critical decisions and discussion of the draft manuscript. All authors critically revised the manuscript and approved the final version.

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Conflict of interest

J.R.V., T.V. and M.Z.E. have patents ZL910050-PCT/EP2011/060211-WO/2011/157846 ('Methods for haplotyping single cells') with royalties paid and ZL913096-PCT/EP2014/068315-WO/2015/028576 ('Haplotyping and copy-number typing using polymorphic variant allelic frequencies') with royalties paid, licensed to Cartagenia (Agilent technologies). J.R.V. also has a patent ZL91 2076-PCT/EP20 one 3/070858 ('High throughout genotyping by sequencing') with royalties paid.

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