CLINICAL TRIAL

Hereditary breast and ovarian cancer and reproduction: an observational study on the suitability of preimplantation genetic diagnosis for both asymptomatic carriers and breast cancer survivors

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Abstract Preimplantation genetic diagnosis (PGD) is a reproductive option for *BRCA1/2* mutation carriers wishing to avoid transmission of the predisposition for hereditary breast and ovarian cancer (HBOC) to their offspring. Embryos obtained by in vitro fertilisation (IVF/ICSI) are tested for the presence of the mutation. Only *BRCA*-negative embryos are transferred into the uterus. The suitability and outcome of PGD for HBOC are evaluated in an observational cohort study on treatments carried out in two of Western-Europe's largest PGD centres from 2006 until 2012. Male carriers, asymptomatic female carriers and breast cancer survivors were eligible. If available, PGD on embryos cryopreserved before chemotherapy was possible. Generic PGD-PCR tests were developed based on haplotyping, if necessary combined with mutation detection. 70 Couples underwent PGD for *BRCA1/2*. 42/71 carriers (59.2 %) were female, six (14.3 %) of whom have had breast cancer prior to PGD. In total, 145 PGD cycles were performed. 720 embryos were tested, identifying 294 (40.8 %) as *BRCA*-negative. Of fresh IVF/PGD cycles, 23.9 % resulted in a clinical pregnancy. Three cycles involved PGD on embryos cryopreserved before chemotherapy; two of these women delivered a healthy child. Overall, 38 children were liveborn. Two *BRCA1* carriers

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were diagnosed with breast cancer shortly after PGD treatment, despite negative screening prior to PGD. PGD for HBOC proved to be suitable, yielding good pregnancy rates for asymptomatic carriers as well as breast cancer survivors. Because of two cases of breast cancer shortly after treatment, maternal safety of IVF(PGD) in female carriers needs further evaluation.

Keywords Preimplantation genetic diagnosis · Hereditary breast and ovarian cancer · BRCA1 · BRCA2 · Reproductive outcome

Abbreviations
PGD	Preimplantation genetic diagnosis
HBOC	Hereditary breast and ovarian cancer
IVF	In vitro fertilisation
ICSI	Intracytoplasmic sperm injection
BMI	Body mass index
MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
FET	Frozen/thawed embryo transfer cycle

Purpose

Hereditary breast and ovarian cancer (HBOC) is an autosomal dominant cancer predisposition syndrome caused by mutations in tumour suppressor genes Breast Cancer 1 (*BRCA1*, 17q21.31, MIM 113705) or Breast Cancer 2 (*BRCA2*, 13q13.1, MIM 600185). Female carriers have strongly increased risks for both breast and ovarian cancer, estimated at 57 and 49 % for breast cancer and 40 and 18 % for ovarian cancer for *BRCA1* and *BRCA2*, respectively, at the age of 70 [1]. In comparison, women in the United Kingdom in general have a 12.5 % lifetime risk for invasive breast cancer and 1.9 % for invasive ovarian cancer [2, 3]. The prevalence of germline *BRCA1/2* mutations is estimated at 0.1–1.0 % in the general population, making HBOC one of the more prevalent autosomal dominant genetic disorders [4, 5].

Carriers of a *BRCA1/2* mutation have a 50 % risk of passing this predisposition to their offspring. There are several reproductive options to circumvent this, but only two lead to a child genetically related to both partners: prenatal diagnosis and preimplantation genetic diagnosis (PGD). Prenatal diagnosis on the one hand involves genetic testing of a fetus for the presence of a familial *BRCA1/2* mutation during pregnancy, followed by pregnancy termination in case of an unfavourable result. Although applied on a small scale, reports regarding clinical experience with prenatal diagnosis for HBOC are not available in the literature to date. PGD on the other hand involves in vitro fertilisation (IVF) with intracytoplasmic sperm injection

