J Genet Counsel (2016) 25:1327–1337
DOI 10.1007/s10897-016-9975-4

ORIGINAL RESEARCH

Preimplantation Genetic Diagnosis (PGD) for Monogenic Disorders: the Value of Concurrent Aneuploidy Screening

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Received: 23 December 2015 / Accepted: 16 May 2016 / Published online: 9 June 2016

Abstract Pre-implantation genetic diagnosis (PGD) has changed the landscape of clinical genetics by helping families reduce the transmission of monogenic disorders. However, given the high prevalence of embryonic aneuploidy, particularly in patients of advanced reproductive age, unaffected embryos remain at high risk of implantation failure or pregnancy loss due to aneuploidy. 24-chromosome aneuploidy screening has become widely utilized in routine in vitro fertilization (IVF) to pre-select embryos with greater pregnancy potential, but concurrent 24-chromosome aneuploidy screening has not become standard practice in embryos biopsied for PGD. We performed a retrospective cohort study of patients who underwent PGD with or without 24-chromosome aneuploidy screening to explore the value of concurrent screening. Among the PGD + aneuploidy-screened group (n = 355 blastocysts), only 25.6 % of embryos were *both* Single Gene Disorder (SGD)-negative (or carriers) and euploid; thus the majority of embryos were ineligible for transfer due to the high prevalence of aneuploidy. Despite a young mean age (32.4 ± 5.9y), 49.9 % of Blastocysts were aneuploid. The majority of patients (53.2 %) had ≥1 blastocyst that was Single Gene Disorder (SGD)-unaffected *but* aneuploid; without screening, these unaffected but aneuploid embryos would likely have been transferred resulting in implantation failure, pregnancy loss, or a pregnancy affected by chromosomal aneuploidy. Despite the transfer of nearly half the number of embryos in the aneuploidy-screened group (1.1 ± 0.3 vs. 1.9 ± 0.6, $p < 0.0001$), the implantation rate was higher (75 % vs. 53.3 %) and miscarriage rate lower (20 % vs. 40 %) (although not statistically significant). 24-chromosome aneuploidy screening when performed concurrently with PGD provides valuable information for embryo selection, and notably improves single embryo transfer rates.

Keywords Pre-implantation genetic diagnosis (PGD) · 24-chromosome aneuploidy screening · Single gene disorder · Monogenic disorder · Single embryo transfer

Introduction

Pre-implantation genetic diagnosis (PGD) was first performed in the 1990s to reduce the risk of transmitting monogenic disorders to offspring of carriers or affected individuals, and the indications have since evolved to include the selection of euploid embryos for embryo transfer (pre-implantation genetic screening, PGS) (Grifo et al. 1992, Harper and Harton 2010). When traditional in vitro fertilization (IVF) is performed without aneuploidy screening, embryos are selected for transfer using a standard morphologic grading system (Gardner 1999). However, 50 % of blastocysts deemed to be 'top-grade' (grade 5 and 6) by standard morphologic criteria are in fact aneuploid, confirming that morphology is a poor predictor of euploidy (Alfarawati et al. 2011). Non-invasive means such as time-lapse embryo imaging have been unsuccessfully employed to identify morphokinetic parameters correlating with embryo euploidy

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(Campbell et al. 2013, Kramer et al. 2014, Rienzi et al. 2015). However, 24-chromosome aneuploidy screening remains the only way to accurately identify a euploid embryo.

Embryo biopsy techniques for PGD have evolved from the biopsy of a single blastomere cell on day 3 to the biopsy of 5–7 trophectoderm cells from the blastocyst on day 5 or 6 of culture, and the only randomized controlled trials to demonstrate a beneficial effect of embryonic aneuploidy screening on implantation and delivery rates utilized trophectoderm biopsy (Scott et al. 2013a, b; Yang et al. 2013). As biopsy techniques have evolved, so has the ability to amplify greater quantities of DNA thereby allowing the simultaneous testing of both monogenic disorders and aneuploidy. Reports are emerging of simultaneous pre-implantation genetic testing for single gene disorders and aneuploidy utilizing TE biopsy and 24-chromosome aneuploidy screening, but this practice is not yet universally accepted as standard practice (Brezina et al. 2011, Daina et al. 2013, Obradors et al. 2008, 2009, Rechitsky et al. 2013, Shen et al. 2013, Treff et al. 2011, 2013). Rechitsky et al. described their center's experience combining PGD and concurrent aneuploidy screening; however, the analysis included embryos biopsied at both the cleavage and blastocyst stage as well as embryo transfers occurring in both fresh and frozen cycles, variables known to impact pregnancy outcomes and thus muddying the interpretation of results (Rechitsky et al. 2015, Scott et al. 2013a, b; Shapiro et al. 2014).

