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ASSISTED REPRODUCTION TECHNOLOGIES

The impact of preimplantation genetic testing for aneuploidies (PGT-A) on clinical outcomes in high risk patients

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Abstract

Purpose To investigate whether preimplantation genetic testing for aneuploidy (PGT-A) improves the clinical outcome in patients with advanced maternal age (AMA), recurrent miscarriages (RM), and recurrent implantation failure (RIF).

Methods Retrospective cohort study from a single IVF center and a single genetics laboratory. One hundred seventy-six patients undergoing PGT-A were assigned to three groups: an AMA group, an RM group, and a RIF group. Two hundred seventy-nine patients that did not undergo PGT-A were used as controls and subgrouped similarly to the PGT-A cohort. For the PGT-A groups, trophectoderm biopsy was performed and array comparative genomic hybridization was used for PGT-A. Clinical outcomes were compared with the control groups.

Results In the RM group, we observed a significant decrease of early pregnancy loss rates in the PGT-A group (18.1% vs 75%) and a significant increase in live birth rate per transfer (50% vs 12.5%) and live birth rate per patient (36% vs 12.5%). In the RIF group, a statistically significant increase in the implantation rate per transfer (69.5% vs 33.3%) as well as the live birth rate per embryo transfer (47.8% vs 19%) was observed. In the AMA group, a statistically significant reduction in biochemical pregnancy loss was observed (3.7% vs 31.5%); however, live birth rates per embryo transfer and per patient were not significantly higher than the control group.

Conclusion Our results agree with recently published studies, which suggest caution in the universal application of PGT-A in women with infertility. Instead, a more personalized approach by choosing the right candidates for PGT-A intervention should be followed.

Keywords Preimplantation genetic testing for aneuploidies (PGT-A) · aCGH · Advanced maternal age (AMA) · Recurrent miscarriages (RM) · Recurrent implantation failure (RIF)

Introduction

Aneuploidy, defined as an abnormal number of chromosomes in a cell, is considered the main cause of early pregnancy loss and implantation failure, with a reported prevalence as high as 76% in first trimester spontaneous abortions [1]. Embryonic aneuploidy mainly originates from meiotic chromosomal segregation errors and is positively correlated with increased maternal age, usually resulting in loss of implantation potential [2, 3].

Preimplantation genetic testing for aneuploidies (PGT-A) is the procedure used to screen the chromosomal composition of embryos produced by assisted reproduction technology (ART) to select those that do not exhibit chromosomal abnormalities. By selecting euploid embryos,

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PGT-A aims to eliminate the maternal age effect [4] and increase implantation and live birth rates per embryo transfer, reduce miscarriage rates and abnormal pregnancies, as well as reduce time to pregnancy and enable elective single embryo transfer (eSET) [5]. Thus, PGT-A is indicated for couples with advanced maternal age (AMA), recurrent implantation failure (RIF), and recurrent miscarriages (RM) when the parental karyotypes are normal, as well as severe male factor (SMF) infertility [1, 5].

One of the initial methodologies used was fluorescent in situ hybridization (FISH) performed on polar body biopsies or blastomeres that were obtained from cleavage stage biopsy. FISH was used to screen for the most common chromosomal aneuploidies, affecting chromosomes 13, 18, 21, X, and Y [6], expanding to cover more chromosomes as the technology advanced. This approach, however, failed to deliver on its promise of improving IVF outcomes [7], due to a multitude of reasons including embryo biopsy techniques and limitations of FISH analysis, as well as the high prevalence of mosaicism at this stage of the developing embryo.

The advancement of techniques such as blastocyst stage biopsy [8] and embryo cryopreservation through vitrification [9] along with the development of novel molecular methodologies allowing the comprehensive screening of all 24 chromosomes, such as array comparative genomic hybridization (aCGH), led to a revised approach [10].

This second iteration of PGT-A has since been augmented by enhanced whole genome amplification methods and the introduction of oligonucleotide probe arrays for aCGH and, more recently, next generation sequencing (NGS) technology [9], all of which additionally enabled segmental chromosomal aberrations and mosaicism detection [11].

A number of randomized controlled trials (RCTs) [12–16] and studies [17–20] have attempted to assess the efficacy of this revised PGT-A strategy, with mixed results fueling an ongoing debate. In order to conclusively decide, more data on the outcomes of PGT-A on trophectoderm biopsy with comprehensive chromosome screening molecular methods are required [21].