(ICSI), followed by genetic testing of the embryos for the presence of a familial *BRCA1/2* mutation before intrauterine transfer. PGD has been successfully applied since 1990 for an expanding list of monogenic disorders and chromosomal abnormalities [6]. In 2003, the Ethics Taskforce of the European Society of Human Reproduction and Embryology stated that it is acceptable to perform PGD for late onset and multifactorial diseases, including HBOC [7]. In 2005, a survey amongst *BRCA1/2* carriers was carried out to investigate the public attitude towards PGD for HBOC, an important step in the legalisation of PGD for hereditary cancer syndromes in the United Kingdom [8, 9]. This study and other opinion surveys have shown that most *BRCA* carriers consider PGD for HBOC as an acceptable reproductive option, although only a minority of them would consider using PGD personally [9, 10]. However, appliance of both prenatal diagnosis as well as PGD for HBOC remains controversial, considering the reduced penetrance of the condition, its late onset, and availability of prophylactic and therapeutic options [11]. Previous research has shown that safety of ovarian stimulation for IVF is an important consideration for female *BRCA* carriers when deciding on PGD [12]. This topic has not extensively been studied in female *BRCA1/2* carriers to date, although one case–control study did not find a significant adverse effect on the incidence of breast cancer [13]. Up to now, some case reports and small case series have been reported on the clinical experience with PGD for HBOC, with Jasper and colleagues as the first to report a pregnancy in 2008 [14–18].

In 2006, PGD for HBOC was started at the Universitair Ziekenhuis Brussel, Belgium (hereafter named centre A) and in 2008 at Maastricht University Medical Centre +, The Netherlands (hereafter centre B), two large centres for PGD in Western Europe [19]. In this study, we aim to determine the suitability of this treatment, for both asymptomatic male and female *BRCA1/2* carriers as well as *BRCA*-positive female breast cancer survivors, in terms of genetic results, pregnancy rates, and successful deliveries. Additionally, we report on cancer outcome of female mutation carriers.

Methods

Patients

Observational cohort study on PGD cycles performed for *BRCA1/2* mutations from the onset in 2006 until 1-1-2012. Couples of whom at least one partner was known to have a *BRCA1/2* mutation were referred for PGD counselling to our centres. We provided them with verbal and written information regarding the PGD procedure (including IVF

and ICSI, embryo biopsy, single cell analysis, chance of pregnancy, and risk of misdiagnosis). We considered female age >40 years and female body mass index (BMI) >30 kg/m^2 as relative contra-indications for PGD, whereas female age ≥43 years and female BMI ≥35 kg/m^2 were absolute contra-indications.

Gynaecological screening procedures

We performed gynaecological and andrological examination, including sperm analysis, female hormonal assessment and virology tests of both partners, to ensure suitability of the couple for IVF/ICSI treatment. In cases where embryos were harvested by IVF/ICSI prior to chemotherapy, appliance of PGD on these cryopreserved embryos was possible.

Oncological screening procedures

We screened female carriers without a prophylactic mastectomy in the past for the presence of occult breast cancer before admission to the PGD programme. In addition to annual screening procedures, at least a magnetic resonance imaging (MRI) of the breasts was performed prior to the start of PGD [20, 21]. BRCA-positive women with a history of breast cancer were eligible for PGD, if they had been free of malignant disease for at least 2 years after their oncologic treatment. Depending on age and familial phenotype, we screened female carriers for the presence of occult ovarian carcinoma prior to admission to the PGD programme by gynaecological and ultrasound examination and CA-125 determination in blood.

PGD procedures

Prior to the introduction of the PGD programme, we obtained medical ethical approval of the institutional review boards at both centres. All couples gave their informed consent before PGD was started. We performed IVF and PGD according to international guidelines [22, 23] and used ICSI for fertilisation to avoid contamination of the zona pellucida with spermatozoa, which may disturb the PGD analysis. We biopsied obtained embryos three days after fertilisation. Single cell analysis of the blastomeres was performed using polymerase chain reaction (PCR), based on haplotyping of at least two informative flanking microsatellite markers on each side of the BRCA1/2 loci. In a minority of cases, this generic test was not informative. In these cases we set up a mutation-specific protocol, based on identifying the private mutation in combination with at least one informative marker (Table 1) [15, 24]. After single cell analysis, we classified the embryos as affected (BRCA1/2 mutation present), unaffected (BRCA1/2 mutation absent),

