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Original Article

Reproductive genetics

Implicit bias in diagnosing mosaicism amongst preimplantation genetic testing providers: results from a multicenter study of 36 395 blastocysts

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ABSTRACT

STUDY QUESTION: Does the diagnosis of mosaicism affect ploidy rates across different providers offering preimplantation genetic testing for aneuploidies (PGT-A)?

SUMMARY ANSWER: Our analysis of 36 395 blastocyst biopsies across eight genetic testing laboratories revealed that euploidy rates were significantly higher in providers reporting low rates of mosaicism.

WHAT IS KNOWN ALREADY: Diagnoses consistent with chromosomal mosaicism have emerged as a third category of possible embryo ploidy outcomes following PGT-A. However, in the era of mosaicism, embryo selection has become increasingly complex. Biological, technical, analytical, and clinical complexities in interpreting such results have led to substantial variability in mosaicism rates across PGT-A providers and clinics. Critically, it remains unknown whether these differences impact the number of euploid embryos available for transfer. Ultimately, this may significantly affect clinical outcomes, with important implications for PGT-A patients.

STUDY DESIGN, SIZE, DURATION: In this international, multicenter cohort study, we reviewed 36 395 consecutive PGT-A results, obtained from 10 035 patients across 11 867 treatment cycles, conducted between October 2015 and October 2021. A total of 17 IVF centers, across eight PGT-A providers, five countries and three continents participated in the study. All blastocysts were tested using trophectoderm biopsy and next-generation sequencing. Both autologous and donation cycles were assessed. Cycles using preimplantation genetic testing for structural rearrangements were excluded from the analysis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The PGT-A providers were randomly categorized (A to H). Providers B, C, D, E, F, G, and H all reported mosaicism, whereas Provider A reported embryos as either euploid or aneuploid. Ploidy rates were analyzed using multilevel mixed linear regression. Analyses were adjusted for maternal age, paternal age, oocyte source, number of embryos biopsied, day of biopsy, and PGT-A provider, as appropriate. We compared associations between genetic testing providers and PGT-A outcomes, including the number of chromosomally normal (euploid) embryos determined to be suitable for transfer.

MAIN RESULTS AND THE ROLE OF CHANCE: The mean maternal age (±SD) across all providers was 36.2 (±5.2). Our findings reveal a strong association between PGT-A provider and the diagnosis of euploidy and mosaicism. Amongst the seven providers that reported mosaicism, the rates varied from 3.1% to 25.0%. After adjusting for confounders, we observed a significant difference in the likelihood of diagnosing mosaicism across providers ($P < 0.001$), ranging from 6.5% (95% CI: 5.2–7.4%) for Provider B to 35.6% (95% CI: 32.6–38.7%) for Provider E. Notably, adjusted euploidy rates were highest for providers that reported the lowest rates of mosaicism (Provider B: euploidy, 55.7% (95% CI: 54.1–57.4%), mosaicism, 6.5% (95% CI: 5.2–7.4%); Provider H: euploidy, 44.5% (95% CI: 43.6–45.4%), mosaicism, 9.9% (95% CI: 9.2–10.6%)); and Provider D: euploidy, 43.8% (95% CI: 39.2–48.4%), mosaicism, 11.0% (95% CI: 7.5–14.5%)). Moreover, the overall chance of having at least one euploid blastocyst available for transfer was significantly higher when mosaicism

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was not reported, when we compared Provider A to all other providers (OR = 1.30, 95% CI: 1.13–1.50). Differences in diagnosing and interpreting mosaic results across PGT-A laboratories raise further concerns regarding the accuracy and relevance of mosaicism predictions. While we confirmed equivalent clinical outcomes following the transfer of mosaic and euploid blastocysts, we found that a significant proportion of mosaic embryos are not used for IVF treatment.

LIMITATIONS, REASONS FOR CAUTION: Due to the retrospective nature of the study, associations can be ascertained, however, causality cannot be established. Certain parameters such as blastocyst grade were not available in the dataset. Furthermore, certain platform-related and clinic-specific factors may not be readily quantifiable or explicitly captured in our dataset. As such, a full elucidation of all potential confounders accounting for variability may not be possible.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings highlight the strong need for standardization and quality assurance in the industry. The decision not to transfer mosaic embryos may ultimately reduce the chance of success of a PGT-A cycle by limiting the pool of available embryos. Until we can be certain that mosaic diagnoses accurately reflect biological variability, reporting mosaicism warrants utmost caution. A prudent approach is imperative, as it may determine the difference between success or failure for some patients.