The objective of this study was to better understand the advantages of simultaneous testing for both monogenic disorders and aneuploidy in a relatively homogenous population of patients undergoing blastocyst culture, trophectoderm biopsy, and single gene testing, with and without aneuploidy screening. The aims are two-fold: first, to quantify the incidence of aneuploidy in a population of patients undergoing trophectoderm biopsy and PGD for a single gene disorder, in order to better understand the advantages of simultaneous testing and the possible risk of not performing concomitant aneuploidy screening. We then sought to compare pregnancy outcomes of patients with single gene-tested embryos with and without aneuploidy screening to characterize any improvement in outcomes following 'dual-screening.'

Methods

Participants

A retrospective cohort study was performed at the Fertility Center at New York University Langone Medical Center (NYUFC). Approval was obtained by the Institutional Review Board (IRB) of the New York University (NYU) School of Medicine. Cycles analyzed included those performed between July 2010 and August 2014 in which patients underwent TE biopsy for PGD of a single gene disorder with simultaneous 24-chromosome aneuploidy screening using array comparative genomic hybridization (aCGH). In patients who underwent multiple cycles of PGD with aCGH, only their first cycle was analyzed. Patients were excluded if embryos from multiple cycles were biopsied or if biopsies were performed for gender selection, HLA typing or translocation.

Cycle parameters analyzed include patient age at the time of oocyte retrieval and biopsy, number of oocytes and metaphase-II (M-II) oocytes retrieved, total units of gonadotropin required during stimulation, peak estradiol on the day of ovulation trigger, number of two pro-nuclear (2PN) zygotes, indication for embryo biopsy, total number of blastocysts and good-quality blastocysts available for biopsy, and total number of embryos biopsied on day 5 and day 6 (and rarely day 7). Single gene results were then analyzed for all blastocysts biopsied, as well as results of 24-chromosome aneuploidy screening. For patients who returned for frozen embryo transfer, additional parameters analyzed included number of embryos transferred, implantation rate (number of intrauterine sacs per total embryos transferred), spontaneous abortion rate (number of spontaneous abortions per pregnancy with at least one intrauterine sac) and live birth or ongoing pregnancy rate.

Procedures

Ovarian Stimulation

Before initiation of treatment, menstrual day 2 or 3 serum estradiol (E2) and follicle-stimulating hormone (FSH) levels were assessed. Patients with acceptable parameters were then stimulated using injectable gonadotropins (Follitropin beta, Schering Plough, NJ; Serono Pharmaceuticals, Rockland, MA; Menotropins, Parsippany, NJ), with LH suppression achieved using either a GnRH agonist (leuprolide acetate, TAP Pharmaceuticals, Lake Forest, IL) or antagonist (ganirelix acetate, Organon; cetrorelix, Serono). Ovulation was triggered when ≥2 follicles reached ≥17 mm in diameter; ultrasound-guided transvaginal oocyte retrieval was performed 34–36 h later.

Pre-Implantation Genetic Diagnosis

Embryos intended for trophectoderm biopsy underwent laser ablation to create a small breech in the zona pellucida on day 3 of embryo development (Saturn, Research Instruments, Falmouth, United Kingdom). Embryos were cultured to day 5, and embryos unsuitable for day 5 biopsy were cultured to day 6 or rarely day 7. On the day of trophectoderm biopsy, a piece of extruded trophectoderm was isolated and cut using the Cronus laser. The biopsied cells were placed in Eppendorf tubes, cryopreserved in dry ice, and transported to an outside facility for PGD/PGS analysis. For PGD analysis, a patient-

specific test was developed using linkage analysis via short tandem repeats (STRs) using multiplex PCR along with direct mutation testing. Aneuploidy screening was performed using aCGH as described (Gutierrez-Mateo et al. 2011). Single Gene Disorder (SGD) testing was performed on the same whole genome amplified DNA (Repli-G MIDI from Qiagen, 150,045), employing an isothermal whole genome amplification method (multiple displacement amplification [MDA]), for non-specific amplification of the genome of the biopsied sample. Polymerase chain reaction (PCR) was then performed to amplify cells to a detectable level of DNA fragments encompassing the mutation site and/or the linked polymorphic loci assessed. This technique has an amplification failure rate of 1.6 %. Published studies utilizing MDA to amplify single cell samples showed overall allele drop-out rates ranging from 9 to 31 %, and allele drop-out rates of individual loci range from 0 to 60 % (Burlet et al. 2006, Glentis et al. 2009, Handyside et al. 2004, Hellani et al. 2005, Lledo et al. 2006, Ren et al. 2007, Renwick et al. 2007, Spits et al. 2006).