Table 1 PGD strategies for BRCA1/2 mutations

	Centre A[a]	Centre B[b]
Indirect testing BRCA1 mutations[c]	BRCA1STR24CA, BRCA1STR20TG, BRCA1STR16GA, BRCA1STR4, BRCA1STR21CA, D17S2249, D17S1323, D17S855	D17S932, BRCA1_dis24AC, D17S950, D17S1814, D17S800, D17S1787
Indirect testing BRCA2 mutations[c]	BRCA2STR19TG, BRCA2STR20GT, BRCA2STR18AC, D13S260, D13S171	D13S171, D13S1695, BRCA2_dist18AC, D13S267, D13S289, D13S260, D13S1698, BRCA2STR19
Alkaline lysis buffer	50 mM DTT, 200 mM NaOH	50 mM DTT, 200 mM NaOH
Freezing post tubing	30' −20 °C	30' −20 °C
Decontamination	UV-C	UV-C
Polymerase	Qiagen Multiplex PCR KIT	Qiagen Multiplex PCR KIT
Split for multiplex PCR	No	No
Genetic analyser	ABI3730xl	ABI3730xl

PGD preimplantation genetic diagnosis, BRCA1 breast cancer gene 1, BRCA2 breast cancer gene 2, PCR polymerase chain reaction

[a] Universitair Ziekenhuis Brussel, Belgium

[b] Maastricht University Medical Centre +, The Netherlands

[c] Many of the markers have not been published; the primer sequences were designed in-house and are available upon request. In case indirect testing was not possible due to either non-informativity of markers or availability of family members, mutation-specific tests were developed including the typical familial mutation combined with at least 2 markers

abnormal (abnormal genotype, e.g. haploidy or triploidy) or no diagnosis (no test result or inconclusive BRCA1/2 status). Subsequently, one or two unaffected embryos were transferred into the uterus at day four or five postfertilisation. The number of transferred embryos depended on embryo quality, female age, number of previous unsuccessful attempts and the couples' preference for transferring only one embryo. Supernumerary unaffected embryos of sufficient quality were cryopreserved and transferred in a subsequent cycle after thawing (defined as 'frozen/thawed embryo transfer cycle, FET') [25]. FETs were included in the survey, if they followed a fresh IVF/PGD cycle during the study period, and in case the embryo transfer was performed before 1-10-2012.

Pregnancy rates are reported as positive hCG tests as well as clinical pregnancy rates. The clinical pregnancy rate was diagnosed according to the standard definition, i.e. a pregnancy diagnosed by transvaginal ultrasonographic visualisation of one or more gestational sacs or definite

clinical signs of pregnancy, including ectopic pregnancy. A delivery was defined as the birth of one or more fetuses after at least 20 completed weeks of gestational age [25].

Couples were given the option of prenatal diagnosis to confirm PGD outcome. Follow-up of pregnancies and children was carried out at centre A as described earlier [26] and at centre B using a questionnaire. At the end of the study time (i.e. 1-10-2012), all female *BRCA1/2* carriers were contacted by telephone and asked for their health status, including diagnosis of breast cancer since the last PGD treatment and prophylactic surgeries performed in the meantime.

Statistical analysis

Statistical analyses were performed using SPSS 18.0.0. Data are presented as mean and standard deviation (for continuous variables) or number of cases and percentages (for categorical variables).

Results

Patients

70 Couples underwent PGD for HBOC. In one couple, the male and female partner were both a *BRCA1* carrier. Of 71 carriers, 42 were female (59.2 %). Of the female carriers, 28 (66.7 %) had a *BRCA1* mutation and fourteen (33.3 %) had a *BRCA2* mutation. Of 29 male carriers, 21 (72.4 %) had a *BRCA1* mutation and eight (27.6 %) had a *BRCA2* mutation. Over a quarter of female carriers (11/42, 26.2 %) had undergone prophylactic breast surgery before PGD. Six out of 42 female carriers (14.3 %) had a history of breast cancer (Table 2).

Outcome

In total, 145 PGD cycles were carried out (Table 3). Three of these cycles involved PGD on embryos cryopreserved before chemotherapy because of breast cancer. Overall, 720 embryos were tested for *BRCA1/2*, identifying 294 (40.8 %) as unaffected, 311 (43.2 %) as affected, 70 (9.7 %) as abnormal, and 45 (6.3 %) as having no diagnosis. In 87 out of 142 fresh IVF/PGD cycles (61.3 %), one or two embryos were transferred, resulting in 37 positive hCG tests and 34 clinical pregnancies. Clinical pregnancy rates were 23.9 % per cycle started and 39.1 % per embryo transfer. Subsequently to these fresh IVF/PGD cycles, 34 frozen/thawed embryo transfer cycles (FETs) were performed, resulting in ten positive hCG tests and nine clinical pregnancies (clinical pregnancy rate 26.5 % per embryo transfer, Table 3).