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Keywords: PGT-A / PGT laboratory / chromosomal mosaicism / euploidy / intermediate copy number / aneuploidy

Introduction

The high prevalence of chromosomal abnormalities in human embryos is a prominent factor contributing to reproductive failure (Gruhn *et al.*, 2019). Embryonic aneuploidies largely originate during oocyte meiosis and increase progressively with advancing maternal age (Franasiak *et al.*, 2014; Capalbo *et al.*, 2017). Accordingly, the success of IVF treatment rapidly declines for women over the age of 35 years. Yet, if a euploid embryo is transferred, implantation, ongoing pregnancy, and live birth rates remain similar across all age groups (Harton *et al.*, 2013; Irani *et al.*, 2019). In line with this premise, preimplantation genetic testing for aneuploidies (PGT-A) seeks to screen a patient's cohort of embryos to select those that are chromosomally normal, with an aim of achieving the highest chance of live birth per embryo transfer. Given its inherent appeal, the use of PGT-A has increased steadily over the past decade. In 2017, over 30% of all IVF cycles performed in the USA included PGT-A (Roche *et al.*, 2021). Nevertheless, the value of this add-on treatment remains unclear, with many challenging its routine clinical application without reliable evidence attesting to its effectiveness (Mastenbroek *et al.*, 2021; Barad *et al.*, 2022; Gleicher *et al.*, 2022).

PGT-A currently relies on a trophectoderm (TE) biopsy of 5–10 cells, analyzed by next-generation sequencing (NGS). The reduced cost and the high sensitivity of NGS have delivered an enhanced platform for evaluating chromosomal abnormalities at the blastocyst stage of development (Fiorentino *et al.*, 2014). This is particularly true for whole chromosome abnormalities of meiotic origin. Meiotic aberrations affect all embryonic cells uniformly and are largely predictive of adverse clinical outcomes (Marin *et al.*, 2021; Tiegs *et al.*, 2021). However, the interpretation of chromosomal profiles with intermediate copy number values remains a challenge. Such profiles predict mosaicism, i.e. the presence of chromosomally distinct cells within a TE biopsy, and have recently emerged as a third category of possible outcomes in PGT-A (Cram *et al.*, 2019; Leigh *et al.*, 2022). The most clinically relevant diagnoses include the mix of euploid and aneuploid cells (with either whole chromosome or segmental aberrations) (Viotti, 2020), hereafter referred to as mosaic embryos.

Chromosomal mosaicism is largely attributed to mitotic errors during preimplantation development (Delhanty *et al.*, 1993, 1997). Although well-recognized, the higher frequency of mosaic diagnoses following the implementation of NGS casts doubts on the clinical relevance of these findings (Sachdev *et al.*, 2017). The developmental potential of mosaic embryos also remains contentious. Several studies have suggested that embryos diagnosed as mosaic can lead to normal live births (Popovic *et al.*, 2020; Capalbo *et al.*, 2021; Viotti *et al.*, 2021). The selective elimination of aneuploid cells through the competitive growth of euploid cells has been proposed as a mechanism by which human mosaic embryos tolerate chromosomal instability (Yang *et al.*, 2021). These findings have led to recommendations to transfer mosaic embryos in the absence of a euploid alternative (Cram *et al.*, 2019; Leigh *et al.*, 2022). However, retrospective studies have suggested that mosaic diagnoses are associated with reduced reproductive potential (Munné *et al.*, 2017b; Viotti *et al.*, 2021). Although these conclusions were founded on inherently poor prognosis patient cohorts, mosaic embryos were ultimately given low priority and have been routinely classified as unsuitable for clinical use. Prospective non-selection studies have now shown similar reproductive outcomes of euploid and low-level (50%) mosaic embryos (Capalbo *et al.*, 2021; Gill *et al.*, 2022), yet prevailing concerns regarding the potential risks of mosaicism on development continue to complicate clinical decision making following PGT-A. Thus, despite recommendations to transfer mosaic embryos, it has been proposed that fewer than 3% are used for IVF treatment (American Society for Reproductive Medicine, 2020; Capalbo *et al.*, 2021), limiting, in practice, the pool of embryos available for transfer.