Blastocyst Vitrification

Embryos were cryopreserved using vitrification by first equilibrating in media containing the lowest concentration of cryoprotectants (7.5 % ethylene glycol (EG) and 7.5 % dimethyl sulfoxide (DMSO)) to achieve the first level of dehydration. Embryos were then placed in vitrification solution with cryoprotectants (15 % EG and 15 % DMSO) and subsequently loaded onto the Cryolock vitrification device (Cummings, GA) and plunged directly into liquid nitrogen.

Frozen Embryo Transfer

Patients scheduled for frozen embryo transfer underwent uterine preparation using sequentially increasing doses of oral estradiol until endometrial diameter reached ≥7 mm in greatest diameter. Progesterone was then added (Progesterone in oil 50 mg/day; Watson Pharmaceuticals, Corona, CA). Blastocyst transfer occurred on the sixth day of Progesterone.

Data Analysis

Univariate analyses were performed using student's t-test and fisher's exact test or chi-square where appropriate. Data are presented in averages (%) or mean ± standard deviation (SD).

Results

Forty-seven patients who met inclusion criteria underwent their first cycle of TE biopsy and PGD for a single gene disorder with concurrent aneuploidy screening between July 2010 and August 2014; 355 blastocysts were biopsied (day-5 = 199; day-6 = 148; day-7 = 8). Ten patients with 64 blastocysts (day-5 = 38; day-6 = 26) underwent TE biopsy and PGD without 24-chromosome screening during the same time period. There were no differences between groups when comparing age (32.4 ± 5.9 vs. 34.4 ± 4.6, $p = 0.3$), baseline ovarian reserve testing, units of gonadotropin required during ovarian stimulation, number of oocytes retrieved, number of 2PN zygotes, total number of blastocysts, and number of good-quality blastocysts available for biopsy (7.6 ± 5.4 vs. 6.4 ± 3, $p = 0.5$) (Table 1). The estradiol (E2) on the day of ovulation trigger was lower in the dual-screening group compared to the group undergoing SGD testing without aneuploidy screening (2712 ± 1005 vs. 3832 ± 1782, $p = 0.01$), but there were otherwise no differences between groups (Table 1).

Among patients pursuing PGD + aCGH, PGD was performed for the following indications: Fragile X ($n = 7$), BRCA ($n = 5$), Huntington's disease ($n = 2$), Cystic Fibrosis ($n = 2$), Tay Sachs ($n = 2$), Alpha Thalassemia ($n = 1$), Gauchers ($n = 2$), Sickle Cell ($n = 1$), Muscular Dystrophy ($n = 3$), Multiple Endocrine Neoplasia ($n = 1$), Hemophilia ($n = 2$), Hereditary Angioedema ($n = 1$), Charcot Marie Tooth ($n = 1$), other ($n = 17$). For the PGD alone group, the following indications applied ($n = 1$): Charcot Marie Tooth, Li Fraumeni Syndrome, Hemophilia, Muscular Dystrophy, Sickle Cell, Gauchers, Beta Thalassemia, CF, BRCA, Tay Sachs (Tables 2 and 3).

There were no differences between the SGD + aneuploidy-screened group and SGD-alone group when comparing the percentage of blastocysts affected by the single gene disorder of interest (37.0 % vs. 32.8 %, $p = 0.57$). Of all blastocysts that underwent testing for SGD + aCGH, 49.9 % were aneuploid. Notably, among blastocysts biopsied for SGD + aCGH, 16.3 % were unaffected by a single gene disorder but aneuploid. Only 25.6 % of blastocysts biopsied tested negative or were carriers of the single gene disorder and also euploid; therefore, only one quarter of blastocysts were ultimately eligible for transfer following dual-screening. This compares to 54.7 % of embryos in the SGD-alone group that were deemed eligible for transfer based on their unaffected or carrier status after SGD testing ($p = .001$). Notably, 53.2 % of patients had at least one embryo that was unaffected by the SGD but aneuploid; in other words, greater than half of all patients were at risk of transferring an embryo that that was deemed 'unaffected' but was in fact aneuploid (Table 4). Of note, SGD biopsy results were available for 88.1 % of blastocysts in the SGD + aneuploidy-screened group and 87.5 % of the SGD-alone group. A small number of patient outliers were responsible for the relatively high percentage of blastocysts with no SGD-result, including four patients in the SGD + aneuploidy group who had ≥3 blastocysts without biopsy results, and 2 patients in the SGD-alone group who had ≥3 blastocysts without data. One patient in the SGD + aneuploidy group had 17 blastocysts tested for Huntington's