Table 2 Couples' characteristics

	n = 70
Nulliparity prior to PGD	60 (85.7 %)
At-risk person	
Male	28 (40.0 %)
Female	41 (58.6 %)
Both partners	1 (1.4 %)[a]
Mutation	
BRCA1	48 (68.6 %)
BRCA2	22 (31.4 %)
Mean female age in years (SD)	29.5 (3.6)
Female carriers	29.6 (3.7)
Mean female BMI (SD)	23.1 (3.4)
Female carriers with prophylactic mastectomy	11 (26.2 %)
Female carriers with history of breast cancer	6 (14.3 %)

PGD preimplantation genetic diagnosis, *BRCA1* breast cancer gene 1, *BRCA2*, breast cancer gene 2, *SD* standard deviation, *BMI* body mass index (kg/m^2)

[a] Both partners were *BRCA1* carrier, only unaffected embryos were eligible for embryo transfer

Three out of six women with a history of breast cancer had harvested embryos prior to chemotherapy and underwent PGD on these embryos. Two of them delivered a healthy child after PGD. Subsequently, two of these three women were denied a fresh ovarian stimulation for PGD because of a diminished ovarian reserve after chemotherapy. The third woman and the three women who did not cryopreserve embryos were treated in one or more fresh IVF/PGD cycles. One of them delivered a healthy child (Table 4).

In centre A, four couples pregnant after PGD for *BRCA1* requested prenatal diagnosis to confirm PGD diagnosis. In two cases, a chorionic villus biopsy was performed (one in a twin pregnancy), amniocentesis in the other two. All results were *BRCA*-negative, confirming PGD outcome. None of the pregnant couples treated in centre B opted for prenatal diagnosis to confirm PGD diagnosis.

Out of a total of 45 clinical pregnancies, 36 (80.0 %) proceeded to birth. The other 9 resulted in a miscarriage or concerned ectopic pregnancies. Of 41 children (31 singletons and five twins), 38 (92.7 %) were born alive. One singleton pregnancy was terminated at 23 weeks of gestation because of multiple congenital malformations based on a de novo chromosomal abnormality (deletion 3q26.2 and duplication 15q11.2). Two other children were stillborn: one member of twins died in utero at 24 weeks of gestation for unknown reasons, the other sibling was born alive at 33 weeks. One member of another twin died in utero due to abruption of the placenta at 35 weeks of gestation. The other sibling was born alive. One singleton

Table 3 Reproductive outcome of PGD for HBOC (n = 70 couples)

	IVF/PGD cycles	PGD on embryos cryopreserved before chemotherapy
PGD treatments started	142	3
Mean treatments started per couple (SD)	2.1 (1.3)	1
Biopsied embryos	720	
Unaffected	294 (40.8 %)	
Affected	311 (43.2 %)	
Abnormal	70 (9.7 %)	
No diagnosis	45 (6.3 %)	
Ovarian stimulations to embryo transfer[a]	87 (61.3 %)	N/A
Positive hCG tests	37	N/A
% Per oocyte retrieval	30.3 %	
% Per embryo transfer	42.5 %	
Clinical pregnancies	34	
% Per oocyte retrieval	27.9 %	
% Per embryo transfer	39.1 %	
FET	34	3
Positive hCG tests after FET	10	2
% Per FET after IVF/PGD	29.4 %	
% Per FET of embryo(s) cryopreserved before chemotherapy		66.7 %
Clinical pregnancies after FET	9	2
% Per FET after IVF/PGD	26.5 %	
% Per FET of embryo(s) cryopreserved before chemotherapy		66.7 %
Pregnancies (total)	49	
Pregnancies ongoing ≥12 weeks	36	
Lost to follow-up <12 weeks	1	
Deliveries (≥20 weeks)	36	
Singletons	31	
Twins	5 (10 children)	
Lost to follow-up ≥20 weeks	0	

