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Preimplantation genetic testing for more than one genetic condition: clinical and ethical considerations and dilemmas

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STUDY QUESTION: Which clinical and ethical aspects of preimplantation genetic testing for monogenic disorders or structural rearrangements (PGT-M, PGT-SR) should be considered when accepting requests and counselling couples for PGT when applied for more than one condition (combination-PGT; cPGT-M/SR)?

SUMMARY ANSWER: cPGT is a feasible extension of the practice of PGT-M/SR that may require adapting the criteria many countries have in place with regard to indications-setting for PGT-M/SR, while leading to complex choices that require timely counselling and information.

WHAT IS KNOWN ALREADY: Although PGT-M/SR is usually performed to prevent transmission of one disorder, requests for PGT-M/SR for more than one condition (cPGT-M/SR) are becoming less exceptional. However, knowledge about implications for a responsible application of such treatments is lacking.

STUDY DESIGN, SIZE, DURATION: Retrospective review of all (40) PGT-M/SR applications concerning more than one genetic condition over the period 1995–2018 in the files of the Dutch national PGT centre. This comprises all relevant national data since the start of PGT in the Netherlands.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: Data regarding cPGT-M/SR cases were collected by means of reviewing medical files of couples applying for cPGT-M/SR. Ethical challenges arising with cPGT-M/SR were explored against the background of PGT-M/SR regulations in several European countries, as well as of relevant ESHRE-guidance regarding both indications-setting and transfer-decisions.

MAIN RESULTS AND THE ROLE OF CHANCE: We report 40 couples applying for cPGT-M/SR of which 16 couples started their IVF treatment. Together they underwent 39 IVF cycles leading to the birth of five healthy children. Of the couples applying for cPGT, 45% differentiated between a primary and secondary condition in terms of perceived severity. In the light of an altered balance of benefits and drawbacks, we argue the 'high risk of a serious condition' standard that many countries uphold as governing indications-setting, should be lowered for secondary conditions in couples who already have an indication for PGT-M/SR. As a consequence of cPGT, professionals will more often be confronted with requests for transferring embryos known to be affected with a condition that they were tested for. In line with ESHRE guidance, such transfers may well be acceptable, on the condition of avoiding a high risk of a child with a seriously diminished quality of life.

LIMITATIONS, REASONS FOR CAUTION: We are the first to give an overview of cPGT-M/SR treatments. Retrospective analysis was performed using national data, possibly not reflecting current trends worldwide.

WIDER IMPLICATIONS OF THE FINDINGS: Our observations have led to recommendations for cPGT-M/SR that may add to centre policy making and to the formulation of professional guidelines. Given that the introduction of generic methods for genomic analysis in PGT will regularly yield incidental findings leading to transfer requests with these same challenges, the importance of our discussion exceeds the present discussion of cPGT.

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Key words: preimplantation genetic testing/combination PGT/ethics/indications/transfer decisions

Introduction

Over the past decades, preimplantation genetic testing for monogenic disorders (PGT-M) or structural rearrangements (PGT-SR) has become an established technique allowing couples at high risk of having affected offspring to avoid the birth of a child with a serious genetic disorder or of losing the pregnancy as a result of an unbalanced chromosomal abnormality. PGT as offered for these indications (PGT-M/SR) comprises an in vitro fertilization (IVF)-treatment combined with a blastomere biopsy at the cleavage stage or trophectoderm (TE) biopsy at the blastocyst stage of the *in vitro* embryo. The cell or cells thus obtained are tested for the relevant mutation(s) and/or chromosomal anomalies. This then allows for the selective transfer of embryos in which the targeted genetic condition is absent, thereby enabling at risk couples to reproduce with confidence (Harton et al., 2011). Although usually performed to avoid the transmission of a single disease, PGT-M/SR allows for simultaneous testing for more disorders. Nowadays, it is no longer rare for centres to be confronted with applicants asking for such 'combination PGT' (cPGT-M/SR). Two case reports describe cPGT-M for Tay–Sachs and Gaucher disease and a cPGT-M/SR for a reciprocal translocation and alfa-thalassemia (Altarescu et al., 2007; Lee et al., 2014). Kuliev et al. (2014) listed a small series of cPGT-M treatments including combinations of single-gene disorders like Charcot–Marie–Tooth and Fabry disease; HBOC (hereditary breast and ovarian cancer) and SMA (spinal muscular atrophy) and HBOC and MEN1 (multiple endocrine neoplasms type 1). Rechitsky et al. (2013) reported 11 cycles of combined testing for cystic fibrosis (CF) mutations and another monogenic disorder.