In addition to biological considerations, several technical factors hinder the diagnosis of mosaicism. These reflect challenges associated with the genetic analysis of very few cells, such as amplification bias, contamination, and mitotic state (American Society for Reproductive Medicine, 2020; Treff and Marin, 2021). These difficulties, coupled to the complex task of interpreting intermediate copy number values, have led to varying practices in diagnosing and reporting mosaic calls amongst providers. For instance, different intermediate copy number values have been proposed to predict mosaicism. These range between 20% and 80% or 30% and 70% abnormal cells within the TE biopsy (García-Pascual *et al.*, 2020; Marin *et al.*, 2021; Leigh *et al.*, 2022). Thresholds specifying the level of mosaicism also differ amongst PGT-A providers, with 20–40% or <50% abnormal cells, classified as low range, and >40% or >50% aberrant cells indicating high level mosaicism. Some providers choose not to report mosaicism,

classifying an embryo as euploid if the TE biopsy shows <30%, 40%, or 50% abnormal cells. All of these factors, in addition to changing perceptions toward embryo mosaicism, have led to substantial variability in mosaicism rates amongst clinics and genetic testing laboratories (Munné *et al.*, 2019; Viotti *et al.*, 2021).

Arbitrary differences in threshold values may have significant clinical implications, as they ultimately determine whether an embryo is considered suitable for clinical use or how it is prioritized. It remains unknown whether disparities in diagnosing and reporting mosaicism affect PGT-A results, including the availability of euploid embryos for transfer. To compare associations between providers and PGT-A outcomes, we reviewed 37 205 TE biopsy results obtained from eight leading genetic testing laboratories. We further assessed mosaic embryo transfer rates across 15 clinics, including clinical outcomes following the transfer of 245 mosaic blastocysts. Ultimately, our findings shed light on the current clinical utility of PGT-A as a means of improved embryo selection.

Materials and methods

Study design

This is a retrospective, international, multicenter consecutive cohort study of 37 205 PGT-A results, obtained from 10 051 patients across 11 879 cycles conducted between October 2015 and October 2021. Of the 37 205 PGT-A results, 810 TE biopsies (2.2%) were non-informative and were thus excluded from further analyses. Ultimately, we included 11 867 PGT-A cycles, encompassing 36 395 TE biopsy results with known chromosomal status (Fig. 1). A total of 17 IVF clinics, across eight PGT-A providers, five countries and three continents (Europe, North America, and South America) participated in the study (Supplementary Table S1). Providers were randomly categorized from A to H.

The study was approved by the Ethics Committee for Clinical Research of the participating clinics, as per local laws and regulations.

Study population

All patients underwent IVF, using either conventional insemination or ICSI, with PGT-A performed using a TE biopsy and NGS. Both autologous oocyte and oocyte donation cycles were assessed. Preimplantation genetic testing for structural rearrangements (PGT-SR) cycles, which evaluate the presence of specific segmental rearrangements in embryos for which patients have a predetermined risk, were excluded from the analysis. The variables assessed included patient demographics, oocyte source (autologous versus donor oocytes), number of embryos biopsied per cycle, day of biopsy, and PGT-A results per chromosome. All providers biopsied only good quality blastocysts applying similar morphological criteria for biopsy (Gardner score 3CC and above; Gardner and Schoolcraft, 1999). Patient data were de-identified and the results were compiled for analysis.

Embryo classifications

All PGT-A providers performed shallow whole-genome sequencing following their own laboratory protocols, proprietary diagnostic algorithms, and criteria for classifying embryos.

The majority of the providers, B, C, F, G, and H used automated mosaicism calling, with copy number thresholds set between 30% and 70%. Providers D and E used intermediate copy number values ranging between 20% and 80% abnormal cells, however, results were reviewed and called by a certified clinical laboratory supervisor. Providers B, C, D, E, F, G, and H all reported mosaicism; however, Provider D classified all mosaic embryos as unsuitable for transfer. Providers B, C, F, G, and H recommended mosaic embryos for transfer in the absence of a euploid embryo, however, they applied different criteria depending on the chromosome affected, in accordance with current recommendations (Grati *et al.*, 2018; Cram *et al.*, 2019; Leigh *et al.*, 2022). Provider A did not report mosaicism nor did they adhere to stringent thresholds for intermediate copy number values. Here, each intermediate copy number call was reviewed and evaluated in the context of the remaining genome, combining both NGS- and SNP-based assays.