PGD preimplantation genetic diagnosis, *HBOC* hereditary breast and ovarian cancer, *IVF* in vitro fertilisation, *SD* standard deviation, *hCG* human chorionic gonadotropin, *FET* frozen/thawed embryo transfer cycle, *N/A* not applicable

[a] One couple, who underwent two IVF/PGD cycles, requested cryopreservation of unaffected embryos because of prophylactic salpingo-oophorectomy. In five other IVF/PGD cycles, unaffected embryos were cryopreserved to postpone embryo transfer for different reasons (ovarian hyperstimulation syndrome (n = 2), insufficient endometrial buildup (n = 2), and delay of PGD results (n = 1)

pregnancy was complicated by premature labour at 26 weeks of gestation. At the end of study time, at age 2.5 years, the girl born was doing well.

Follow-up of female *BRCA1/2* carriers

Two *BRCA1* carriers (one in each PGD centre) were diagnosed with early stage triple negative breast cancer within two, respectively, three months after their first ovarian stimulation for IVF/PGD, despite having a negative breast screening shortly before (Table 5). One of them had a history of contralateral breast cancer. Both women did not become pregnant after PGD.

One female carrier did not want to be contacted to check on her medical condition after PGD for personal reasons; all other female carriers were contacted. None of them have been diagnosed with breast cancer after PGD. Mean exposed follow-up time (from ovarian stimulation until end of follow-up or until prophylactic breast surgery) was 27.5 months (range 2–68 months).

Conclusions

This study establishes the clinical suitability of PGD for *BRCA1/2* mutations in both asymptomatic carriers and *BRCA*-positive female breast cancer survivors, either in a fresh IVF/PGD cycle as well as on embryos harvested before chemotherapy. A series of 145 consecutive PGD cycles are presented, the first large series of PGD for HBOC. When compared to the outcome of PGD for autosomal dominant disorders as reported by the European Society of Human Reproduction and Embryology PGD consortium, our clinical pregnancy rates are in line with

Table 4 Characteristics of women with a history of breast cancer before PGD treatment

	Centre A[a]			Centre B[b]		
	Patient A[c]	Patient B	Patient C	Patient D	Patient E	Patient F
Reproductive history	None	None	None	None	2005 healthy daughter, 2007 miscarriage, 2007 molar pregnancy	None
Gynaecologic history	None	None	None	2007 unilateral salpingo-oophorectomy (inflammation)	2007 dilatation and curettage	None
Gene mutation	*BRCA1*	*BRCA1*	*BRCA1*	*BRCA2*	*BRCA1*	*BRCA2*
Age at breast cancer diagnosis	31	29	26	33	33	32
Oncologic treatment						
Surgery	Mastectomy	Mastectomy	Skin sparing mastectomy	Mastectomy	Modified radical mastectomy	Lumpectomy
Chemotherapy	Yes	Yes	Yes	Yes	Yes	No
Irradiation	No	Yes	Yes	No	Yes	Yes
Contralateral prophylactic mastectomy	No	Yes	Yes	Yes	Yes	No
PGD treatment						
Embryos cryopreserved before chemotherapy	Yes	No	Yes	Yes	Yes	N/A
Disease free interval before first PGD cycle (years)	2.5	4.0	2.0	3.8	2.8	4.5
PGD on embryos cryopreserved before chemotherapy	No	N/A	Yes	Yes	Yes	N/A
Outcome	N/A	N/A	Healthy son born	Not pregnant	Healthy daughter born	N/A
IVF/PGD after recovery from cancer	Yes	Yes	Yes	No (denied because of chemotherapy induced infertility)	No (denied because of chemotherapy induced infertility)	Yes
Outcome	No embryo transfer	Not pregnant	Ectopic pregnancy	N/A	N/A	Healthy son born

PGD preimplantation genetic diagnosis, *BRCA1* breast cancer gene 1, *BRCA2* breast cancer gene 2, *IVF* in vitro fertilisation, *N/A* not applicable