Several factors may have led to a growing number of requests for cPGT-M/SR, including an increased familiarity with the role of genetics in disease, and a greater awareness of personal reproductive risks as a result of more frequent genomic testing in families. The possible future introduction of a routine offer to the general population of expanded preconception carrier screening for recessive disorders may further add to this effect (Henneman et al., 2016; Sallevelt et al., 2017). Finally, possibilities to diagnose genetic disorders at a single-cell level are expanding. For instance, comprehensive methods such as genome wide single nucleotide polymorphism (SNP) haplotyping (karyomapping) or next generation sequencing (NGS)-based techniques (Natesan et al., 2014; Zamani et al., 2015) enable simultaneous testing for multiple or even an unlimited number of genetic disorders without the need for extensive customization of PGT protocols.

Materials and Methods

We retrospectively reviewed all PGT-M/SR applications concerning more than one genetic condition in the files of the Dutch national PGT centre at the Maastricht University Medical Centre since the start of PGT in the Netherlands (1995–2018). All data were collected prospectively. This review was limited to applications involving monogenic/mitochondrial or structural chromosomal abnormalities for which the applicants were known to be at risk, thus excluding PGT for '*de novo*' aneuploidy (i.e. PGT-A) or HLA-typing. Data concerning applications and counselling, protocol development, IVF-treatment, results of PGT-analysis, and obstetric outcome were evaluated. Ethical challenges arising with cPGT-M/SR were explored against the background of PGT-M/SR regulations in several European countries, as well as of relevant ESHRE-guidance regarding both indications-setting and transfer-decisions.

Results

In this section, we present both our clinical data and the findings of our ethical exploration with regard to how cPGT/M-SR relates to criteria for indications-setting and transfer-decisions.

Clinical data

Applications and counselling

We reviewed requests for cPGT-M/SR from 40 couples (Table I). These involved either two structural chromosomal anomalies ($n = 10$), two monogenic disorders ($n = 7$), or combinations of both (also including mitochondrial diseases) ($n = 23$). Three out of four couples applying for cPGT for two autosomal recessive diseases were consanguineous.

When asked, eighteen couples (45%) identified a 'primary' and 'secondary' condition in terms of perceived severity. Neurofibromatosis type 1 (NF1) was perceived as the primary condition for all three couples requesting cPGT for NF1. HBOC was perceived as the secondary indication for five out of seven couples asking for cPGT. CF was perceived as the secondary condition in three out of four requests involving this disease. Twenty-two couples (55%) perceived both conditions for which they requested cPGT as equally severe or could not make a differentiation because of the nature of the disorders (for example two structural chromosomal anomalies, both with a high risk of miscarriage) (Table I).

Table I Indications for combination PGD according to mode of inheritance (*n* = 40) and started cycles (*n* = 16).

Couple number	AD	AR	C	XL	M
AD (*n* = 20)					
1 **NF 1**	Aniridia*	-	-	-	-
2	HME 1*				
3	Myoclonus dystrophy				
4 DM 1	-	**-**	Translocation (n=2)	-	-
5			22q11deletion*		
6					
7 HBOC (BRCA2)	**Retinoblastoma***		-		
8	Hereditary diffuse gastric cancer				
9		**Deafness type 22**			
10				**Lujan–Fryns syndrome***	
11				**Duchenne muscular dystrophy**	
12					**MELAS***
13 **HBOC (BRCA 1)**	Noonan syndrome	-	-	**-**	-
14 Peutz–Jeghers syndrome	**Porencephaly***	-	-	-	-
15 Familial paraganglioma	**Saethre Chotzen syndrome**	-	-	-	-
16 Ehlers–Danlos type VI	-	MPS VII	-	-	-
17 SCA 6	-	CF	-	-	-
18 FAP	-	PAE		-	-
19			**Translocation**		
20 **HME 1**	-	-	-	Retinitis pigmentosa*	-
AR (*n* = 9)	-	-	-	-	-
21 CF		**Zellweger**			-
22			**Translocation**		
23				**Fragile X syndrome**	
24 GM1 gangliosidose		MADD	-	-	-
25 AOA 1		SMA 1*	-	-	-
26 Cockayne syndrome	**-**	Limb Girdle Muscular Dystrophy 2C	-	-	-
27 **Sickle cell disease**		-	Translocation	-	-
28 **Pompe disease**		-	Translocation	-	-
29 **Alpers syndrome**		-	-	-	MELAS*
C (*n* = 11)	**-**	-	-	-	
30 Translocation	-		Translocation		-
-			(*n* = 10)******		
39					
40				Fragile X syndrome	