In this retrospective study, we report on the pregnancy outcomes for three groups of patients with advanced maternal age (AMA), recurrent miscarriages (RM), and recurrent implantation failure (RIF) as their main indication, undergoing IVF with PGT-A. The study used oligonucleotide aCGH analysis after blastocyst biopsy. Three patient groups comprising couples with the same IVF indication and demographics that did not undergo PGT-A were used as controls to quantify any potential benefits.

Materials and methods

Patient groups

All couples included in this study were Caucasian and underwent IVF treatment at Genesis Athens Clinic between January 2017 and September 2019. One hundred seventy-six couples were referred to Genesis-Genoma laboratory for PGT-A. Specifically, 121 couples had advanced maternal age (AMA) as main indication for testing, 25 couples had recurrent miscarriages (RM) and normal parental karyotypes, and 30 couples had recurrent implantation failure (RIF). A total of 690 embryo biopsies were analyzed for chromosomal aberrations with PGT-A.

Women included in the AMA group were ≥ 37 years old, whereas the RM group was defined as couples with ≥ 3 miscarriages, and the RIF group as couples that failed to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in ≥ 3 IVF cycles when the maternal age was < 40 years [22].

Couples undergoing IVF treatment without PGT-A for the same indication during the same time period were used as controls. Specifically, 197 couples with AMA, 40 with a history of RM, and 42 with RIF were included in the study. For the control groups, embryos for implantation were selected based on morphological criteria, using the consensus scoring system of the Istanbul workshop on embryo assessment [23]. This scoring system consists of a numerical interpretation of the Gardner scale [24], where each blastocyst is scored with a 3-digit number that provides information on the stage of development as well as the grade of the inner cell mass (ICM) and the trophectoderm (TE). All embryos transferred in both the PGT-A and control groups comprised equally high-quality blastocysts, i.e., expanded blastocysts with either good or fair ICM and TE scores.

For the PGT-A groups, all patient karyotypes were normal, with the exception of the presence of low-level mosaicism for X chromosome monosomy (46, XX [92–98]/46, X [2–8]) in 18 women from the AMA group, 2 from the RM and 3 from the RIF group. Couples with chromosomal abnormalities were also excluded from the control groups.

Ethics statement

Informed written consent was obtained from all couples. The study protocol was carried out in accordance with the guidelines of the Bioethics Committee of the National and Kapodistrian University of Athens.

Methods

IVF procedures

Ovarian stimulation protocols were given to all patients, including long and short gonadotropin-releasing hormone (GnRH) agonist, GnRH antagonist and clomiphene citrate (CC) protocol. Depending on the protocol, human chorionic gonadotropin (hCG) was administered for oocyte maturation, after follicle measurement by transvaginal ultrasonography. Oocyte retrieval was performed 36 h after the hCG injection and following the removal of cumulus cells, all MII stage oocytes were fertilized by intracytoplasmic sperm injection (ICSI) and embryos were cultured to the blastocyst stage.

Embryo biopsy

Embryo biopsy was performed at the blastocyst stage, which for all embryos was reached by the 5th day after fertilization. A small number of cells (5–10) were dissected from the trophectoderm cell mass that herniated after laser-assisted hatching of the *zona pelucida*. The cells were then transferred to a sterile 0.2 ml PCR tube with ~ 2 µl of phosphate buffered saline (PBS) 1X (Gibco, Thermo Fisher Scientific).

Whole genome amplification (WGA)

WGA was performed with the RepliG Single Cell kit (Qiagen, Hilden, Germany), which is based on the multiple displacement amplification (MDA) method, a non-PCR-based amplification method utilizing the Φ29 enzyme. In short, the biopsied cells were lysed with gentle alkaline incubation and isothermal amplification was performed with the Φ29 polymerase at 31 °C for 85 min. Whole genome amplification of a male and female Human Reference DNA (Agilent Technologies, Inc., CA, USA), diluted to 1 ng/µl concentration, was performed at the same time. The efficiency of the WGA reaction was determined with the Qubit 2.0 Fluorometer (Thermo Fisher Scientific), using the dsDNA Broad Range kit. Negative controls were used to exclude contamination from both the embryology as well as the genetics laboratory.

Array comparative genomic hybridization (aCGH) analysis

aCGH analysis was performed using the GenetiSure Prescreen 8 × 60 K microarray platform (G5963A, Agilent Technologies, Inc., CA, USA) that contains 55,000 unique oligonucleotide probes with a median probe spacing of ~ 50 kb, offering genome-wide coverage with an increased probe density on chromosomes 13, 18, 20, 21, 22, X, and Y.

