Are blastocyst aneuploidy rates different between fertile and infertile populations?

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Abstract

Purpose This study aimed to determine if patients with infertility or recurrent pregnancy loss have higher rates of embryo aneuploidy than fertile controls.

Methods This was a retrospective review of all pre-implantation genetic screening (PGS) cases processed by a single reference lab prior to March 2014 after a blastocyst biopsy. Cases were excluded if no indication for PGS was designated or patients were translocation carriers. The fertile control group consisted of patients undergoing IVF with PGS for sex selection only. The comparison cohorts included those with recurrent pregnancy loss, male factor infertility, unexplained infertility, prior failed IVF, or previous aneuploid conceptions. A quasi-binomial regression model was used to assess the relationship between the dependent variable, aneuploidy rate and the independent variables, maternal age and reason for PGS. A quasi-Poisson regression model was used to evaluate the relationship between similar independent variables and the number of blastocyst biopsies per case.

Results The initial study population consisted of 3378 IVF-PGS cycles and 18,387 analyzed trophoctoderm samples. Controlling for maternal age, we observed an increased rate of aneuploidy among patients with recurrent pregnancy loss (OR 1.330, $p < 0.001$), prior aneuploid pregnancy (OR

1.439, $p < 0.001$), or previous failed IVF cycles (OR 1.356, $p = 0.0012$) compared to fertile controls. Patients with unexplained and male factor infertility did not have a significantly different aneuploidy rate than controls ($p > 0.05$). The increase in aneuploidy in patients with RPL and prior IVF failure was driven by both an increase in meiotic (OR 1.488 and 1.508, $p < 0.05$) and mitotic errors (1.269 and 1.393, $p < 0.05$) relative to fertile controls, while patients with prior aneuploid pregnancies had only an increased risk of meiotic error aneuploidies (OR 1.650, $p < 0.05$).

Conclusions Patients with recurrent pregnancy loss, previous IVF failures, and prior aneuploid pregnancies have a significantly higher, age-independent, aneuploidy rate compared to patients without infertility.

Keywords Pre-implantation genetic screening (PGS) · Aneuploidy · Infertility · Sex selection

Introduction

Embryo aneuploidy is a significant cause of early pregnancy loss and in vitro fertilization (IVF) failure [1, 2]. As pre-implantation genetic screening (PGS) of embryos has become an increasingly utilized embryo selection method, the likelihood of obtaining euploid blastocysts has become a focus point of counseling between providers and patients. Because blastocyst aneuploidy rates correlate strongly with maternal age [3], age-based extrapolations are frequently used to make projections regarding the expected number of euploid blastocysts available to transfer for patients undergoing IVF-PGS.

While the correlation between age and aneuploidy has been consistently demonstrated, it is unknown whether an infertility

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Table 1 Designated indication for IVF-PGS

Indication for IVF-PGS	Number of cases with designation	Mean age (years)
Fertile controls (gender selection only)	<i>n</i> = 233	35.0 ± 4.8
Male factor infertility	<i>n</i> = 265	34.7 ± 4.2
Previous aneuploidy	<i>n</i> = 213	38.2 ± 4.1
Recurrent pregnancy loss	<i>n</i> = 644	37.2 ± 4.2
Unexplained infertility	<i>n</i> = 268	36.3 ± 4.1
Previous IVF failure	<i>n</i> = 392	37.0 ± 4.3

diagnosis, or specific diagnoses, conveys an increased risk of embryo aneuploidy independent of maternal age. Studies characterizing aneuploidy at the cleavage and blastocyst stages by necessity consist of embryos from patients undergoing IVF, the majority of whom are doing so because of an infertility diagnosis. It is unclear whether the incidence of embryo aneuploidy in this population is applicable to a fertile population—a subgroup that is increasingly undergoing IVF-PGS whether it be for fertility preservation, pre-implantation genetic diagnosis (PGD), or nonmedical sex selection. A previous study by Taylor et al. [4] investigated this question, failing to detect a difference in the number of euploid blastocysts; however in this study, patient age was only stratified by < 35 or ≥ 35 years old, and analysis was limited to 93 patients. The difference in aneuploidy rates changes dramatically with increasing maternal age > 35 years old [5], and this study's negative findings may be due to insufficient stratification and/or power. Another study by Shahine et al. [6] did find significantly higher aneuploidy rates and a greater likelihood of not having a euploid embryo available for transfer among younger patients (≤ 37 years old) with diminished ovarian reserve (DOR) and recurrent pregnancy loss (RPL) compared to their control group of patients with only unexplained RPL. This may reflect the pathologic nature of DOR at a young age compared to the more physiologic nature of DOR with increasing age. Furthermore, these results suggest that DOR may affect oocyte ploidy in addition to ovarian reserve, a notion which has been previously suggested by other studies [7].