In our study, results were considered as euploid if no aberrations were identified; aneuploid, if they were diagnosed with a single uniform abnormality (single aneuploid), two uniform abnormalities (double aneuploid), or three or more uniform abnormalities (complex aneuploid) (Fragouli *et al.*, 2013), or aneuploid and mosaic (uniform abnormalities in addition to mosaic aberrations). Diagnoses consistent with mosaicism were those containing only mosaic abnormalities, including TE biopsies with one (single mosaic), two (double mosaic), or three or more mosaic aberrations (complex mosaic). We considered mosaic diagnoses as low level, if they predicted <50% abnormal cells.

Clinical outcomes

We evaluated mosaic embryo transfer rates (% of all mosaic embryos transferred) across 15 clinics (A1, C1, C2, D1, E1, F1–F4, G1, H1–H5), including clinical outcomes following the transfer of 245 mosaic blastocysts. We further compared clinical outcomes following the transfer of mosaic embryos to those of euploid embryo transfers, performed across 10 clinics (C1, E1, F1-F4, G1, H1, H4, H5). Our analysis included the assessment of clinical pregnancy rates (the presence of a fetal sac detected by ultrasound at 6–10 weeks per embryos transferred), clinical miscarriage rates (the spontaneous loss of an intra-uterine pregnancy prior to 22 weeks of gestational age per embryos transferred), ongoing pregnancy rates (clinical pregnancy rate minus clinical miscarriage rate), and live birth rates (live births per embryos transferred), according to the International Committee for Monitoring Assisted Reproductive Technologies International Glossary on Infertility and Fertility Care (Zegers-Hochschild *et al.*, 2017). A positive pregnancy test was defined as the presence of positive serum hCG.

Statistical analysis

Considering the hierarchical structure of the data, analyses were performed on three levels: Level 1, patients; Level 2, cycles; and Level 3, embryos. Multilevel mixed regression (linear with robust estimation of variances and logistic) was performed with random intercepts for each level. For logistic models, the coefficients were expressed as odds ratios. The proportion of variance at the provider level was expressed as the percentage relative to the total variance.

The analysis per individual chromosomes was based on results that included a single whole chromosome aneuploidy or single mosaic whole chromosome aberration. A one-sample binomial test was used to evaluate the observed probability for an aberration in each of the 22 autosomal chromosomes against the expected probability (1/22).

Both maternal and paternal age were analyzed as continuous and categorical variables, with the first category (youngest) selected as the reference. Maternal age was categorized in groups according to the Society for Assisted Reproductive Technology (SART). Paternal age data were missing for 2295 cycles. When