The amplified DNA samples were differentially labeled using Cyanine3 (Cy3) and Cyanine5 (Cy5) fluorescent dyes after random priming, for 45 min at 37 °C with the Klenow frag. Exo(-) enzyme (SureTag Labeling Kit, 5190–4240, Agilent Technologies, Inc., CA, USA). The labeled products were then combined and purified by centrifugation using the SureTag Purification Columns (Agilent Technologies, Inc., CA, USA). The purified-labeled pairs were hybridized on the microarray in a rotating hybridization oven at 67 °C for 12 h. The microarray slides were then washed and scanned on a SureScan Dx Microarray Scanner (G5761A, Agilent Technologies, Inc., CA, USA), at 3 um resolution. The resulting tiff image was subsequently imported for both feature extraction and analysis of the resulting data into the CytoGenomics v.5.0.2 software suite. The aberrations were inferred using the ADM-2 algorithm, with a $\log_2$ ratio threshold of 0.45 for the deletions and 0.35 for the duplications and a minimum size cut-off value of 10 Mb. Embryos were classified as euploid, aneuploid, or undiagnosed. In the case of an undiagnosed embryo, either a re-biopsy was performed or the embryo was not used. Only euploid embryos were transferred.

Embryo transfer

Fresh embryo transfer was performed on the 6th day after fertilization. When more than one embryo was available, 1–3 embryos were transferred. Specifically, for the AMA and RM PGT-A groups, an average of 1.6 embryos were transferred, while an average of 2 embryos were transferred in the RIF PGT-A group. For the control groups, a slightly higher average of 2.1 embryos were transferred in the AMA group, 2.3 embryos in the RM group, and 2.2 embryos in the RIF group. A total of 586 embryos were transferred in the control groups while 158 embryos were transferred in the PGT-A groups.

Outcome variables

The primary outcome measure was live birth rate per patient that was included in the cohort, while secondary outcomes were live birth rate per ET, implantation rate, biochemical pregnancy loss rate, and miscarriage rate. Biochemical pregnancy loss was defined as the coexistence of maternal hCG serum level > 4 mIU/ml with the absence of a gestational sac, diagnosed by ultrasound. Miscarriage was defined as the loss of pregnancy following the identification of a gestational sac by ultrasound.

Statistical analysis

Statistical analysis of the results was performed on R with the fmsb statistical package [25]. The default settings were used, i.e., the probability for confidence intervals was 0.95 and the *p*-value calculation was done by testing the null-hypothesis of independence between exposure and disease. The *p* value, calculated point estimate of odds ratio, and upper and lower limit of confidence intervals are reported in the relevant tables. Any differences observed were considered statistically significant at $p < 0.05$.

Results

AMA

PGT-A was performed on a total of 425 embryo biopsies from 121 couples due to advanced maternal age (mean age 41.3 years, range 37–49). Diagnosis was achieved for 382/425 embryos (287 aneuploid, 95 euploid). Of the aneuploid embryos, 28.2% had one aberration, 22.3% had two, and 49.5% had more than 3. From the 51 cases with available embryos for transfer, 27 resulted in successful implantation. Of those 11 resulted in single pregnancy, 6 in twin pregnancy and 1 in triplet. In total from the 18 live birth cases, a total of 26 babies were born (Table 1). For the AMA PGT-A group, the diagnosis rate was 89.9% with an aneuploidy incidence of 75.2%.

One hundred ninety-seven couples undergoing IVF because of AMA that were not subjected to PGT-A were used as control group (mean age 41.4 years, range 38–50). A total of 197 embryo transfers were performed, 92 of which led to successful implantation with 48 cases resulting in live birth. Early abortion occurred in 14 cases, biochemical pregnancy loss in 29, and an ectopic pregnancy was observed in 1 case. In total, 72 babies were born (Table 2).

RM

A total of 112 embryo biopsies from 25 couples with recurrent first trimester miscarriages were subjected to PGT-A (mean age of 35.9 years, range 31–45). Of the 112 embryos tested, diagnosis was possible for 106 (62 aneuploid and 44 euploid). Of the aneuploid embryos, 40.3% had a single chromosomal aberration, 21% had two while 38.7% had ≥ 3 aberrations. Embryo transfer was performed in 18 cases, resulting in 11 successful implantations. Two cases resulted in early spontaneous abortions. The total number of successful pregnancies was 9 (6 single and 3 twin), resulting in 12 babies born (Table 1). In the RM PGT-A group, the diagnosis rate was 94.6%, the incidence of aneuploidy was 58.5%, and 72% of the patients proceeded with ET.