The objective of this study was to determine whether fertile patients, who presumably otherwise would not need to undergo IVF-PGS, have significantly lower blastocyst aneuploidy rates than patients with infertility-related diagnoses.

Materials and methods

This study was a retrospective cohort study of all pre-implantation genetic screening (PGS) cases processed by a single reference lab prior to March 2014. While a previous analysis using the same database investigated differences between mitotic and meiotic error aneuploidies at different stages of embryo development [8], this analysis investigated differences in aneuploidy rates between fertile and infertile or RPL patients. Only cases using 24-chromosome analysis analyzing a trophectoderm biopsy were included. Cases involving a known translocation carrier, cases without an indication for PGS, and those in which DNA was not detected or a reliable ploidy diagnosis was unable to be made were excluded from analysis.

The presumed fertile control group included patients whose sole indication for PGS was nonmedical sex selection. The comparison cohorts included patients who included “male factor infertility,” “unexplained infertility,” “recurrent pregnancy loss,” “previous IVF failure,” and/or “previous aneuploidy” on the reference lab's indication form.

Embryo biopsy and ploidy determination

Embryos were biopsied by IVF clinics using Natera as the reference lab. Genetic material was obtained from the oocyte source, sperm source (peripheral blood or buccal swabs), and blastocysts (multi-cell day-5 or day-6 trophectoderm biopsy) and sent to Natera. Multiple displacement amplification (MDA) with proteinase K buffer (PKB) was used for this procedure. Cells were placed in 5 µl PKB (Arcturus PicoPure Lysis Buffer, 100 mM DTT, 187.5 mM KCl, 3.75 mM MgCl₂, 3.75 mM Tris-HCl) incubated at 56 °C for

Table 2 Aneuploidy OR vs. “fertile control group” by IVF indication (all cases)

Diagnosis	Adj. OR (aneuploidy)	95% CI	<i>p</i> value	Mean blastocysts per patient cycle
Male factor	1.036	(0.854, 1.257)	0.721	4.54
Unexplained infertility	1.061	(0.869, 1.295)	0.563	5.47
Recurrent pregnancy loss	1.33	(1.132, 1.565)	< 0.001	5.46
Previous IVF failure	1.356	(1.129, 1.629)	0.0012	5.39
Previous aneuploidy	1.439	(1.170, 1.772)	< 0.001	4.88

Fig. 1 Aneuploidy rate by maternal age for PGS indication

1 h, followed by heat inactivation at 95 °C for 10 min, and held at 25 °C for 15 min. MDA reactions were incubated at 30 °C for 2.5 h and then 65 °C for 10 min. Genomic DNA from buccal tissue was isolated using the QuickExtract DNA extract solution (Epicentre; Madison, WI). Template controls were included for the amplification method. Bulk parental tissues were genotyped using the Infinium II (Illumina; San Diego, CA) genome-wide SNP arrays (HumanCytoSNP12 chip). The standard Infinium II protocol was used for parent samples (bulk tissue), and Genome Studio was used for allele calling. Whole chromosome aneuploidies were detected and classified using the Parental Support algorithm previously described by Johnson et al. [9]. This approach uses high-quality genotype data from the sperm and oocyte source to infer the presence or absence of homologs in embryo genotype data.

Meiotic origin of aneuploidy was inferred based on the observed transmission of both maternal—or rarely, paternal—haplotypes for any region of an embryonic chromosome (see [8–10] for further description of this “both parental homologs” signature). Excluding rare paternal meiotic trisomies, aneuploidies involving extra or missing paternal chromosomes were

assigned as putative mitotic errors. Such aneuploidies affect ~ 10% of blastocyst biopsies and show no significant associations with maternal or paternal age, further supporting their mitotic origin [8, 10].

Statistical analysis

Statistical analysis was performed using the *R* statistical computing environment. A quasi-binomial regression model was used to assess the relationship between the dependent variable, aneuploidy rate (proportion of aneuploid blastocysts per case), and the independent variables, maternal age and reason for PGS. Age was included as a quadratic covariate to model its non-linear association with aneuploidy. A quasi-Poisson regression model was used to evaluate the relationship between the same independent variables, maternal age and indication for PGS, and the number of blastocyst biopsies per case—a proxy for the number of good-morphology embryos surviving to the blastocyst stage and thus available for biopsy. This analysis was then repeated for only cases with a single indication for PGS in order to see

Table 3 Aneuploidy OR vs. “fertile control group” by IVF indication (only cases with single indication)