Bold indicates primary condition as indicated by the couple (*n* = 18 (45%); see text for explanation); * indicates started cycles.
Conditions are listed according to mode of inheritance (rows 1–20 combination with AD condition; rows 21–29 combination with AR condition excluding AD conditions; rows 30–40 combination with chromosomal anomaly excluding AD and AR conditions.)

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked; C, chromosomal; M, mitochondrial; s. indicates 'syndrome'; XL indicates 'X-Linked'; d. indicates 'disease' NF1, neurofibromatosis type 1; HME 1, hereditary multiple exostoses type 1; DM 1, myotonic dystrophy type 1; HBOC, hereditary breast and ovarium cancer; BRCA, breast cancer; MELAS, mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; DFNB 22, DeaFNess – autosomal recessive type 2; MPSV II, mucopolysaccharidosis type 7; SCA 6, spino cerebellar ataxia type 6; CF, cystic fibrosis; FAP, familial adenomatous polyposis; PAE, pyridoxine dependent epilepsy; MADD, multiple acyl CoA dehydrogenase deficiency; AOA1, ataxia-oculomotor apraxia type 1; SMA 1, spinal muscular atrophy type 1.

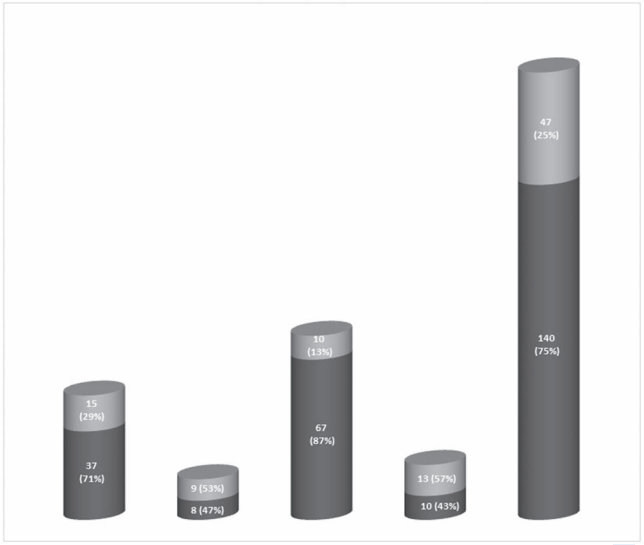

Figure 1 Outcome embryo analysis in cPGT-M/SR (n=187). Number of embryos (% of total in category) not affected for both conditions (lighter shade - top) and affected for one or both conditions (darker shade - bottom), for two autosomal dominant conditions (column 1), autosomal recessive conditions (column 2), chromosomal anomalies (column 3), other combinations of conditions (column 4), and the total of embryos analyzed (column 5).

Twenty-one couples (52%) proceeded with cPGT treatment. Combinations of disorders comprised two chromosomal abnormalities ($n = 6$), monogenic disorders ($n = 8$), or a combination of the above and mitochondrial diseases ($n = 7$). Five of these couples are still awaiting protocol development. Nineteen couples (48%) refrained after intake. Reasons to refrain were low chance of success ($n = 4$), opting for PGT for one disorder ($n = 3$), preference for prenatal testing (PNT) ($n = 3$), medical contra-indication for IVF-treatment ($n = 2$), ending of the relationship ($n = 2$), PGT-treatment in a hospital abroad ($n = 2$), treatment not possible because of technical reasons ($n = 1$), religion ($n = 1$), and spontaneous pregnancy ($n = 1$).

cPGT-M/SR treatments

For 16 couples, treatment protocols were developed; six for combinations of chromosomal anomalies by either FISH or array comparative genomic hybridization (CGH) analysis, seven for two monogenic disorders by marker and/or mutation analysis, two for a monogenic and a mitochondrial disorder by quantative analysis and one for a combination of a monogenic disorder and a chromosomal anomaly (Table II). All treatment protocols were based on blastomere biopsies (Day 3).