Table 1 Patient and cycle characteristics

	PGD + 24-chromosome aneuploidy screening $n = 47$ patients	PGD alone $n = 10$ patients	p-value
Age (years)	32.4 ± 5.9	34.4 ± 4.6	0.3
Day 2 E2 (pg/ml)	44.1 ± 21	47.1 ± 19	0.7
Day 2 FSH (IU/ml)	6.9 ± 8.3	5.5 ± 2.5	0.6
Total gonadotropins (units)	3541 ± 1733	2981 ± 1505	0.3
E2 on day of ovulation trigger (pg/ml)	2712 ± 1005	3832 ± 1782	.01
No. oocytes retrieved	18.6 ± 10.8	20.5 ± 6.7	0.6
No. 2PN zygotes	11.8 ± 7.4	14 ± 5.6	0.4
Mean Blastocysts per cycle	8.7 ± 5.9	8.2 ± 3.9	0.8
Mean Blastocysts biopsied per cycle (total blastocysts biopsied)	7.6 ± 5.4 (355)	6.4 ± 3.0 (64)	0.5

Data are presented in mean ± standard deviation. Data were analyzed with student's t-test, $p < 0.05$

Disease but only 3 blastocysts for which a result was available; this highly unusual outcome represents a rare exception but certainly contributed to the high percentage of 'no-result' outcomes. In total, there were 43 blastocysts with no result in the SGD + aneuploidy group and 15 blastocysts with no result in the SGD-alone group. Of the 43 blastocysts in the SGD + aneuploidy group, the following reasons were identified for no result: recombinant (11.6 %), failed amplification (9.3 %), unable to determine single gene result due to degraded DNA or other reason (51.2 %), excluded from analysis due to aneuploidy or chaotic profile (27.9 %). Of the 15 blastocysts without a result in the SGD-alone group, the following reasons were identified: recombinant (6.7 %), failed amplification (13.3 %), unable to determine single gene result (46.7 %), or contaminated (13.3 %). Outcomes were re-analyzed after excluding the above outliers, and again half as many embryos were 'eligible for transfer' based on the available testing in the SGD + aneuploidy group compared to the SGD-alone group (29.1 % vs. 62.5 %, $p = .0001$). However, given that 56.5 % of blastocysts biopsied in the SGD + aneuploidy group were aneuploid, it is likely that a similar percentage of blastocysts in the SGD-alone were aneuploid and could have resulted in the transfer of an 'SGD-unaffected' but aneuploid embryo (Table 5).

Thirty-two patients in the PGD + aCGH group underwent frozen embryo transfer (FET) compared with 8 in the PGD alone group. Of those who underwent FET, significantly more embryos were transferred among the patients pursuing SGD alone (1.1 ± 0.3 vs. 1.9 ± 0.6, $p = 0.0001$), or in other words, 87.5 % of patients in the PGD + aCGH group underwent single embryo transfer compared to only 25 % in the PGD-alone group ($p = 0.001$) (Table 6). The implantation rate was 75 % in the PGD + aCGH group compared to 53.3 % in the PGD alone group ($p = 0.19$), the spontaneous abortion rate was 20 % in the PGD + aCGH group compared to 40 % in the PGD alone group ($p = 0.56$), and the live birth rate was 59.4 % in the dual-screening group compared to 37.5 % in the PGD alone group ($p = 1$).

Discussion

Pre-implantation genetic diagnosis, the ultimate goal of which is to select an embryo unaffected by a monogenic disorder, comes with substantial physical, emotional, and financial costs (Karatas et al. 2010, 2011). Despite the significant effort required by patients, genetic counselors, physicians, and laboratory staff to guide patients through the process of identifying embryos unaffected by a monogenic disorder, transferring an SGD-negative but possibly aneuploid blastocyst may still result in an unintended outcome such as IVF failure, miscarriage, pregnancy termination, or a child affected by chromosomal aneuploidy. We investigated outcomes of patients who pursued PGD with or without 24-chromsome aneuploidy screening to better understand the advantages of concomitant screening. In our study, 50 % of embryos biopsied concurrently for single gene disorders and aneuploidy were aneuploid, and three quarters of embryos screened concurrently with PGD and aneuploidy screening were ineligible for transfer due to either SGD status or aneuploidy. This data is consistent with data from Alfarawati et al. demonstrating that 50 % of blastocysts deemed to be 'top-grade' (grade 5 and 6) by standard morphologic criteria are in fact aneuploid, (Alfarawati et al. 2011). Understanding the high prevalence of embryonic aneuploidy even in a young patient population, we theorize that more than half of the embryos in the SGD-alone group would also have been aneuploid if tested, and embryo transfer of these 'unaffected' but aneuploid embryos could therefore have resulted in unintended, and sometimes devastating, consequences for families.

