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ASSISTED REPRODUCTION TECHNOLOGIES

Do patients who achieve pregnancy using IVF-PGS do the recommended genetic diagnostic testing in pregnancy?

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Abstract

Purpose Patients undergoing in-vitro fertilization (IVF) with preimplantation genetic screening (PGS) are counseled about the limitations of this technique. As part of the consent process for PGS, physicians recommend diagnostic genetic testing performed in early pregnancy to definitively rule out chromosomal abnormalities. We have noted anecdotally, however, that few patients undergo the recommended diagnostic testing. In this study, we are examining if women who conceived using IVF-PGS did early pregnancy chromosomal testing, and if they did, what type of testing they had.

Methods This study was performed from 2015 to 2017 in the Division of Reproductive Endocrinology and Infertility at Northwestern University. We included patients who became pregnant after IVF-PGS who were seen by the Division of Reproductive Genetics and non-PGS control group.

Results Sixty-eight patients were included. A total of 50 patients (73.5%) opted for non-invasive prenatal screening; 5 (7.4%) had invasive testing (4 had chorionic villus sampling and 1 had amniocentesis). A total of 13 patients (19%) declined further genetic testing. When comparing demographic data, the mean age was significantly higher in the group of patients who pursued non-invasive testing than in the group who declined further testing (37.15 vs 34.05 years old, $p < 0.05$). Control group declined invasive diagnostic testing.

Conclusions Most patients who conceive using IVF-PGS do not pursue diagnostic prenatal chromosomal testing. Future studies focusing on decision making in this patient group are warranted to further elucidate why a small percentage of patients opt for diagnostic testing, even when adequately counseled about the inherent limitations of PGS.

Keywords IVF · PGS · Aneuploidy · Chromosomal testing · NIPT · CVS · NGS

Introduction

Aneuploidy is the most common abnormality in human embryos derived from in vitro fertilization (IVF) [1–7]. It is widely recognized that the prevalence of aneuploidy in human embryos further increases with advanced female age [8].

It has been shown that selecting genetically normal embryos by pre-implantation genetic screening (PGS) can decrease the chance of becoming pregnant with a genetically abnormal fetus [9]. Even though PGS decreases the chances of aneuploidy and may improve pregnancy outcomes, this technique is inherently imperfect. Errors may occur during the genetic analysis of the small amount of DNA collected. More importantly, mosaicism may occur in which mitotic errors during embryo development result in chromosomally distinct cell populations [10]. Mosaicism in the preimplantation embryo may lead to sampling errors due to the intentionally limited collection of cells from the trophectoderm (TE) that will become the placenta. Results may not be representative of the entire embryo, the unbiopsied TE cells, or the inner cell mass (embryo structure that will become the fetus) [10]. In this manner, abnormal cells may be collected in an otherwise euploid embryo and vice-versa [11].

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There are different options for genetic testing and screening in early pregnancy. Screening tests can determine whether an individual is at increased risk of having a pregnancy affected by a specific aneuploidy while diagnostic tests determine whether a specific condition or aneuploidy is present in the embryo or fetus. Screening tests during early pregnancy include sequential screening (maternal serum biochemical tests and fetal ultrasound markers associated with maternal age) and non-invasive prenatal testing (NIPT) that uses fetal genetic material, namely cell-free DNA (cfDNA). These screening methods are not able to definitively diagnose or rule out chromosomal abnormalities. Chorionic villus sampling (CVS) and amniocentesis are both diagnostic tests for fetal aneuploidy. These procedures carry risks, including the increased risk for procedure-related miscarriage (approximately 1–2/1000 over the baseline risk) [12]. There is also an approximately 1% chance of finding mosaicism with a CVS sample, which would require that the patient undergo an amniocentesis around 15 weeks for clarification of fetal chromosomes. It is established that all women who conceive after an IVF-PGS cycle should be offered prenatal diagnostic testing for aneuploidy early in pregnancy [13, 14]. PGS does not guarantee the birth of a chromosomally normal child because of the chance of undetected mosaicism and the investigational nature of PGS. Therefore, patients with ongoing pregnancies resulting from IVF-PGS should be counseled that CVS (10–12 weeks) or amniocentesis (15–18 weeks) are the tests available to confirm a chromosomally normal fetus [14].