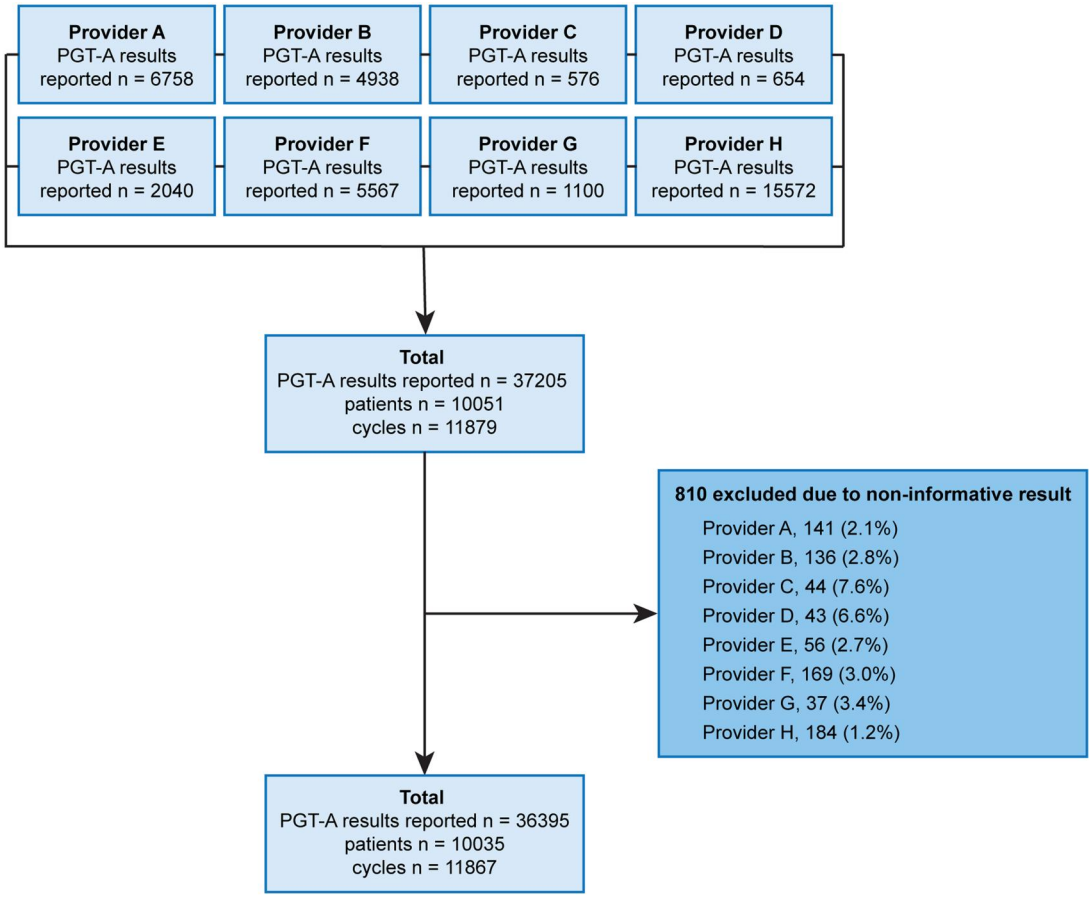

Figure 1. **Total number of preimplantation genetic testing for aneuploidy (PGT-A) results included in the study.**

paternal age was a covariate, separate analyses were performed with the exclusion of these cycles and with missing data imputation. Multiple imputation (five datasets) was performed by linear regression, separately for oocyte donation cycles (year and country as predictors) and for autologous oocyte cycles (maternal age, year, and country as predictors).

We calculated adjusted estimates (rates) per provider by using the marginal predictions of the model. These were adjusted for maternal and paternal age, oocyte source, number of embryos biopsied, and day of biopsy. To test whether the provider or clinic significantly adds to the model explaining the occurrence of the event (euploidy, aneuploidy, and mosaicism), we ran constrained models (with all other predictors as covariables) and full models where the provider or clinic was additionally entered. A likelihood ratio test (chi-square) was then performed to compare both models. The overall significance of categorical variables in the regression models was also tested using a likelihood ratio test (chi-square). Further comparisons between groups with categorical outcome variables were performed using a two-tailed Fisher's exact test. P-values <0.05 were considered significant.

To test whether miscarriage and live birth rates following mosaic embryo transfers were not inferior to those following euploid embryo transfers, we calculated the mean difference in proportions and its 95% confidence intervals (CI). As per previous reports (Capalbo *et al.*, 2021), we set the relevant differences in proportions (delta values) at 7.5% and 2.5% for live birth and miscarriage, respectively. When the 95% CI of the difference of proportion crossed 0 but did not cross the delta value, non-inferiority was assumed.

Results

Baseline characteristics

The demographics of the patient population and baseline embryo characteristics were stratified by PGT-A provider and clinic, as illustrated in Table 1 and Supplementary Tables S1 and S2. Mean maternal age (±SD) ranged from 33.7 (±6.5) to 38.0 (±5.1) years across providers, while mean paternal age (±SD) ranged from 39.0 (±7.3) to 41.8 (±6.3) years. Our study included a total of 4237 (11.6%) PGT-A results obtained from oocyte donor cycles. All donors were <35 years of age. The frequency of blastocysts from oocyte donors varied across providers, ranging from 0% to 34.5%. The number of embryos biopsied per cycle differed across providers, from 4.4 (±2.2) to 6.8 (±3.3). A considerable proportion of blastocysts (59.2%) were biopsied 5 days post-fertilization (Day 5), 38.6% were biopsied at 6 days post-fertilization (Day 6), and 2.2% were biopsied at 7 days post-fertilization (Day 7). These characteristics were largely consistent amongst providers, with the exception of Provider E. Moreover, Clinic D1 (Provider D) and Clinic G1 (Provider G) did not routinely perform biopsies on Day 7 (Supplementary Table S2).