The non PGT-A control group included 40 couples (mean age 33.5 years, range 28–36). All cases proceeded with embryo transfer and 28 achieved implantation resulting in 5 live births (13 babies born), 21 first trimester spontaneous abortions, and 2 cases with biochemical pregnancy loss (Table 2).

RIF

A total of 153 embryo biopsies from 30 couples with recurrent implantation failure (mean age 34.7 years, range 29–39) were subjected to PGT-A. Out of the 153 embryos analyzed, 140 were successfully diagnosed, with 81 embryos being aneuploid and 59 euploid. Of the aneuploid embryos, 40.7% had a single chromosomal aberration, 24.7% had two, and 34.6% had ≥ 3 aberrations. Embryo transfer was performed in 23 cases, resulting in 16 successful implantations, leading to 7 single pregnancies and 4 twin pregnancies. Early spontaneous abortion was observed in 3 cases and biochemical pregnancy loss in 2 cases. The live birth rate was 11/23 (47.8%), with a total of 15 babies being born (Table 1). For the RIF PGT-A group, the diagnosis rate was 91.5% and the incidence of aneuploidy was 57.9%. Twenty-three couples (76.6%) proceeded with ET.

The control group consisted of 42 couples with RIF that proceeded with IVF without PGT-A (mean age 33.4 years, range 28–36). Implantation was achieved in 14 cases, resulting in 3 spontaneous early abortions, 1 ectopic pregnancy, 2 biochemical pregnancy losses, and 8 live births (Table 2).

All patients that were included in the control groups proceeded with embryo transfer, while, as expected, there was a significant decrease in the percentage of cycles reaching ET in the PGT-A groups with 42.1% of the AMA PGT-A group, 72% of the RM PGT-A group and 76.6% of the RIF group having at least one euploid blastocyst and proceeding with ET.

Discussion

In this study, we attempt to evaluate the potential benefits of applying preimplantation genetic testing for aneuploidies to couples with advanced maternal age, recurrent miscarriages, and recurrent implantation failure as their main reason for referral by the IVF clinic. To this end, the clinical outcomes of these three patient groups undergoing PGT-A with an oligonucleotide aCGH platform were compared with three control groups comprising couples with the same IVF indication that proceeded with embryo transfer based on morphological criteria.

Table 1 Results for the AMA, RM, and RIF PGT-A groups

	AMA PGT-A	RM PGT-A	RIF PGT-A
Number of cases	121	25	30
Mean age (range)	41.3 (37–49)	35.9 (31–45)	34.7 (29–39)
Total no of embryos	425	112	153
No of diagnosed embryos	382	106	140
No of embryos without diagnosis	43	6	13
No of euploid embryos	95	44	59
No of aneuploid embryos	287	62	81
No of embryos with 1 aberration	81	25	33
No of embryos with 2 aberrations	64	13	20
No of embryos with ≥ 3 aberrations	142	24	28
Embryo transfers	51 (82 embryos transferred)	18 (29 embryos transferred)	23 (47 embryos transferred)
Couples with 1 embryo transferred	25 (49%)	7 (39%)	3 (13%)
Couples with 2 embryos transferred	21 (41%)	11 (61%)	16 (70%)
Couples with 3 embryos transferred	5 (10%)	-	4 (17%)
Implantation	27	11	16
Biochemical pregnancy loss	1	-	2
Clinical pregnancy	26	11	14
Early abortion (< 12 weeks)	8	2	3
Single pregnancy	11	6	7
Twin pregnancy	6	3	4
Triple pregnancy	1	-	-
Live births	18	9	11
Total no of babies born	26	12	15

In the AMA PGT-A group, live birth rate and implantation rate per ET were not significantly higher from the control group (35.2% vs 24.3% and 52.9% vs 46.7% respectively), but a significant reduction in biochemical pregnancy loss was observed (3.7% vs 31.5%) (Table 3). Additionally, an increase in the percentage of babies born out of the total number of embryos transferred was observed, indicating a positive impact of embryo selection through PGT-A. However, a reduction in live birth rate per patient was observed at 14.8% vs 24.3% in the control group.