Because mosaicism is one of the most important concerns regarding the use of PGS, many IVF programs biopsy at the blastocyst stage when mosaicism is reduced [15–18]. Techniques such as next-generation sequencing (NGS) now enable the diagnosis of mosaicism in a group of TE cells, allowing for the possible transfer of potentially mosaic embryos and ability to follow these pregnancies. However, studies addressing the clinical outcome after transfer of mosaic embryos remain scarce [10].

Our objective was to analyze which, if any, first trimester screening or diagnostic test was selected by the IVF-PGS population at our institution. The primary endpoint was to examine if patients that achieved pregnancy had further first trimester genetic screening or testing. In those that did, we examined what type of screening or testing patients selected. We also included a control group of 52 patients who achieved pregnancy during this same period with IVF but without doing PGS and looked at what kind of first trimester genetic screening or testing they chose.

Materials and methods

Patients and data collection

Our study was approved by the Northwestern Institutional Review Board. We reviewed medical records of women who became pregnant after IVF-PGS cycles from 1/2015–12/2017 in the Division of Reproductive Endocrinology and Infertility at Northwestern University who were then seen by a faculty physician at our institution for obstetric care records from a control group of patients undergoing IVF without PGS screening who were then seen by a faculty physician were also reviewed. We chose to limit the study to these patients because the faculty physicians routinely refer all patients to genetics for counseling and the offering of first trimester screening or testing. Patients who had preimplantation genetic diagnosis (PGD) testing which did not include PGS were excluded. No mosaic embryos were transferred. The following information was retrieved from the patient's medical records: age, BMI, AMH, stimulation protocol, number of mature eggs retrieved, number of eggs fertilized, number of blastocysts PGS tested, PGS results, and type of early pregnancy genetic testing (if they had). The demographic data was placed on an excel spreadsheet for analyses. PGS was done using either NGS or SNP technology, depending on ordering physician's preference.

Counseling

Prior to undergoing IVF-PGS, all patients were counseled by a reproductive endocrinology and infertility physician and subsequently signed a consent form for PGS where the limitations of this testing were discussed. Specifically, the consent form states that PGS may fail to correctly diagnose embryos as being genetically normal; therefore, if pregnancy is achieved, diagnostic testing should optimally be performed for chromosomal analysis during pregnancy.

During their genetic consultation, all patients were counseled based on their individual risk of chromosomal abnormalities related to age, personal, and family history as well as the family history of their partner. Specifically, for the patients who had undergone PGS, the genetic counselors also reviewed the limitations of PGS screening with patients. Available options for genetic screening and testing were then discussed. The benefits and limitations of sequential screening and cfDNA were discussed, including the fact that it is a screening test and is not diagnostic for trisomy 18, 21, 13, and/or sex chromosome abnormalities. Patients were also informed that if the screening test resulted in a positive result, then the patient would be offered prenatal diagnostic testing through CVS or amniocentesis. Because screening methods, including PGS, are not able to definitively diagnose or rule out chromosome abnormality, they also discussed the option of diagnostic testing through CVS or amniocentesis which are both diagnostic for fetal aneuploidy. The risks, benefits, and limitations of both procedures were discussed including the increased risk for procedure-related miscarriage and chance of mosaicism with CVS samples, requiring amniocentesis. Both CVS and amniocentesis offer the option of single nucleotide

polymorphism (SNP) array analysis which can detect small, clinically significant chromosome imbalances not detected by screening. Patients were also informed about the 3 to 5% baseline risk for any pregnancy to result in a baby with a birth defect and or mental retardation [19], that prenatal screening and diagnostic testing cannot detect all abnormalities, and that normal results cannot guarantee a normal pregnancy outcome.

We then examined whether patients underwent first trimester screening or diagnostic testing; in those that did, we recorded which screening or diagnostic test was performed, including first trimester combined screening, NIPT, amniocentesis, or CVS, and if the screening/diagnostic test performed corresponded to PGS results.

Statistical analysis

Our data was normally distributed thus parametric testing was used. The one-way analyses of variance (ANOVA) followed by Tukey's multiple comparison test were used to determine statistically significant differences between means of the three independent groups (Table 1). Statistical analyses were performed with GraphPad software; a P value of < 0.05 was considered statistically significant.