Predictors of diagnoses in PGT-A

Of the eight genetic testing providers, seven (Providers B–H) reported mosaicism as a third category of possible PGT-A outcomes, while Provider A exclusively reported results as either euploid or aneuploid. Overall, 44.9% (n = 16 350) of blastocysts were diagnosed as euploid and 5.7% (n = 2079) were diagnosed as mosaic. The remaining preimplantation embryos (n = 17 966)

Table 1. Patient and embryo characteristics across eight preimplantation genetic testing providers.

	Provider A	Provider B	Provider C	Provider D	Provider E	Provider F	Provider G	Provider H	Total
Patients, n	1846	1149	112	144	527	1509	180	4568	10 035
Cycles, n	1847	1620	135	179	624	1745	210	5507	11 867
Maternal age (years)									
Mean (±SD)	35.7 (±4.6)	37.3 (±5.5)	38.0 (±5.1)	34.2 (±4.8)	33.7 (±6.5)	35.0 (±6.5)	37.3 (±4.4)	36.8 (±4.3)	36.2 (±5.2)
Median	36	39	40	34	35	37	38	38	37
Range	21–47	19–48	22–47	24–45	25–46	18–46	27–47	20–49	18–49
Oocyte donation									
Patients, n (%)	78 (4.2)	129 (11.2)	12 (10.7)	0 (0.0)	153 (29.0)	351 (23.3)	13 (7.2)	303 (6.6)	1039 (10.4)
Paternal age (years)*									
Mean (±SD)	Not available	41.1 (5.2)	40.3 (7.8)	36.8 (6.3)	41.8 (6.3)	39.0 (7.3)	40.1 (6.0)	39.9 (6.2)	40.0 (6.4)
Median	Not available	38	41	43	41	39	41	39	39
Range	Not available	25–67	21–68	24–53	27–78	18–72	28–86	24–82	18–86
Blastocysts, n	6617	4802	532	611	1984	5398	1063	15 388	36 395
Blastocysts biopsied per cycle									
Mean (±SD)	6.2 (±4.6)	4.4 (±2.6)	6.2 (±3.6)	4.6 (±2.3)	4.3 (±2.1)	4.4 (±2.5)	6.8 (±3.3)	4.4 (±3.1)	4.8 (±3.4)
Day of biopsy[1]									
Day 5, n (%)	4218 (63.7)	2283 (47.4)	413 (77.6)	468 (76.6)	355 (17.9)	2945 (54.6)	909 (85.5)	9970 (64.8)	21 561 (59.2)
Day 6, n (%)	2313 (35.0)	2239 (46.5)	114 (21.4)	143 (23.4)	1616 (81.5)	2336 (43.3)	154 (14.5)	5132 (33.4)	14 047 (38.6)
Day 7, n (%)	86 (1.3)	280 (5.8)	5 (0.9)	0 (0.0)	13 (0.7)	117 (2.2)	0 (0.0)	286 (1.9)	787 (2.2)
Oocyte donation									
Blastocysts, n (%)	372 (5.6)	665 (13.9)	49 (9.2)	0 (0.0)	685 (34.5)	1372 (25.4)	65 (6.1)	1029 (6.7)	4237 (11.6)
Blastocyst classification by PGT-A[1]									
Euploid, n (%)	3543 (53.5)	2230 (46.4)	166 (31.2)	336 (55.0)	860 (43.3)	2028 (37.6)	389 (36.6)	6798 (44.2)	16 350 (44.9)
Aneuploid, n (%)	3074 (46.5)	2425 (50.5)	334 (62.8)	238 (39.0)	628 (31.7)	2819 (52.2)	607 (57.1)	7841 (51.0)	17 966 (49.4)
Mosaic, n (%)	Not reported	147 (3.1)	32 (6.0)	37 (6.1)	496 (25.0)	551 (10.2)	67 (6.3)	749 (4.9)	2079 (5.7)
Types of aneuploidies diagnosed by PGT-A[2]									