Although most studies thus far show a relative benefit in applying PGT-A to women with AMA, there are instances where this has not proven to be the case. In a recent study [26] on very poor prognosis AMA patients (44–47 years) undergoing PGT-A with qPCR and single embryo transfer, the authors reported a delivery rate of 57.1% per ET

Table 2 Results for the AMA, RM and RIF control groups

	AMA control	RM control	RIF control
Number of cases	197	40	42
Mean age (range)	41.4 (38–50)	33.5 (28–36)	33.4 (28–36)
Embryo transfers	197 (401 embryos transferred)	40 (92 embryos transferred)	42 (93 embryos transferred)
Implantation	92	28	14
Biochemical pregnancy loss	29	2	2
Clinical pregnancy	63	26	12
Ectopic pregnancy	1	-	1
Early abortion (< 12 weeks)	14	21	3
Live births	48	5	8
Total no of babies born	72	13	18

Table 3 Comparison of clinical outcomes and statistical analysis for the AMA PGT-A and control groups

	AMA PGT-A	AMA control	*P* value	Odds ratio (confidence intervals)
Diagnosed embryos/total no of embryos	382/425 (89.9%)	N/A	N/A	N/A
Euploid embryos/diagnosed embryos	95/382 (24.8%)	N/A	N/A	N/A
Aneuploid embryos/diagnosed embryos	287/382 (75.2%)	N/A	N/A	N/A
Embryo transfers/total no of cases	51/121 (42.1%)	197/197 (100%)	N/A	N/A
Successful implantations/embryo transfers	27/51 (52.9%)	92/197 (46.7%)	0.427	0.778 (0.420–1.443)
Biochemical pregnancy losses/implantations	**1/27 (3.7%)**	**29/92 (31.5%)**	**0.003**	0.083 (0.010–0.645)
Early abortions/implantations	8/27 (29.6%)	14/92 (15.2%)	0.091	2.345 (0.860–6.395)
Live births/embryo transfers	18/51 (35.2%)	48/197 (24.3%)	0.116	0.590 (0.305–1.142)
Babies born/total no of embryos transfered	**26/82 (31.7%)**	**72/401 (17.9%)**	**0.004**	0.471 (0.277–0.801)
Live births/clinical pregnancies	18/26 (69.2%)	48/63 (76.1%)	0.497	1.422 (0.515–3.923)
Live births/total no of cases	**18/121 (14.8%)**	**48/197 (24.3%)**	**0.043**	1.843 (1.014–3.349)

Statistically significant results are shown in bold

and 8.8% per patient. Euploidy rate was 14%, but declined with increasing female age, with women over 45 having no euploid blastocysts. PGT-A seemed to have a positive impact only in women aged 44 or less and with a good ovarian reserve, illustrating the fact that only very specific subgroups of patients with AMA can benefit from the application of PGT-A. Furthermore, in the highly anticipated STAR trial, Munné and colleagues reported no statistically significant benefit of applying PGT-A with NGS and frozen-thawed SET in good prognosis patients aged 25–40 years [16].

In the RM PGT-A group, a statistically significant decrease of early pregnancy loss rates for the PGT-A group versus the control group was observed (18.1% vs 75%). Moreover, a significant increase in live birth rate per transfer (50% vs 12.5%), as well as an increase in live birth rate per pregnancy (81.8% vs 19.2%) and per patient (36% vs 12.5%) were observed (Table 4).

Sato and colleagues recently reported on the outcomes of PGT-A for patients with recurrent miscarriage after a randomized pilot study [20], with very similar results to our own study. Specifically, they reported a diagnosis rate of 92.5%, a 29.2% euploidy rate, with 51.2% of the patients proceeding with embryo transfer. The live birth rate per embryo transfer for this patient group was 52.4% and 26.8% per patient. The miscarriage rate reported was 14.3%. In another study that included 112 RM patients that underwent PGT-A [27], a live birth rate per ET of 57% was reported, in agreement with our results.

Clinical outcomes in the RIF PGT-A group showed a significantly increased 47.8% live birth rate per embryo transfer versus 19% observed in the control group. Furthermore, a statistically significant increase in the implantation rate per transfer was observed (69.5% vs 33.3% in the control). The live birth rate per patient was 36.6% and miscarriage rate was 18.75% (Table 5).