The 16 couples underwent 39 cycles and 187 embryos were analyzed (Fig. **1**). Of these, 47 (47/187 = 25%) displayed the wild-type or normal/balanced genotype of both tested disorders and thus were genetically suitable for transfer. These concerned 15 out of 52 embryos analyzed for two autosomal dominant disorders (15/52=29%), 9 embryos out of 17 tested for two autosomal recessive disorders (9/17 = 53%), 10 embryos of 77 analyzed for two chromosomal anomalies (10/77 = 13%) and 13 of the 23 embryos analyzed for other combinations than mentioned above (13/23 = 57%). Of the other 140 analyzed embryos, 46 were free from both conditions tested for and 59 embryos were affected by both. Of 35 embryos no conclusive results could be obtained.

Thirteen couples underwent one or more embryo transfer(s). A fresh embryo transfer was performed in 21/39 (54%) of the cPGT cycles. An additional seven frozen embryo transfers were performed, adding up to a total of 28 transfers (28/39 cycles = 72%). Three double embryo transfers were performed (3/28 = 11%). In three consecutive PGT cycles of couple no. 14, no embryos free from both disorders were available. Although in the counselling before treatment, they clearly stated that they opted for exclusion of both disorders, the couple requested transfer of an embryo affected with Peutz–Jeghers syndrome in all three cycles. No pregnancy was achieved.

Six single embryo transfers (four fresh and two frozen) resulted in a positive HCG test (6/28 = 21%). In four of these cases, two blastomeres were biopsied (Table II). One pregnancy ended in a miscarriage at 6^{+4} weeks gestational age, genotype unknown. Five children were born, three boys (Table II no. 1, 9, 20) and two girls (Table II no. 9, 37). Prenatal or postnatal testing to confirm cPGT diagnosis was performed for two couples (no. 9 and no. 37). In both cases, the cPGT results were confirmed. Couple 9 obtained two healthy PGT children, one from a fresh transfer and the second resulting from a frozen cycle.

Couple no. 10 requested analysis for only the MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) mutation after two unsuccessful PGT treatments for both the MELAS and the BRCA2 mutation. This third cycle resulted in an ongoing bpregnancy.

Ethical exploration

Indications setting for cPGT-M/SR

In countries where PGT-M/SR is available, its lawful application is often limited to couples with a 'significant' or 'high' risk of transmitting a 'serious' genetic disorder to their offspring (see Fig. **2**). In this paper, we will refer to this as 'the high risk of a serious disorder' standard. The reasoning behind this requirement often remains unspecified. However, ESHRE's Task Force Ethics & Law has suggested that the standard should be understood as reflecting the 'proportionality' of PGT-M/SR (De Wert et al., 2014). This notion refers to the balance between the benefits that PGT may have for the applicants on the one hand and the various aspects that make it a morally sensitive technology on the other. If this interpretation is correct, then a significant change on the 'issues and concerns' side of the proportionality balance may lead to a different range of acceptable indications (De Wert et al., 2014). As argued by the Task Force, there are two situations where this would apply. One is PGT-M/SR for applicants with a fertility problem that gives them a separate indication for IVF or intracytoplasmatic sperm injection (ICSI). The second is where people have an indication for PGT-M/SR and want to add testing for a further disorder for which they are also at risk. In both situations, a significant part of the burdens and (moral) costs have already been taken account of, either with respect to IVF or ICSI as fertility treatment, or for doing PGT for the primary disorder. Adding PGT-M/SR to fertility treatment or doing PGT-M/SR for a further condition could therefore be considered also for lower risk or

Table II Combination PGD treatments according to method of analysis (*n* = 16).