Diagnosis	Adj. OR (aneuploidy)	95% CI	<i>p</i> value	Mean blastocysts per patient cycle
Male factor	1.036	(0.854, 1.257)	0.721	4.48
Unexplained infertility	1.061	(0.869, 1.295)	0.563	5.47
Recurrent pregnancy loss	1.330	(1.132, 1.565)	< 0.001	5.49
Previous IVF failure	1.356	(1.129, 1.629)	0.00118	5.41
Previous aneuploidy	1.439	(1.170, 1.772)	< 0.001	4.93

Table 4 Meiotic error (maternal meiotic trisomy) aneuploidies compared to fertile controls

Diagnosis	Adj. OR (meiotic error)	95% CI	<i>p</i> value
Male factor	0.969	(0.742, 1.035)	0.8230
Unexplained infertility	1.306	(1.005, 1.704)	0.0477
Recurrent pregnancy loss	1.488	(1.195, 1.865)	0.0005
Previous IVF failure	1.508	(1.191, 1.919)	0.0008
Previous aneuploidy	1.65	(1.268, 2.150)	0.0002

if the initial results were confounded by having multiple indications for a single case.

Results

The study included 18,387 five- to ten-cell trophectoderm biopsies from 3605 IVF-PGS cycles (mean 5.1 blastocysts per cycle). After excluding cases without a designated indication for PGS or with either the sperm or oocyte source being a known translocation carrier, 10,711 blastocysts from 2015 total cycles were included for analysis. Table 1 provides the distribution of cycles by designated PGS indication among the study cohorts.

Patients with a history of recurrent pregnancy loss, previous IVF failure, and previous aneuploid pregnancy, had significantly higher rates of blastocyst aneuploidy than fertile controls both compared collectively against fertile controls (OR 1.230, 95% CI 1.062–1.425) and individually (Table 2, Fig. 1), even when including maternal age as a covariate in the regression analysis. In contrast, patients with unexplained infertility and male factor indications did not have significantly different blastocyst aneuploidy rates than the fertile control group. When the analysis was repeated for only cases with a single PGS indication, the findings were similar (Table 3). This observed difference in aneuploidy rates was primarily driven by meiotic error aneuploidies (maternal meiotic trisomies), while putative mitotic error aneuploidies were similar between cohorts (Tables 4 and 5).

After controlling for age, patients designated to have “male factor” as the indication for PGS had significantly fewer blastocysts available for biopsy than any of the other cohorts, while there was no observed difference in the number of blastocysts biopsied between any of the other PGS indication cohorts and the fertile control group (Table 6, Fig. 2).

Discussion

This study demonstrates an age-independent increased risk for blastocyst aneuploidy after IVF for patients with a history of recurrent pregnancy loss, a previous aneuploid pregnancy, or previous IVF failures, compared to fertile controls. Additionally, an increased age-independent increased rate of meiotic error aneuploidies is the principal etiology of the increased prevalence of blastocyst aneuploidies among these cohorts. Interestingly, not all infertility patients had a significantly increased rate of blastocyst aneuploidy; patients with male factor infertility or unexplained infertility had similar aneuploidy rates. When considering that the other cohorts’ increased aneuploidy risk was predominantly driven by meiotic error aneuploidies, and that oocytes rather than sperm have a higher prevalence of meiotic error aneuploidies, it is logical that patient couples with a presumably fertile female from an oocyte perspective would have a similar incidence of blastocyst aneuploidy as fertile couples. This result also suggests that while sperm aneuploidy has been demonstrated to be significantly higher among patients with male factor infertility [11], this was not observed to manifest as an increased rate of blastocyst aneuploidy in this study. Given the relatively low rate of paternal origin meiotic aneuploidy observed in blastocysts, oocytes fertilized by aneuploid sperm may be more likely to fail development to the blastocyst stage and not be represented in our analysis of aneuploidy rates. This would also explain the observation that couples with a male factor designation had fewer blastocysts available to biopsy. Similarly, many patients with unexplained infertility may have yet-undiagnosed uterine or male factor infertility, and these non-oocyte related etiologies of infertility do not manifest in an increased rate of embryo aneuploidy.

As all blastocysts included in this study resulted from in vitro fertilization cycles, it is unclear whether the observed

Table 5 Putative mitotic error aneuploidies compared to fertile controls

Diagnosis	Adj. OR (mitotic error)	95% CI	<i>p</i> value
Male factor	1.038	(0.773, 1.3994)	0.8050
Unexplained infertility	1.028	(0.763, 1.388)	0.8550
Recurrent pregnancy loss	1.269	(1.002, 1.619)	0.0516
Previous IVF failure	1.393	(1.065, 1.837)	0.0173
Previous aneuploidy	1.303	(0.969, 1.753)	0.0803