The study by Sato and colleagues on a group of RIF patients of similar size ($n = 42$) that undertook PGT-A

Table 4 Comparison of clinical outcomes and statistical analysis for the RM PGT-A and control groups

	RM PGT-A	RM control	*P* value	Odds ratio (confidence intervals)
Diagnosed embryos/total no of embryos	106/112 (94.6%)	N/A	N/A	N/A
Euploid embryos/diagnosed embryos	44/106 (41.5%)	N/A	N/A	N/A
Aneuploid embryos/diagnosed embryos	62/106 (58.5%)	N/A	N/A	N/A
Embryo transfers/total no of cases	18/25 (72%)	40/40 (100%)	N/A	N/A
Successful implantations/embryo transfers	11/18 (61%)	28/40 (70%)	0.508	1.484 (0.463–4.756)
Biochemical pregnancy losses/implantations	0/11 (0%)	2/28 (7.1%)	0.369	0
Early abortions/implantations	**2/11 (18.1%)**	**21/28 (75%)**	**0.001**	0.074 (0.012–0.428)
Live births/embryo transfers	**9/18 (50%)**	**5/40 (12.5%)**	**0.002**	0.142 (0.038–0.532)
Babies born/total no of embryos transfered	**12/29 (41.4%)**	**13/92 (14.1%)**	**0.001**	0.233 (0.090–0.598)
Live births/clinical pregnancies	**9/11 (81.8%)**	**5/26 (19.2%)**	**0.0004**	0.052 (0.008–0.325)
Live births/total no of cases	**9/25 (36%)**	**5/40 (12.5%)**	**0.026**	0.253 (0.073–0.880)

Statistically significant results are shown in bold

reported 95.7% diagnosis rate, with 21.1% of blastocysts tested being euploid. Twenty-four patients proceeded with embryo transfer (57.1%), while 70.8% achieved clinical pregnancy. Live birth rate for this group was 62.5% when compared to the total number of embryo transfers and 35.7% when compared to the total number of patients in the group.

In a 2014 study by Greco and colleagues [28] on a group of RIF patients, undergoing PGT-A with aCGH on blastocyst biopsy and single embryo transfer reported 68.3% clinical pregnancy rate in the PGT-A group compared to 21.25% in the control group. Additionally, they observed similar implantation rates between the RIF PGT-A group and a group that undertook PGT-A without RIF, concluding that PGT-A can provide a benefit for patients with RIF.

Overall, our data showed a clear benefit of applying PGT-A in patients with recurrent miscarriages and recurrent implantation failure, as chromosomal aneuploidies appear to be the most common factor leading to early pregnancy loss and loss of implantation potential. However, not all women with difficulty in achieving implantation share the same underlying causes and thus a personalized approach to each case by the attending ART specialist is crucial to decide on whether an intervention with PGT-A can be beneficial. The coexistence of higher rates of euploid embryos with lower successful pregnancy rates that we observed in the AMA group could be a result of conditions other than aneuploidy, such as endometrium receptivity, which should also be evaluated after failed implantations following PGT-A. The relatively poor results observed in the AMA group could also be associated with the lower average number of embryos transferred (1.6 vs 2.1 in the control group); however, this cannot be ascertained. When assessing embryo morphology data, no indication for lower quality embryos for inner cell mass (ICM) or trophectoderm cells was observed in any one of the groups compared to the others.

The study carries some limitations, mainly associated with its retrospective non-randomized nature, even though care was given to assign similar profile patients as control groups. As a result, the PGT-A and control groups may have slightly different clinical characteristics, introducing a possible source of bias. Furthermore, couples that did not proceed with embryo transfer were excluded from the control groups, to provide a more robust frame of comparison, although this could impact outcome results and possibly explain the subpar results achieved by the AMA PGT-A group, although at the same time making results obtained from the RM and RIF groups more robust. Finally, some baseline characteristics of the couples included in the study apart from age and ethnicity (e.g., BMI) were not available.

Most aneuploid embryos in our cohort carried uniform chromosomal aneuploidies, which are considered to originate from meiotic errors while segmental aneuploidies, which are predominantly of mitotic origin [29], were less frequent. These mitotic events are not uniformly distributed throughout the blastocyst thus often resulting in chromosomal mosaicism. The chromosomes most observed to be aneuploid in our cohort were 22, 16, 15, and 21 while the higher incidence of segmental aberrations was observed in chromosomes 8 and X.

In a recent randomized study [30] of young infertile patients (≤ 35 years), single blastocyst frozen embryo transfer after PGT-A did not increase live birth rates when compared to morphological assessment in the control group. Still, the authors argued that the hypothesis that PGT-A can improve outcomes cannot be completely dismissed as the inability to demonstrate improvement in live birth rates can be a result of the effectivity of TE biopsy, which can lead to misdiagnosis due to undetected mosaicism and the discordance between the trophectoderm biopsy and the inner cell mass.