Couple nr. (table I)	Condition 1		Condition 2		Cycle number	Embryos biopsied	No. blastomeres biopsied	No. unaffected embryo's cond	Transfer fresh/cryo	Transfer nr.	hcg	Outcome pregnancy
FISH (n=3)												
30	46, XY, t(4;7)(q21.3;q21.2)		46, XX, t(4;22)(p11;p11.2)		1	Overstim. cancel						
					2	Poor response cancel						
					3	4	1	0				
					4	Poor response cancel						
31	45,XY,der(14;21)(q10;q10)		45,XX,der(13:14)(q10;q10)		1	Poor response cancel						
					2	6	1	0				
					3	Follic. <8						
33	46,XX,t(1;2) (q44;q33)		45,XYder(13;14)(q10;q10)		1	Follic. <8						
					2	Follic. <8						
					3	4	2	0				
					4	Follic. <8						
A CGH (*n* = 3)												
35	45,XX,t(3;4)(q22;q31.3)		45,XX,der(13;14)(q10;q10)		1	7	1	0				
					2	7	1	1	F	1	-	
					3	9	1	1	F	1	-	
					4	5	1	2	F	2	-	
37	45,XX,der(13;14)(q10;q10)		46,XY,t(6;14)(q25.3;q24.3)		1	9	1	2	F	1	+	Healthy daughter NIPT normal
38	45,XX,der(14;22)(q10;q10)		46,XY,t(3;16)(q29;q24)		1	24	1	4	F	1	-	
PCR - 1 protocol for the two conditions (n = 4)												
2	NF 1	Markers + mutation	HME 1	Markers	1	5	1	1	F	1	-	
					2	10	1	3	F	1	+	*Miscarriage 6+4 w AD*
					3	8	1	5	F	1	-	
									C	1	-	
7	HBOC	Markers + mutation	Retino-blastoma	Markers	1	3	1	1	F	1	-	
					2	4	1	0				
					3	7	1	1	F	1	-	
14	Peuts–Jeghers syndrome	Markers	Porencephaly	Markers + mutation	1	3	1	0	F	1	-	
					2	4	1	0	F	1	-	
					3	6	1	0	F	1	-	
25	AOA 1	Markers + mutation	SMA 1	Markers + mutation	1	5	1	1	F	1	-	
					2	23	1	5	F	1	-	
									C	1	-	
									C	1	-	
PCR - 2 protocols for the two conditions (n = 5)												
1	NF 1	Markers + mutation	Aniridia	Markers + mutation	1	9	2	4	F	2	-	
									C	2	+	Healthy boy, no genetic analysis

Continued

Table II Continued:

Couple nr. (table 1)	Condition 1		Condition 2		Cycle number	Embryos biopsied	No. blastomeres biopsied	No. unaffected embryo's cond	Transfer fresh/cryo	Transfer nr.	hcg	Outcome pregnancy
9	Alpers syndrome	Markers + mutation	MELAS	Mutation % *	1	6	2	4	F	1	+	Healthy boy CarrierAlpers, no MELAS
									C	1	+	Healthy girl No carrier Alpers, no MELAS
10	HBOC	Markers	MELAS	Mutation % *	1	7	2	1	F	1	-	
					2	8	2	4	C	1	-	
									C	1	-	
20	HME 1	Markers + mutation	XL Retinitis Pigmentosa	Markers	1	8	2	2	F	1	+	Healthy boy no genetic analysis
29	HBOC	Markers	Lujan-Fryns syndrome	Markers	1	5	2	2	F	1	-	
PCR + FISH - *2 protocols (n = 1)*												
6	DM1	Markers + repeat	22q11.2 deletion	FISH	1	Overstim.						
					2	Overstim.						
					3	4	2	0				
					4	10	2	3	F	1	-	

FISH, fluorescence in situ hybridization; Array Comparative Genemoic Analysis; Nr., number; ins., insufficient; mut., mutation load s. indicates 'syndrome'; XL indicates 'X-Linked'; NF 1, neurofibromatosis type 1; HME, hereditary multiple exostoses; HBOC, hereditary breast and ovarian cancer; AOA 1, ataxia-oculomotor apraxia type 1; SMA1, spinal muscular atrophy type 1 ; Alpers., Alpers syndrome; MELAS, mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; Lujan–Fryns s., Lujan–Fryns syndrome.
*The amplification conditions for the mitochondrial MELAS mutation were as described in Sallevelt, et al. (2013) with a mutational threshold set on <15%.

less serious conditions. As we have suggested elsewhere (Dondorp and De Wert, 2019), a possible example is cleidocranial dysostosis (CCD), an autosomal dominant skeletal spectrum disorder involving bone deformities (collarbone, skull) and abnormal teeth (Machol et al., 2017). While this disorder comes with a high penetrance, the clinical features are relatively mild. As CCD only moderately affects the quality of life in most patients, it seems that a 'stand alone' PGT-M procedure for this disorder would be at odds with the 'high risk of a serious disorder' standard. However, should the applicants have an indication for IVF or ICSI as fertility treatment, or if they are already having PGT for an accepted (M/SR) indication, holding on to that same standard would seem too strict.