Results

A total of 68 patients who underwent IVF-PGS met our inclusion criteria and were included in our study. Demographic data of this cohort are shown in Table 1. The mean age of this cohort was 36.7 years. A total of 55 (80.9%) opted for either screening or diagnostic testing. Of these 55 patients, 50 (73.5%) opted for screening and 5 patients (7.4%) opted for diagnostic testing. Specifically, 48 patients (70.6%) opted for NIPT (one of these patients also had CVS because of a positive NIPT result that was not confirmed by CVS) and 2 had sequential screening. A total of 4 patients (5.9%) had CVS testing, and one patient opted for an amniocentesis at 15-week gestation. We also found that 13 patients (19.1%) declined further genetic screening or testing after doing PGS (Fig. 1a).

When comparing demographic data between groups, we found no difference between BMI, AMH, stimulation duration, gonadotropin dosage, and number of eggs fertilized. However, the mean age was significantly higher in the group of patients who pursued non-invasive screening than in the group of patients who declined further screening or testing (37.15 vs 34.05 years old) (Table 1).

In addition, we examined 52 patients who achieved pregnancy during this same time period with IVF but without doing PGS. The mean age of this control group was 35.29 years. In this group of patients, 92% (48 patients) opted for NIPT, 2 patients (4%) pursued first trimester sequential screening, and 2 patients (4%) declined any kind of first trimester genetic testing or screening (Fig. 1b). Interestingly, none of the patients from this control group opted for invasive diagnostic testing. When compared to the group who had IVF-PGS, significantly fewer patients declined genetic screening or testing ($p < 0.01$) (Fig. 1b).

Discussion

In this study, we found that most women who have IVF-PGS did not undergo recommended diagnostic testing

Table 1 Demographic features and ovarian stimulation characteristics of patients who achieved pregnancy with IVF-PGS

	Non-Invasive testing		Invasive testing		Declined further testing		
	Mean ± SD	SE	Mean ± SD	SE	Mean ± SD	SE	p value
Maternal age at retrieval	37.15 (± 3.94)	0.56	38.72 (± 4.40)	1.97	34.05 (± 2.95)	0.82	0.020**
BMI	24.46 (± 4.2)	0.6	22.06 (± 2.40)	1.07	26.69 (± 5.63)	1.56	0.110
AMH	3.98 (± 3.24)	0.47	2.94 (± 1.85)	0.83	3.39 (± 2.40)	0.67	0.670
Gravidity	1.16 (± 1.39)	1.20	2 (± 2.55)	1.14	1.23 (± 1.30)	0.36	> 0.99
Parity	0.22 (± 0.46)	0.07	0.4 (± 0.55)	0.24	0.15 (± 0.38)	0.10	0.48
Stimulation duration (days)	10.53 (± 1.32)	0.19	9.6 (± 0.55)	0.24	10.15 (± 0.80)	0.22	0.199
Gonadotropin dosage	3287.61 (± 1188)	247.73	4000 (± 2291)	1322.88	3390 (± 1543)	690	0.563
No. of mature eggs retrieved	15.14 (± 8.19)	1.17	10.2 (± 4.44)	1.98	9.92 (± 3.20)	0.89	0.045***
No. of eggs fertilized	11.63 (± 6.96)	0.99	8.2 (± 4.76)	2.13	8.75 (± 3.14)	0.91	0.237
No. of blastocysts	4.96 (± 2.74)	0.40	3 (± 1.23)	0.55	4.5 (± 1.85)	0.51	0.252
Number of patients (n) per group	50		5		13		

One-way ANOVA analysis with Tukey's multiple comparisons test. Avg ± SD. Comparison of demographic data from three groups: Non-invasive testing, invasive testing, and the patients who declined further genetic testing

*$p < 0.05$; **non-invasive vs declined further testing $p = 0.0303$; ***non-invasive vs declined $p = 0.064$