Concurrent screening demonstrably aided in embryo selection, as evidenced by the significant improvement in single

Table 2 Indications for PGD/PGS screening

Disease	Gene	Inheritance Mode	No. Patients	No. Embryos Tested for SGD	No. Affected by SGD	No. unaffected by SGD	No. Carriers	No. Inconclusive / other result
Alpha Thalassemia	HBA1	AR	1	10	0	2	2	6
Angioedema	SERPING1	AD	1	3	1	2	0	0
Beta Thalassemia	HBB	AR	1	10	3	1	3	3
Breast cancer, familial	BRCA	AD	6	37	20	11	0	6
Charcot Marie Tooth	PMP22	AD	2	13	4	7	0	2
Congenital Adrenal Hyperplasia	CYP21A2	AR	1	3	0	0	3	0
Cystic Fibrosis	CFTR	AR	3	34	7	9	17	1
1q21.1 microdeletion	GJA5	AD	1	6	4	2	0	0
Dominant Creutzfeldt Jakob Disease	PRNP	AD	1	8	4	3	0	1
Dystonia	DTY1	AD	1	4	2	2	0	0
Family Dysautonomia	IKBKAP	AR	1	6	0	1	5	0
Fanconi Anemia	FANCA	AR	1	3	1	2	0	0
Familial Mediterranean Fever	MEFV	AR	1	12	6	6	0	0
Fragile X	FMR1	XL	7	59	23	26	2	8
Gaucher Disease	GBA	AR	3	25	4	5	11	5
Factor II and Factor V Leiden deficiency	F2, F5	AD	1	9	6	3	0	0
Hemophilia A	F8	XL	3	22	3	9	6	4
Holt Oram Syndrome	TBX5	AD	1	8	5	2	0	1
Huntingtons	HTT	AD	2	21	3	4	0	14
Incontinentia pigmenti	IKBKG	XL	1	13	7	6	0	0
Li Fraumeni	TP53	AD	1	2	1	1	0	0
Long QT Syndrome	KCNQ1	AD	1	14	9	5	0	0
Multiple Endocrine Neoplasia, Type 1	MEN1	AD	1	4	1	3	0	0
Metachromatic leukodystrophy	ARSA	AR	1	1	1	0	0	0
Muscular Dystrophy, Duchenne	DMD	XL	1	6	1	3	2	0
Muscular dystrophy, Emery Dreifuss	LMNA	XL	1	16	9	5	0	2
Muscular dystrophy, Facioscapulohumeral	D4Z4	AD	1	4	3	0	0	1
Myotonic Dystrophy	DMPK	AD	1	7	2	5	0	0
Neurofibromatosis	NF1	AD	1	3	3	0	0	0
Rhesus blood group, D Antigen	RHD	AR	1	6	5	1	0	0
Sickle Cell	HBB	AR	2	7	2	1	3	1
Spondyloepiphseal dysplasia	SEDL	XL	1	1	1	0	0	0
Stargart Macular Degeneration	ABCA4	AR	1	4	2	0	2	0
Tay Sachs	HEXA	AR	3	23	6	7	10	0
Sheldon Hall Syndrome	TNNT3	AD	1	13	4	8	0	1

AR Autosomal recessive, *AD* Autosomal dominant, *XL* X-linked inheritance

Table 3 FET Outcomes based on indication for PGD/PGS screening

Disease	No. Patients	No. Blastocysts screened for aneuploidy	No. Euploid	No. Aneuploid	Inconclusive/ No result	No. Embryos Eligible for Transfer	No. of ET	OP/LB	SAB	BC
Alpha Thalassemia	1	10	4	2	4	4	1	1	0	0
Angioedema	1	3	2	1	0	2	1	0	0	1
Beta Thalassemia	1	0			0	4	0			
Breast cancer, familial	6	34	16	18	0	7	3	1	0	0
Charcot Marie Tooth	2	9	5	4	0	6	3	1	0	0
Congenital Adrenal Hyperplasia	1	3	2	1	0	2				
Cystic Fibrosis	3	25	6	19	0	6	2	1	0	1
1q21.1 microdeletion	1	6	2	4	0	0	0			
Dominant Creutzfeldt Jakob Disease	1	8	7	1	0	2	1	0	1	0
Dystonia	1	4	1	3	0	1	1	1	0	0
Family Dysautonomia	1	6	0	6	0	0	0			
Fanconi Anemia	1	3	1	2	0	1	1	1	0	0
Familial Mediterranean Fever	1	12	3	9	0	1	1	0	0	1
Fragile X	7	62	27	35	8	14	6	3	1	1
Gaucher Disease	3	18	8	8	2	9	2	1	1	0
Factor II and Factor V Leiden deficiency	1	9	2	7	0	1	1	1	0	0
Hemophilia A	3	16	11	4	1	12	4	1	0	1
Holt Oram Syndrome	1	8	5	2	1	2	2	1	0	0
Huntingtons	2	21	6	15	0	2	1	1		
Incontinentia pigmenti	1	13	8	5	0	3	1	1	0	0
Li Fraumeni	1	0			0	1	1	0	1	0
Long QT Syndrome	1	14	10	4	0	3	1	1	0	0
Multiple Endocrine Neoplasia, Type 1	1	4	2	2	0	1	1	0	0	0
Metachromatic leukodystrophy	1	1	1	0	0	0	0			
Muscular Dystrophy, Duchenne	1	6	4	2	0	4	2	0	0	0
Muscular dystrophy, Emery Dreifuss	1	16	12	1	3	3	1	1	0	0
Muscular dystrophy, Facioscapulohumeral	1	6	1	5	0	0	0			
Myotonic Dystrophy	1	0			0	5	3	1	0	0
Neurofibromatosis	1	3	3	0	0	0	0			
Rhesus blood group, D Antigen	1	6	4	2	0	0	0			
Sickle Cell	2	2	1	1	0	3	3	1	0	
Spondyloepiphseal dysplasia	1	1	0	1	0	0	0			
Stargart Macular Degeneration	1	4	0	4	0	0	0			
Tay Sachs	3	14	6	8	0	7	4	1	1	0
Sheldon Hall Syndrome	1	13	12	0	1	5	2	1	0	0