Transfer decisions after cPGT-M/SR

According to a classical rule of PGT-practice, 'affected embryos' (meaning, embryos with the very mutation or abnormality targeted in PGT-M/SR), should not be transferred to the womb (Thornhill et al., 2005). An important argument for this is that in medically assisted reproduction, professionals should take account of the welfare of the child that they are causally involved in creating (Pennings et al., 2007). As pointed out by ESHRE, there is a broad international consensus that requests for reproductive assistance should not be granted if chances are high that the resulting child will have a seriously diminished quality of life. However, for this to lead to the 'do-not-transfer' rule, it must be the case that PGT-M/SR is only done for conditions that clearly fall in the range of 'high risk and serious'. Given that in the past decennium, the scope of accepted indications has widened beyond the

Several European countries have legislation limiting the scope of acceptable PGT-M/SR indications. For instance in Denmark, France, Germany, the Netherlands, Norway, Sweden, and the U.K., PGT-M/SR is only allowed in situations where there is a 'significant' or 'high' risk of bearing a child with a 'serious' genetic disorder. Of the countries upholding this standard, some (Germany and Norway) require that each individual case must have the prior approval of a multidisciplinary ethics committee. Some other countries (the Netherlands and the U.K.) have a national committee or authority determining on a more general level which conditions are sufficiently 'high risk' and 'serious'. In yet other countries, it is left to individual centres and practitioners to determine which requests are in line with those criteria.

Adapted from: Dondorp and De Wert 2019 (references in original).

Figure 2 Legislation limiting the scope of acceptable PGT-M/SR indications.

limited range of classical disorders to also include conditions marked by a less than complete penetrance, a later time of onset, and at least some treatment or surveillance options (e.g. hereditary breast and ovary cancer (HBOC) or hypertrophic cardiomyopathy), it has become less obvious that the 'do-not-transfer' rule would not allow for exceptions. In a recent focus group study about professional views about these issues, some participants said requests for transferring affected embryos were difficult to swallow. In their view, such requests signalled a capricious attitude on the part of the couple, out of tune with professional efforts to help them avoid the reproductive risk for which they had PGT in the first place. However, others regarded such requests more favorably as an understandable adjustment of priorities in the light of a reassessment of what is realistically feasible (Soto Lafontaine et al., 2018). This, they said, was especially understandable in cases where no further hormone stimulation cycles were realistically possible and where, because of their fertility problem, the alternative option of natural reproduction was not available to the couple. When in such cases the only otherwise good quality embryos available happen to be affected, these represent the couple's last chance to have a genetically related child. In the UK, regulations allow considering such last chance affected embryos for transfer at the patients request, but permission is required from a clinical ethics committee on a case-by-case basis (Human Fertilisation & Embryology Authority, 2019). This has been done for example for BRCA1 carrier embryos.

Assuming, for the sake of debate, that cPGT-M/SR is offered to couples that are normally fertile, it would seem that talk of 'last chance embryos' does not apply. However, this impression is mistaken, especially with regard to those couples who distinguish between a primary and a secondary condition in terms of perceived severity. Faced with the message that no embryos free of either condition are found, then in cases where trying a further hormone-stimulation cycle is not an option, they may request the transfer of embryos affected by what in their view is the secondary target condition. These are 'last chance embryos' in the wider sense of enabling the couple to start a pregnancy with the confidence that the resulting child will not be affected by the disorder that they want to avoid most. Clearly, making this request entails accepting that the child has a high chance of developing the secondary condition, the one that they had preferred to be able to avoid as well, albeit with a lower priority. Allowing cPGT-M/SR may thus lead to an increased number of requests for transferring affected embryos.

Discussion

Clinical feasibility

We report 40 applications for cPGT-M/SR. Sixteen couples started their treatment. Thirty-nine IVF cycles led to the analysis of 187 embryos and 28 embryo transfers, resulting in five healthy children. Pregnancy rate per transfer was 21%. This is in line with the figure reported by the PGT-consortium (Harper et al., 2012). In half of the started cycles, we could at least identify one embryo suitable for transfer. Our results clearly demonstrate the feasibility of cPGT-M/SR.

Bearing these results in mind, we expect an even better outcome after full implementation of comprehensive methods as SNP or NGS-based haplotyping that allow simultaneous testing for an unlimited number of genetic disorders hence avoiding multiple biopsies.