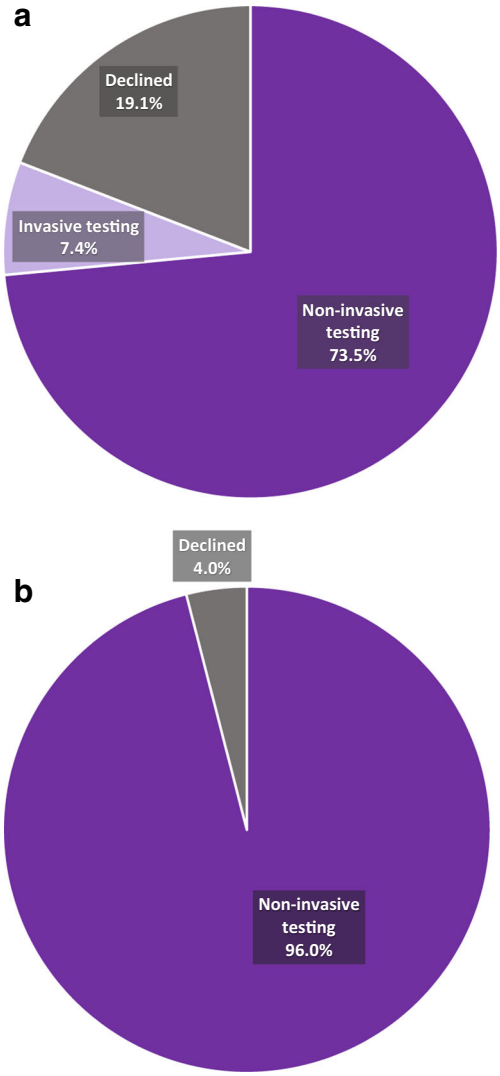

Fig. 1 **a** Type of testing or screening that was selected in patients who achieved pregnancy using IVF-PGS. **b** Type of testing or screening selected by patients who achieved pregnancy with IVF alone

(CVS or amniocentesis), even when counseled by a genetic counselor. We found that most patients (70.6%) opted for NIPT with cfDNA. Only 5 patients (7.4%) opted for diagnostic testing. Surprisingly, almost 20% of patients opted to forgo first trimester screening or testing (Fig. 1a). When demographic data were compared between these three groups (non-invasive screening, invasive testing, and declined further genetic screening or testing), we found a significant difference in the mean age between the group that pursued non-invasive screening versus the group of patients that declined further screening or testing. Since age is the main risk factor for aneuploidies, normal PGS results may give younger patients a greater sense of reassurance that motivates them to decline further testing or screening, even when counseled about the inherent limitations of PGS. We also found that among patients achieving pregnancy using IVF without PGS, 92% chose to undergo non-invasive (48 patients NIPT and 2 patients first trimester sequential screening) screening during pregnancy (Fig. 1b). When comparing this cohort to IVF patients who achieved pregnancy without doing PGS, we noticed that there were significantly less patients in the IVF-PGS group who declined first trimester genetic screening or testing after doing PGS ($p < 0.01$) (Fig. 1b).

PGS is increasingly used to select embryos for transfer. Similar to our study results, Arian and colleagues presented an abstract where they examined what type of genetic screening or testing patients are opting for after IVF-PGS cycles. They found that most of their patients opted for NIPT and only a few of them had amniocentesis or CVS [20]. Takyi et al. [21] recently published a commentary where they emphasized the need for a revised algorithm for prenatal screening and testing for chromosomal abnormalities in IVF-PGS patients. In this paper, they proposed that free fetal DNA in maternal circulation would be the best first trimester prenatal screening test for chromosomal abnormalities in IVF-PGS patients because risk estimations from all other prenatal screening algorithms depend heavily on maternal age, which becomes irrelevant in patients who had PGS [21].

The introduction and incorporation of NIPT into routine obstetrical care has shifted the paradigm of prenatal diagnosis and screening for all women and has had a profound impact on the prenatal screening paradigm for fetal aneuploidy [13]. While NIPT has proven effective in detecting fetuses with aneuploidy, results are not considered diagnostic. Because NIPT does not screen for all chromosomal or genetic conditions, and because it provides an alteration in risk rather than it does not replace prenatal diagnostic testing. Any result suggestive of an increased likelihood of a fetal chromosomal abnormality should receive genetic counseling and given the option of a prenatal diagnostic procedure such as CVS or amniocentesis [13].

The strengths of our study include the fact that all patients were seen by genetic counselors and the availability of complete clinical follow-up. The weakness of this study includes its retrospective design and that this is a single-center study. We also intentionally limited our study to patients who were seen by faculty physicians because all these patients were seen by genetic counselors.

Future studies in patient decision making should be done to see why such a small percentage of patients opt for diagnostic testing, even when adequately counseled about the inherent limitations of PGS testing. In addition, given that most patients pursue cfDNA as a primary screening test after IVF-PGS cycles, studies should focus on outcomes of pregnancies achieved with IVF-PGS to see if invasive testing is still routinely warranted.

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