[a] Universitair Ziekenhuis Brussel, Belgium

[b] Maastricht University Medical Centre +, The Netherlands

[c] Same patient as patient G in Table 5. This patient was diagnosed with breast cancer and underwent one IVF cycle prior to chemotherapy to cryopreserve embryos ($n = 6$). After recovery, she chose for a new ovarian stimulation for IVF/PGD instead of using her cryopreserved embryos first. After IVF/PGD, she was diagnosed with contralateral breast cancer and needed chemotherapy again (see Table 5). She underwent two IVF cycles to cryopreserve embryos ($n = 1$). Besides, a laparoscopic unilateral oophorectomy took place to cryopreserve the ovary. After recovery of the second breast cancer, she conceived spontaneously and gave birth to a twin

these data (39.1 vs. 26.7 % per embryo transfer respectively) [6]. Two factors known to influence reproductive outcome in *BRCA1/2* carriers were present in our series: on the one hand, the women included in our survey were younger than those reported by the PGD consortium (29.6 vs. 34 years), which is a favourable factor for reproductive outcome. On the other hand, it was hypothesised that *BRCA1/2* mutations may unfavourably reduce ovarian reserve due to accumulated DNA damage secondary to inadequate DNA repair [27]. In total, 49 pregnancies were established, resulting in the birth of 31 singletons and five twins. The observation of two perinatal deaths and one pregnancy termination because of major malformations in our cohort of 41 children is presumed to be an coincidence; PGD is not associated with an increased risk for perinatal deaths or major congenital malformations [26]. However, the health of children born after PGD (for HBOC) needs to be subject to further research and longer follow-up.

Analysis of the blastomeres for the presence of *BRCA1/2* reflected the suspected 50/50 distribution of unaffected

Table 5 Overview of women who were diagnosed with breast cancer after PGD treatment

	Patient G[a] Centre A[b]	Patient H Centre B[c]
PGD Centre Gene mutation	*BRCA 1*	*BRCA 1*
Oncologic history prior to PGD	Diagnosed with breast cancer in 2006: invasive ductal carcinoma, T2mN0M0, triple negative. Treatment consisted of mastectomy and chemotherapy	None
Reproductive history prior to PGD cycle after which breast cancer was diagnosed	One IVF cycle for fertility preservation prior to chemotherapy in 2006 (6 embryos). It was the patient's choice to undergo a new ovarian stimulation for PGD instead of using the cryopreserved embryos first	None
Age at breast cancer diagnosis after PGD	34	28
Last breast screening before PGD	1 month before PGD, MRI: no abnormalities	2 months before PGD, MRI: no abnormalities
Number of IVF/PGD cycles	1	1
Outcome IVF/PGD cycle	No embryo transfer	Not pregnant
Breast cancer diagnosis	2 months after PGD, MRI	3 months after PGD, MRI
Pathology	Invasive ductal carcinoma, T1cN0M0, triple negative	Invasive ductal carcinoma, T1bN1aM0, triple negative
Treatment		
Oncologic surgery	Mastectomy, SNP	Lumpectomy, axillary lymph node dissection
Systemic therapy	Yes	Yes
Irradiation	No	Yes
Prophylactic mastectomy	N/A	Planned
Current status	No evidence of disease, delivered twin after spontaneous conception (see legend Table 4)	No evidence of disease, wishes to continue PGD after recovery of prophylactic surgery

PGD preimplantation genetic diagnosis, *BRCA1* breast cancer gene 1, *IVF* in vitro fertilisation, *MRI* magnetic resonance imaging, *SNP* sentinel node procedure, *N/A* not applicable

[a] Same patient as patient A in Table 4

[b] Universitair Ziekenhuis Brussel, Belgium

[c] Maastricht University Medical Centre +, The Netherlands

(41 %) versus affected (43 %) embryos. Almost 10 % of the embryos showed fertilisation abnormalities (e.g. haploidy or triploidy), which is not an uncommon finding in preimplantation embryos. We presume that the diagnostic accuracy of PGD analyses based on PCR is high; an earlier study in one of our centres reported a false-negative rate of 0.5 % in surplus embryos [28]. This is in accordance to the reported misdiagnosis rate of 0.4 % in pregnancies established after PGD for monogenic disorders detected by PCR analysis [29]. However, since only few of the pregnant couples opted for prenatal diagnosis, definitive confirmation of PGD diagnosis was not possible in the majority of cases. Because genetic testing for adult-onset disorders in childhood is ethically controversial and therefore discouraged [30], postnatal testing after PGD for HBOC was not performed.