Scope for wider indications setting

In the experience of our centre with cPGT-M/SR, a large percentage of couples distinguished between a primary and secondary condition in terms of perceived relative seriousness. However, no cases were listed where the secondary condition would not on its own have met the 'high risk of a serious condition' standard. The lack of requests for cPGT involving a mild condition as secondary to an accepted PGT-M/SR condition is not surprising, given that the Dutch 'PGD regulations' strictly adhere to the 'high risk of a serious condition' standard and do not recognize—or even discuss—the case for making an exception for applications with an altered proportionality profile (Tweede Kamer der Staten Generaal, 2009). If ESHRE's reasoning on this point is sound, as we think it is, this may have as a consequence that in the Netherlands and other countries that also fail to allow for this exception, some are denied without a good reason what might have been a meaningful option for them. However, an important qualification is that the reasoning behind accepting a lower standard is only valid as long as a further hormone stimulation cycle would not be needed in order to successfully complete a cPGT procedure. While trying a further cycle may be necessary to obtain transferable embryos not also affected by the secondary target condition, this inevitably comes with the full array of burdens, risks and (moral) costs of regular PGT-M/SR. For this to be acceptable in terms of the 'high risk of a serious disorder' standard, the secondary condition would have to be sufficiently high risk and serious to qualify as a PGT indication on its own (Dondorp and De Wert 2019). Clearly, in countries upholding this standard, making exceptions for cPGT cases requires adequately informing the applicants about this qualification as part of pre-test counselling.

Requests for transferring affected embryos: three categories

There are three types of situation in which professionals may be confronted with requests to transfer affected last chance embryos in cPGT-M/SR (Dondorp and De Wert 2019). At one end of the spectrum, one may think of cases where, notwithstanding a possible categorization by the applicants in terms of primary and secondary, both conditions are evidently highly serious. In our view, transferring embryos leading to disorders in this category would be difficult to reconcile with the responsibility of professionals to take account of the welfare of the child-to-be. Using an example from the list of cPGT-M/SR cases in our centre (Table I), a potential request by couple #25 to transfer any last chance embryos that would lead to a child with either ataxia with oculomotor apraxia type 1 (AOA1) or spinal muscular atrophy type 1 (SMA1) should be rejected. This scenario is theoretical in so far as couples are not likely to ask for the transfer of affected embryos in such cases.

At the opposite end are cases where the secondary condition is clearly not 'high risk and serious', whereas the primary condition is. If, following the reasoning by ESHRE, cPGT-M/SR is allowed for such cases, this may lead to requests for transferring affected last chance embryos that professionals have no good reason to reject, given that such a transfer would not involve a high risk of a child with a seriously

diminished quality of life. Here again, CCD might be an example of such a secondary condition. In such cases, a problem would rather arise with couples requesting an additional hormone stimulation cycle as a further attempt to obtain transferrable embryos not affected by the secondary condition. As explained in the previous section, such a further attempt would bring the proportionality balance back into to the range where the 'high risk of a serious condition' standard would seem to exclude conditions such as CCD.

Probably the most difficult cases fall in the middle of the spectrum. These comprise requests for transferring last chance embryos where the secondary condition, although an accepted indication, is in the grey area where it can be a matter of debate if the condition qualifies as 'high risk and serious'. As an example, one may think here of BRCA-mutations in female fetuses. As these conditions have been subject to quite some discussion about whether they would qualify as acceptable indications under the 'high risk of a serious condition' standard, it is not obvious what the response should be with regard to requests for transferring 'last chance' embryos affected with one of those conditions (Dondorp and De Wert, 2019). We propose shared decision-making about such 'grey area' cases, in which the particular views of the applicants and the history and context of their experiences with the condition ('the story behind the request'; Soto Lafontaine et al. 2018) are taken into account. The one case (couple #14) where last chance embryos affected with Peutz–Jeghers syndrome were transferred on request from a couple having cPGT-M, can perhaps be argued to fall in this middle category.