The aCGH platform used in this study utilizes high density oligonucleotide probes, leading to an increase in both sensitivity and specificity when compared to older BAC-based platforms. At the same time, the higher resolution afforded can

Table 5 Comparison of clinical outcomes and statistical analysis for the RIF PGT-A and control groups

	RIF PGT-A	RIF control	*P* value	Odds ratio (confidence intervals)
Diagnosed embryos/total no of embryos	140/153 (91.5%)	N/A	N/A	N/A
Euploid embryos/diagnosed embryos	59/140 (42.1%)	N/A	N/A	N/A
Aneuploid embryos/diagnosed embryos	81/140 (57.9%)	N/A	N/A	N/A
Embryo transfers/total no of cases	23/30 (76.6%)	42/42 (100%)	N/A	N/A
Successful implantations/embryo transfers	**16/23 (69.5%)**	**14/42 (33.3%)**	**0.005**	0.218 (0.073–0.654)
Biochemical pregnancy losses/implantations	2/16 (12.5%)	2/14 (14.2%)	0.887	0.857 (0.104–7.042)
Early abortions/implantations	3/16 (18.75%)	3/14 (21.4%)	0.857	0.846 (0.141–5.070)
Live births/embryo transfers	**11/23 (47.8%)**	**8/42 (19%)**	**0.015**	0.256 (0.083–0.789)
Babies born/total no of embryos transfered	15/47 (31.9%)	18/93 (19.3%)	0.099	0.512 (0.229–1.140)
Live births/clinical pregnancies	11/14 (78.5%)	8/12 (66.6%)	0.503	0.545 (0.094–3.145)
Live births/total no of cases	11/30 (36.6%)	8/42 (19%)	0.096	0.406 (0.139–1.184)

Statistically significant results are shown in bold

provide a more detailed investigation of chromosomal content and segmental aneuploidies while potentially increasing aneuploidy rates when compared to lower resolution platforms. However, this detailed investigation of embryonic chromosomal composition could also potentially lead to a decrease in the number of affected live births after PGT-A.

Even though aCGH is still being widely implemented in laboratories worldwide for PGT-A, NGS (next generation sequencing) is considered the preferred platform due to its higher sensitivity towards mosaicism detection. However, it has been argued that this higher dynamic range might not be able to provide any benefit in clinical outcome, due to differences in the chromosomal composition of the TE biopsy and inner cell mass. A recent review of the concordance rates of published biopsy reanalysis data and found a significantly higher degree of discordance in studies that utilized NGS and included the reporting of mosaicism and segmental imbalances when compared to other methods, suggesting caution when adopting new iterations of PGT-A in the clinical setting [31].

The large number of studies that have this far been performed has provided highly discordant results [32–34], leaving the questions of whether PGT-A can improve pregnancy and live birth rates, as well as decrease time and cost to pregnancy, unanswered. Additionally, with the emergence of NGS technology, the incidence of mosaicism and its clinical impact has fueled the ongoing debate on the benefit of PGT-A even more [35].

Our results suggest that PGT-A can be of great benefit to specific groups of patients, mainly couples with recurrent miscarriages and recurrent implantation failure that can be attributed to chromosomal aneuploidy.

The decision on PGT-A intervention should be made on a *per case* basis after careful assessment of each patient's profile. A cautious approach to the universal application of PGT-A to all women undergoing IVF is advised until more data from well-organized multicenter RCTs are available and the biology and impact of mosaicism on preimplantation embryos has been elucidated.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Sciorio R, Dattilo M. PGT-A preimplantation genetic testing for aneuploidies and embryo selection in routine ART cycles: time to step back? Clin Genet. 2020;98:107–15.
2. Hassold T, Hunt P. Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. Curr Opin Pediatr. 2009;21:703–8.
3. Rodrigo L, Mateu E, Mercader A, Cobo AC, Peinado V, Milán M, et al. New tools for embryo selection: comprehensive chromosome screening by array comparative genomic hybridization. Biomed Res Int. 2014;2014.
4. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013;100:1695–703.
5. Coonen E, Rubio C, Christopikou D, Dimitriadou E, Gontar J, Goossens V, et al. ESHRE PGT Consortium good practice recommendations for the detection of structural and numerical chromosomal aberrations†. Hum Reprod Open. 2020;2020:1–20.
6. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod. 1993;8:2185–91.
7. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update. 2011;17:454–66.
8. McArthur SJ, Leigh D, Marshall JT, De Boer KA, Jansen RPS. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril. 2005;84:1628–36.
9. Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genet [Internet]. Nature Publishing Group; 2016;17:643–56. Available from: https://doi.org/10.1038/nrg.2016.97
10. Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril [Internet]. Elsevier Ltd; 2010;94:1700–6. Available from: https://doi.org/10.1016/j.fertnstert.2009.10.015
11. Fragouli E, Munne S, Wells D. The cytogenetic constitution of human blastocysts: Insights from comprehensive chromosome screening strategies. Hum Reprod Update. 2019;25:15–33.
12. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012;5:24.
13. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril [Internet]. Elsevier Inc.; 2013;100:100–107.e1. Available from: https://doi.org/10.1016/j.fertnstert.2013.02.056
14. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril [Internet]. Elsevier Inc.; 2013;100:697–703. Available from: https://doi.org/10.1016/j.fertnstert.2013.04.035
15. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017;107:1122–9.
16. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril. 2019;112:1071-1079.e7.
17. Kushnir VA, Darmon SK, Albertini DF, Barad DH, Gleicher N. Effectiveness of in vitro fertilization with preimplantation genetic screening: a reanalysis of United States assisted reproductive technology data 2011–2012. Fertil Steril [Internet]. Elsevier Inc.; 2016;106:75–9. Available from: https://doi.org/10.1016/j.fertnstert.2016.02.026