In addition to the suitability of PGD for HBOC following a fresh IVF/PGD cycle, we also demonstrated that PGD on embryos harvested prior to chemotherapy is an applicable option: two out of three women treated, delivered a healthy child. These results stress the importance of timely counselling regarding fertility preserving options available for young women with breast cancer [31, 32]. This is important not only to retain an option to reproduce in case oncological treatment would cause infertility but also because PGD can be applied on harvested embryos in case of *BRCA* carriership. Known *BRCA* status at the moment of fertility preservation is not a prerequisite, provided that ICSI is used for fertilisation to keep the possibility of PCR analysis. When cryopreserved embryos of sufficient quality are available, it is preferable to use these first for PGD. This can save the patient a new ovarian stimulation, which may be less successful in case of a diminished ovarian reserve after oncological treatment.

Two women in our cohort were diagnosed with breast cancer after their first IVF/PGD cycle. One of these women

had a history of contralateral breast cancer. Both women were carrier of a *BRCA1* mutation. *BRCA1*-associated tumours are characterised by a higher proportion of interval tumours and a younger age and more often an unfavourable size at diagnosis, when compared with *BRCA2*-associated tumours. Besides, invasive *BRCA1* breast tumours are often high grade and rapidly growing [33, 34]. Whilst a possible linkage between IVF treatment and breast cancer risk has extensively been studied in the general population [35], safety of IVF with regards to the risk for breast cancer has not been systematically studied in female *BRCA1/2* carriers. Gonadotropin use for IVF results in a rise in oestrogens. Several observations suggest an influence of (prolonged) exposure to oestrogens on incidence of *BRCA*-related breast cancers, although ∼80 % of *BRCA1*-tumours are oestrogen and progesterone receptor negative [36]. Kotsopoulos and colleagues conducted a matched case–control study to examine the influence of fertility medications for IVF treatment on breast cancer risk in *BRCA1/2* carriers. They were able to include 26 carriers with a history of gonadotropin use, sixteen of whom were diagnosed with breast cancer (multivariate OR 2.32, 95 % CI 0.91–5.95, $P = 0.08$). The sample size of the study may have been too limited however to detect a significant adverse effect of gonadotropin use on breast cancer risk in *BRCA1/2* carriers [13]. One study reported an association between fertility treatment and an increased risk for breast cancer in women with a positive family history for breast cancer (RR 1.4; 95 % CI 1.0–1.9) [37], whilst others did not find fertility (treatment) and breast cancer to be associated in these women [38, 39]. It is possible, yet unproven that administering gonadotropins may have led to an acceleration in growth of pre-existing, but not yet detectable, tumours in the two affected *BRCA1* carriers in our cohort. Therefore, we stress the importance of screening of the breasts before admission to IVF/PGD treatment, as well as after treatments. Larger studies are needed to elucidate whether our observation is just a coincidental finding in a population with a high a priori risk for breast cancer, or whether a causal relationship exists.

This study has some limitations. Firstly, the sample size is relatively small. Secondly, reliability of PGD diagnosis could not be confirmed; it was ethically impossible to test *BRCA*-status of the children born after PGD, due to the late onset character of the predisposition and the children's autonomy. Finally, our study was not primarily designed to assess maternal safety of IVF in female carriers.

Recommendations

This survey shows that PGD for HBOC is an established and suitable technique with good reproductive outcome, which should be offered as part of a comprehensive approach to the counselling and treatment of all *BRCA1/2* patients. It is important that medical professionals involved in the care for *BRCA* carriers are aware of this reproductive option, in order to inform patients timely and to refer them, at request, to a specialised PGD centre. In case of a newly diagnosed breast cancer in a woman of reproductive age, it is essential to be aware of the possibility of PGD if and when *BRCA* carriership would turn out. Given the complex medical history of female carriers and our observation of two breast cancer cases after PGD treatment, a multidisciplinary approach is a prerequisite in PGD practice. In addition, oncological screening of female carriers before admission to the treatment, as well as careful follow-up, is required.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This study complies with current laws in The Netherlands and Belgium. Medical ethical approval of the institutional review boards was obtained before the start of PGD at both centres. Participants gave their informed consent before they were enrolled in the programme.

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