Scope for reverting to single condition PGT-M/SR

Our conclusion with regard to the first type of cases entails that the couple may end up empty-handed. A possible way out in these cases is to go ahead with a new cycle for one condition only (the condition regarded as primary by the couple). While this would involve a 25–50% chance of embryos with the secondary condition being transferred (in monogenic disorders), professionals (and couples) may feel more comfortable with this alternative as compared with transferring embryos known to be affected. However, in cases with two fully penetrant conditions that are both in the higher range of seriousness, it can be questioned whether testing for just one of those conditions is ethically acceptable, given that a 25 or 50% transmission risk would still amount to a high risk of a child with a seriously diminished quality of life. Ethically, this is similar to doing 'just IVF' when an infertile couple is known to be at a high risk of having a child with a serious disorder (Dondorp and De Wert, 2019). Some centres may find this acceptable on the condition of a clearly indicated intention on the part of the couple to make use of PNT and ask for a termination in case the fetus turns out to be affected (De Wert et al., 2014). Of course, any such understandings about further reproductive decision-making are a matter of trust that cannot be enforced. However, they do relieve professionals from a co-responsibility for the welfare of a seriously affected child that might be born as a result of the couple's backtracking on what was agreed at the pre-treatment stage (Pennings et al., 2003). In cases where the secondary condition is more in a grey-area of risk and seriousness, reverting to PGT-M/SR for the primary condition only may well be acceptable. But if so, solving the dilemma through transferring embryos affected with that grey-area condition, if that is the couple's request, should perhaps be regarded as acceptable as well, also because that would avoid a further cycle with its burdens and costs. It is important that centre-policies with regard to such choices are clearly defined and timely discussed with the couple.

Timely information and counselling

Our data and our analysis underscore the importance of timely information and counselling regarding all relevant aspects of cPGT-M/SR. This includes the inevitably lower number of embryos suitable for transfer, also in the light of other risk factors reducing success rate, such as maternal age. Other options than cPGT-M/SR for both conditions may need to be considered. In addition to the possibility of PGT for one condition and offering PNT for the other, the use of donor gametes to exclude the genetic burden in one of the parents may be an acceptable option for the couple. Moreover, the applicants should be informed already at the pre-test stage of centre policies with regard to the ethically laden choices that may emerge in the process of cPGT-M/SR. This should also include information about the scope for allowing the applicants a change of mind during later stages of the procedure. The one case in our centre where affected embryos were eventually transferred (couple #14), involved strained decision-making at a later stage where professionals had wrongly assumed that there was a clear understanding with the couple that they did not want embryos with either condition to be transferred.

Concluding remarks and recommendations

(i) cPGT-M/SR is feasible.

(ii) cPGT-M/SR should be discussed as a possible treatment option with couples at high risk for offspring with more than one genetic condition.

(iii) Couples applying for cPGT-M/SR should be informed at the stage of pre-treatment informed consent that this procedure entails a lower chance of success than PGT for one condition, and an even further decrease of chances of pregnancy. Pre-test counselling should include a discussion of the couple's views with regard to the relative importance of preventing the birth of a child with either condition. Do they think of those conditions in terms of primary and secondary?

(iv) In countries where PGT-M/SR is only allowed for couples at a high risk of transmitting a serious disorder, this currently limits the scope for the conditions that can be accepted as a secondary condition. However, to the extent that cPGT-M/SR involves an altered proportionality balance, a case can be made for allowing secondary conditions of lower risk and seriousness also in those countries.

(v) Centres allowing cPGT-M/SR may be confronted with requests for transferring embryos affected with what the couple regards as the secondary condition. It is important to have a proactive discussion of all possible outcomes already at the stage of pre-treatment informed consent. This discussion should include the policy of the centre with regard to dealing with such transfer requests. This may lead to a couple-specific proposal for ranking embryos for transfer that takes account of the couple's preferences as well as of the limits to acceptable transfers set by the centre. As we have discussed, professionals should not go ahead with requests for transferring an embryo where there is a high chance of this leading to a child with a seriously diminished quality of life.

(vi) Couples may change their minds with regard to the priority of their preferences during the cPGT-M/SR procedure. A patient-centred policy would require professionals to allow for this as much as reasonably possible, while clearly explaining the inevitable limits to this.

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Authors' roles

C.d.D.-S. and G.d.W. conceived the study. C.d.D.-S. and V.v.d.S designed the background section of the study. V.v.d.S. acquired patient data, executed the study, and wrote the initial version of the manuscript, supervised by C.d.D.-S., J.D., A.P., and E.C. are responsible for the genetic analyses and partly wrote and edited the manuscript. W.D. and G.d.W. designed the ethical reflection that was written by W.D. All authors critically revised the manuscript and approved the final version. V.v.d.S and W.D. contributed equally as first authors.

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Conflict of interest

None to declare.

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