ET Embryo transfer, *SAB* Spontaneous abortion, *OP/LB* Ongoing pregnancy or live birth, *BC* Biochemical pregnancy

Table 4 Outcomes of pre-implantation genetic testing

	PGD + 24-chromosome aneuploidy screening (n = 355 blastocysts)	PGD alone (n = 64 blastocysts)	p-value
SGD-unaffected blastocysts (excluding carriers) [a]	123/355 = 34.6 %	19/64 = 29.7 %	.48
SGD-unaffected blastocysts (including carriers) [a]	173/355 = 48.7 %	35/64 = 54.7 %	.42
SGD-affected [a]	132/355 = 37.0 %	21/64 = 32.8 %	.57
Blastocysts with SGD result	313/355 = 88.1 %	56/64 = 87.5 %	.12
Aneuploid blastocysts	177/355 = 49.9 %		
Euploid blastocysts	169/355 = 47.6 %		
SGD-unaffected and aneuploid	58/355 = 16.3 %		
SGD-unaffected and euploid (eligible for transfer)	73/355 = 20.6 %		
Eligible for transfer based on all testing performed[a] *including SGD-negative and SGD-carriers	91/355 = 25.6 %	35/64 = 54.7 %	.001
Patients with ≥1 SGD-unaffected but aneuploid blastocyst	25/47 = 53.2 %		

Data are presented in percentage (%). [a] Data were analyzed with Fisher's exact test where appropriate, $p < 0.05$

embryo transfer rates in the dual-screening group compared with the PGD-alone group. Specifically, in our study population, significantly fewer embryos were transferred in the PGD + aneuploidy-screened group compared with the PGD-alone group, with almost twice as many embryo transferred in the PGD-alone group. Of the four twin pregnancies in the PGD + aneuploidy-screened group, one pregnancy was a monozygotic pregnancy and the remaining were dizygotic pregnancies resulting from the transfer of two euploid embryos. Given the high implantation potential of euploid embryos, and the increased possibility of multiple gestation, every effort should be made to exclusively perform SET. These data further emphasize the importance of aneuploidy screening as a means to increase the utilization of SET and ultimately decrease the risk of multiple gestation. In patients who underwent aneuploidy screening, although the numbers did not reach statistical significance, overall implantation rates were higher, spontaneous abortion rates were lower, and live birth rates were higher than the patients whose embryos were not subject to 24-chromosome aneuploidy screening. The small sample size likely limited the ability to detect a significant difference, but the outcomes are arguably clinically significant. It is logical to assume that the difference in live birth rate and the low miscarriage rate in the PGD + aneuploidy-screened group was secondary to the selection and transfer of euploid embryos.