Table 6 Number of blastocysts available for biopsy compared to fertile controls

Diagnosis	Adj. OR (# of blastocysts biopsied)	95% CI	<i>p</i> value
Male factor	0.797	(0.702, 0.905)	0.0005
Unexplained infertility	1.009	(0.895, 1.138)	0.881
Recurrent pregnancy loss	1.036	(0.933, 1.152)	0.506
Previous IVF failure	1.032	(0.921, 1.158)	0.585
Previous aneuploidy	1.004	(0.878, 1.146)	0.958

increased blastocyst aneuploidy rates among patients with specific diagnoses seen in this study are applicable to patients' cycles without the use of controlled ovarian stimulation and in vitro embryo culture. Although one study has demonstrated that controlled ovarian stimulation alone does not increase aneuploidy rates [12], it remains unclear whether when trying to conceive spontaneously, these patients also experience higher aneuploidy rates that may contribute to their recurrent pregnancy loss and infertility diagnoses.

One strength of this study is the large sample size of couples undergoing PGS for a variety of reasons and the availability of a sizable gender selection cohort—a practice that is illegal in many countries outside the USA. However, these findings are dependent on the provider-reported diagnosis and limited by the fact that the criteria and indications for PGS on the PGS requisition form may vary from clinic to clinic. However, we assume that the reporting clinics followed ASRM committee guidelines for diagnostic criteria. Additionally, some patients solely undergoing IVF-PGS for sex selection may also have undiagnosed infertility. While some of our findings contradict investigation of a

similar question by Taylor et al. [4], this may reflect sample size and the use of regression to more effectively control for the effect of maternal age. However, these authors did find significantly higher implantation rates for euploid embryos from fertile, compared to infertile, couples—suggesting that euploid blastocysts have greater reproductive potential for fertile patients. This important finding was not tested in our analysis and will be important to investigate to help tailor evidence-based counseling to individual patients. Meanwhile, our findings are consistent with Shahine et al. [6], who observed that specific infertility diagnoses are associated with patient aneuploidy rates independent of maternal age. A limitation of this analysis is that the inclusion of age as a covariate does not entirely remove age from being a potential confounder. Due to variations in age distributions between patient indications for IVF-PGS, there could be a residual effect of maternal age not controlled for in our model. Additionally, since each analysis used the same fertile control group, the “sex selection cohort,” the analyses are not entirely independent and any peculiarity specific to our control group would affect every comparison. Similarly, without patient

Fig. 2 Number of blastocysts available for biopsy, sex selection vs. male factor

identifiers in the database, we are unable to identify repeat cases, and consequently, specific patients who underwent multiple cycles may be overrepresented in our dataset. Finally, this dataset only includes cases in which there were blastocysts available for trophectoderm biopsy, consequently excluding patients who intended to do PGS but had no usable blastocysts. This may have led to patients with infertility affecting oocyte or embryo development being underrepresented in this dataset. However, it has been shown that aneuploidy rates are higher among arrested cleavage stage embryos than those which develop into blastocysts [13]. This may suggest that if these patients and their arrested embryo cohorts were included, it may further affirm our findings. Our finding that patients with an indication of male factor infertility having significantly fewer blastocysts available for biopsy than maternal age-matched cohorts with different indications has been previously observed in both donor and autologous oocytes [14, 15] and may be related to the paternal contribution to transcription after the 8-cell stage [16].

Our method to distinguish meiotic versus mitotic error aneuploidies of maternal and paternal origin is based on the assumption that the vast majority of non-meiotic trisomies and monosomies involving gain/loss of paternal chromosomes are mitotic (post-zygotic) in origin. This assumption is based on a large set of high-confidence meiotic trisomies in which both maternal homologs are observed, which exhibit an expected positive association with maternal age [8]. While paternal meiotic trisomies are similarly identifiable by the presence of both paternal homologs, these are extremely rare (146/14,909 total biopsy samples = 0.98%, 495/342,907 total chromosomes = 0.14%). This leads us to the expectation that paternal monosomies of meiotic origin should be similarly rare as meiotic non-disjunction produces both monosomies and trisomies. Meanwhile, we observe that paternal monosomies are relatively common, suggesting their predominantly mitotic origins. Aneuploid samples that had ambiguous patterns, such as maternal chromosome losses, were not assigned to either the “meiotic” or “putative mitotic” category, as they may frequently arise by either mechanism. While we believe this logic supports the methods used to classify aneuploidy origin, we also recognize it is based on assumptions that may introduce error in our analysis.

Consultations for patients planning treatments with IVF-PGS are often focused on maternal-age-based projections of expected aneuploidy rates and euploid embryos available for transfer. As patients consider the pros and cons of IVF with or without PGS, they may benefit from more personalized estimates of their aneuploidy rates based on their individual infertility diagnoses. We hope these data will assist patients and practitioners understand their projected aneuploidy rates to make informed decisions about the role of PGS in their care.

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