18. Kang HJ, Melnick AP, Stewart JD, Xu K, Rosenwaks Z. Preimplantation genetic screening: who benefits? Fertil Steril. 2016;106:597–602.
19. Murphy LA, Seidler EA, Vaughan DA, Resetkova N, Penzias AS, Toth TL, et al. To test or not to test? A framework for counselling patients on preimplantation genetic testing for aneuploidy (PGT-A). Hum Reprod. 2019;34:268–75.
20. Sato T, Sugiura-Ogasawara M, Ozawa F, Yamamoto T, Kato T, Kurahashi H, et al. Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. Hum Reprod. 2019;34:2340–9.
21. Harper JC. Preimplantation genetic screening. J Med Screen. 2018;25:1–5.
22. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online. 2014;28:14–38.
23. Balaban B, Brison D, Calderón G, Catt J, Conaghan J, Cowan L, et al. Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod Biomed Online. 2011;22:632–46.
24. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. Curr Opin Obstet Gynaecol [Internet]. 1999;11:307–11. Available from: http://journals.lww.com/00001703-199906000-00013
25. Nakazawa M. Functions for medical statistics book with some demographic data [Internet]. Cran. 2021. Available from: http://cran.r-project.org/web/packages/fmsb/fmsb.pdf
26. Ubaldi FM, Cimadomo D, Capalbo A, Vaiarelli A, Buffo L, Trabucco E, et al. Preimplantation genetic diagnosis for aneuploidy testing in women older than 44 years: a multicenter experience. Fertil Steril [Internet]. Elsevier Inc.; 2017;107:1173–80. Available from: https://doi.org/10.1016/j.fertnstert.2017.03.007
27. Murugappan G, Shahine LK, Perfetto CO, Hickok LR, Lathi RB. Intent to treat analysis of in vitro fertilization and preimplantation genetic screening versus expectant management in patients with recurrent pregnancy loss. Hum Reprod. 2016;31:1668–74.
28. Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, et al. Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment : a pilot study. Hindawi Publishing Corporation; 2014;2014.
29. Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroselli S, et al. Incidence, origin, and predictive model for the detection and clinical management of segmental aneuploidies in human embryos. Am J Hum Genet [Internet]. ElsevierCompany; 2020;106:525–34. https://doi.org/10.1016/j.ajhg.2020.03.005
30. Ozgur K, Berkkanoglu M, Bulut H, Yoruk GDA, Candurmaz NN, Coetzee K. Single best euploid versus single best unknown-ploidy blastocyst frozen embryo transfers: a randomized controlled trial. J Assist Reprod Genet. 2019;36:629–36.
31. Marin D, Xu J, Treff NR. Preimplantation genetic testing for aneuploidy: a review of published blastocyst reanalysis concordance data. Prenat Diagn. 2020;1–9.
32. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod. 2015;30:473–83.
33. Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: a meta-analysis. Fertil Steril [Internet]. Elsevier Inc.; 2015;104:1503–12. https://doi.org/10.1016/j.fertnstert.2015.08.038
34. Chen M, Wei S, Hu J, Quan S. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis PLoS One. 2015;10:1–21.
35. Treff NR, Marin D. The "mosaic" embryo: misconceptions and misinterpretations in preimplantation genetic testing for aneuploidy. Fertil Steril [Internet]. Elsevier Inc.; 2021;116:1205–11. https://doi.org/10.1016/j.fertnstert.2021.06.027