These data may have important implications for both the genetic counseling and reproductive medicine communities. In our study population, the majority of patients who underwent PGD with concurrent aneuploidy screening had at least one blastocyst that was unaffected by a single gene disorder but aneuploid. Even in this young group of women with a mean age of 32 years old, 50 % of embryos tested were aneuploid. Despite the fact that 48.7 % of embryos

Table 5 Outcomes of pre-implantation genetic testing excluding blastocysts with no biopsy result

	PGD + 24-chromosome aneuploidy screening (n = 313 blastocysts)	PGD alone (n = 56 blastocysts)	p-value
SGD-unaffected blastocysts (excluding carriers)[a]	123/313 = 39.3 %	19/56 = 33.9 %	.55
SGD-unaffected blastocysts (including carriers)[a]	173/313 = 55.3 %	35/56 = 62.5 %	.38
SGD-affected[a]	132/313 = 42.2 %	21/56 = 37.5 %	.55
Aneuploid blastocysts	177/313 = 56.5 %		
Euploid blastocysts	169/313 = 54.0 %		
SGD-unaffected and aneuploid	58/313 = 18.5 %		
SGD-unaffected and euploid (eligible for transfer)	73/313 = 23.3 %		
Eligible for transfer based on all testing performed[a] *including SGD-negative and SGD-carriers	91/313 = 29.1 %	35/56 = 62.5 %	.0001
Patients with ≥1 SGD-unaffected but aneuploid blastocyst	25/47 = 53.2 %		

Data are presented in percentage (%). [a] Data were analyzed with Fisher's exact test where appropriate, $p < 0.05$

Table 6 Outcomes of frozen embryo transfer

	PGD + 24-chromosome aneuploidy screening (n = 32 patients)	PGD alone (n = 8 patients)	p-value
Mean no. embryos transferred [a]	1.1 ± 0.3	1.9 ± 0.6	.0001
Patients (%) undergoing single ET [b]	87.5 %	25 %	.001
Implantation rate [b]	75 %	53.3 %	.19
Spontaneous abortion rate [b]	20 %	40 %	.56
Multiple gestation rate [b]	12.5 %	12.5 %	1
Live birth rate [b]	59.4 %	37.5 %	1

Data are presented in mean ± standard deviation or percentage (%). Data were analyzed with Student's t-test[a] and Fisher's exact test[b], $p < 0.05$

were 'unaffected' by or carriers of the single gene disorder, only 25.6 % of embryos were eligible for transfer (unaffected or carrier, and euploid) after aneuploidy screening. Assuming consistent rates of aneuploidy in a comparably-aged group of women, around 50 % of embryos in the SGD-alone group would also be aneuploid, and therefore a large number of 'unaffected' but aneuploid embryos may have been transferred. As discussed previously, current morphologic embryo assessment cannot accurately predict euploidy (Alfarawati et al. 2011), and currently no modality exists besides 24-chromosome aneuploidy screening to identify a euploid embryo. Without information regarding the ploidy status of an embryo, patients may unnecessarily increase the likelihood of a poor IVF outcome including a negative pregnancy test, miscarriage, or an aneuploid fetus. When invasive testing is already being performed for purposes of single-gene testing, concurrent aneuploidy screening is a logical means to mitigate risk and improve patient outcomes. This is particularly relevant for families who may already have a child affected by a congenital genetic disorder, and who may strongly value the ability to screen for nonlethal aneuploidies such as trisomy 21 prior to conception.

Intriguingly, a live international poll of reproductive medicine specialists was performed for the journal *Fertility and Sterility* using the *Journal Club Live* ™ platform in February 2015 assessing participants' practice patterns regarding PGD with and without 24-chromosome aneuploidy screening (Fertility and Sterility Journal Club Live 2015). The international convenience sample included over 700 registrants from North America (66 %), Asia (13 %), Europe (9 %), the Middle East (6 %), South America (3 %), and Africa (3 %). When participants were asked if they offer PGD or pre-implantation genetic screening (PGS), 34 % reported that they offer only PGS, 12 % offer only PGD, 48 % offer both, and 6 % do not offer PGD or PGS. Of note, when participants were asked in what percentage of single gene PGD cycles they also perform aneuploidy screening, 27 % reported that they never perform concurrent aneuploidy screening. Only 9 % perform concurrent screening in more than 50 % of cycles, and 64 % of participants perform concurrent screening less than 50 % of the time.

In our practice, patients are counseled regarding the advantages of performing 24-chromosome aneuploidy screening when trophectoderm biopsy is performed for SGD. The ten patients in our study who opted for single gene testing alone did so based on personal preference, primarily related to the added financial burden of aneuploidy screening. A number of young patients felt that their risk of aneuploidy was low enough not to justify the additional cost of screening. The added cost of PGS will likely present a barrier to most patients already faced with significant costs related to PGD, making more affordable options attractive. One such approach could involve sequential testing, in which embryos are first screened for aneuploidy and PGD only performed on euploid embryos. Given the ubiquitously high aneuploidy rates, this could substantially cut down the cost of dual-screening by only screening embryos that would be eligible for transfer based on ploidy.

Patients with single gene disorders that may inherently increase their risk for primary ovarian insufficiency or diminished ovarian reserve are particularly likely to benefit from concurrent aneuploidy screening. Patients with BRCA mutations, and particularly BRCA 1 mutations, have been shown to have diminished ovarian reserve and lower age-adjusted serum AMH levels compared to women without BRCA mutations, as well as an impaired response to ovarian stimulation (Finch et al. 2013, Titus et al. 2013, Wang et al. 2014). It is well established that women with fragile X pre-mutations in the FMR1 gene have an increased risk of POI, and among all women with POI, 6 % will have a pre-mutation in the FMR1 gene (American College of Obstetricians and Gynecologists 2014; Nelson et al. 2005). Given the high likelihood that these women at risk for POI will lose the opportunity to create embryos in the future due to diminished ovarian reserve, it is imperative that they have a realistic understanding of how many of their embryos are not only single-gene-unaffected

but also euploid, and therefore capable of producing a viable pregnancy.

Mounting data suggest that the transfer of a single thawed euploid blastocyst improves IVF pregnancy and miscarriage rates, with implantation rates approaching those attained when donor oocytes are utilized despite maternal age (Grifo et al. 2013). Importantly, transferring a single euploid blastocyst compared to the transfer of two untested blastocysts also decreases multiple gestation rates and therefore improves obstetric and neonatal outcomes including preterm delivery, low birth-weight, and NICU admission (Forman et al. 2013). Increasing the utilization of single embryo transfer is arguably the most impactful intervention to improve obstetric and neonatal outcomes following IVF, and 24-chromosome aneuploidy screening has proven to be the most effective means thus far to select a competent embryo for SET. A review of historical data from our center suggests that patients who previously underwent PGD for monogenic disorders without aneuploidy screening had higher miscarriage rates as well as lower live birth rates compared with present-day patients who are almost exclusively undergoing concurrent aneuploidy screening with PGD (Grifo et al. 2007). However, we acknowledge that practice patterns have changed regarding embryo culture, biopsy, and testing platforms, and for this reason the current study included only those patients pursuing blastocyst culture, trophectoderm biopsy, and frozen embryo transfer in order to minimize confounding where possible.

Study Limitations

The retrospective study design is inherently biased. Decisions regarding which treatment was most appropriate would have been decided by both patients and providers at the time that care was provided, and while patients in our practice are generally counseled toward aneuploidy screening in conjunction with PGD, differences in providers' recommendations in a large group practice may have influenced a patient's treatment group. Given that the providers in our group have similar practice patterns, the patients in the PGD-alone group likely self-selected and opted out of aneuploidy screening.

An important limitation is the small sample size in both groups, and most notably in the PGD-alone group. Sample size particularly limits one's ability to draw extensive conclusions when comparing FET outcomes between groups, and it's possible that statistically significant differences would have been seen in implantation rate and miscarriage rate had the study been larger. In this study we intentionally included only biopsy performed on trophectoderm cells, excluding blastomere biopsy, because of the known differences in implantation potential between the two groups and the likelihood of introducing bias when comparing both modalities. Consequently, the sample size was inherently smaller because of exclusion criteria, but arguably this provided for a cleaner and less biased comparison given that only identical embryo biopsy methods were compared.

Practice Implications

Due to advances in cryopreservation techniques and PGS technology, the landscape of reproductive medicine is rapidly evolving. For many patients, fresh embryo transfer is being replaced by FET, and IVF cycles are more and more frequently accompanied by PGS. As previously discussed, concurrent aneuploidy screening in PGD cycles is not yet considered standard of care, and it is imperative that reproductive medicine specialists understand the benefits of dual-screening. Given the many contributing providers involved in the spectrum of reproductive medicine care, including insurers, genetic counselors, mental health specialists, mid-level providers, and general OB/GYN physicians, these providers must be made aware of the growing application of PGS in patients without a diagnosis of 'infertility.'

Research Recommendations

Larger, prospective studies are needed to support the findings from this small retrospective study. A randomized controlled trial is needed to compare PGD with and without concurrent aneuploidy screening in order to best ascertain the benefits of PGD with aneuploidy screening. As PGD continues to evolve and grow, it is imperative that the genetic counselors, physicians and all providers caring for patients with single gene disorders or who are carriers for single gene disorders provide recommendations that will optimize the likelihood of pregnancy while mitigating risk.

Acknowledgments The authors gratefully acknowledge and thank the physician, nursing, laboratory and ancillary staff at the NYU Fertility Center who all contribute to making the care of patients possible.

Compliance with Ethical Standards

Conflict of Interest Authors K.G., T.N. A.S.B., S.P., and J.G. declare that they have no conflict of interest.

Informed Consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval for this retrospective study was obtained by the Institutional Review Board (IRB) of the New York University (NYU) School of Medicine.

Animal Studies This article does not contain any studies with animals performed by any of the authors.

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