Single autosomal aneuploidy, n (%)	1945 (63.3)	1185 (48.9)	155 (45.1)	138 (58.0)	309 (49.2)	1208 (43.9)	255 (42.0)	4009 (51.1)	9204 (51.2)
Trisomy, n (%)	753 (24.5)	488 (20.1)	63 (18.3)	51 (21.4)	147 (23.4)	475 (16.8)	81 (13.3)	1475 (18.8)	3533 (19.7)
Monosomy, n (%)	865 (28.1)	606 (25.0)	81 (23.5)	62 (26.1)	133 (21.2)	566 (20.1)	129 (21.3)	2012 (25.7)	4454 (24.8)
Segmental, n (%)	327 (10.6)	91 (3.8)	11 (3.2)	25 (10.5)	29 (4.6)	167 (5.9)	45 (7.4)	522 (6.7)	1217 (6.8)
Double aneuploid, n (%)	702 (22.8)	574 (23.7)	95 (27.6)	42 (17.6)	96 (15.3)	649 (23.0)	130 (21.4)	1662 (21.2)	3950 (22.0)
Complex aneuploid, n (%)	340 (11.1)	296 (12.2)	73 (21.2)	41 (17.2)	50 (8.0)	460 (16.3)	168 (27.7)	1105 (14.1)	2533 (14.1)
Sex chromosome abnormalities, n (%)	87 (2.8)	33 (1.4)	3 (0.9)	2 (0.8)	16 (2.5)	36 (1.3)	7 (1.2)	153 (2.0)	337 (1.9)
Aneuploid and mosaic, n (%)	Not reported	337 (13.9)	8 (2.3)	15 (6.3)	157 (25.0)	466 (16.5)	47 (7.7)	912 (11.6)	1942 (10.8)
Types of mosaic abnormalities diagnosed by PGT-A[3]									
Single autosomal mosaic aberration, n (%)	Not reported	117 (79.6)	24 (75.0)	28 (75.7)	311 (62.7)	333 (60.4)	34 (50.7)	526 (70.2)	1373 (66.0)
Mosaic trisomy, n (%)	Not reported	25 (17.0)	5 (15.6)	8 (21.6)	51 (10.3)	111 (20.1)	7 (10.4)	299 (39.9)	506 (24.3)
Mosaic monosomy, n (%)	Not reported	22 (15.0)	11 (34.4)	11 (29.7)	40 (8.1)	79 (14.3)	11 (16.4)	227 (30.3)	401 (19.3)
Mosaic segmental, n (%)	Not reported	70 (47.6)	8 (25.0)	9 (24.3)	220 (44.4)	143 (26.0)	16 (23.9)	0 (0.0)	466 (22.4)
Double mosaic, n (%)	Not reported	16 (10.9)	4 (12.5)	5 (13.5)	79 (15.9)	113 (20.5)	19 (28.4)	121 (16.2)	357 (17.2)
Complex mosaic, n (%)	Not reported	12 (8.2)	4 (12.5)	4 (10.8)	84 (16.9)	83 (15.1)	11 (16.4)	102 (13.6)	300 (14.4)
Mosaic sex chromosome abnormalities, n (%)	Not reported	2 (1.4)	0 (0.0)	0 (0.0)	22 (4.4)	22 (4.0)	3 (4.5)	0 (0.0)	49 (2.4)
Level of mosaicism diagnosed by PGT-A[4]									
Low, n (%)	Not reported	Not reported	17 (70.8)	Not reported	253 (76.0)	235 (66.2)	20 (54.1)	350 (66.5)	875 (68.6)
High, n (%)	Not reported	Not reported	7 (29.2)	Not reported	63 (18.9)	120 (33.8)	13 (35.1)	176 (33.5)	379 (29.7)
Unknown, n (%)	Not reported	Not reported	0	Not reported	17 (5.1)	0 (0.0)	4 (10.8)	0	21 (1.6)

[1] Percent of all biopsies.
[2] Percent of all aneuploid diagnoses.
[3] Percent of all mosaic diagnoses.
[4] Percent of all single mosaic diagnoses (including sex chromosomes).
* Paternal age data were missing for 2295 cycles.

presented with at least one uniform aneuploidy and were classified as aneuploid (49.4%) (Table 1).

Notably, euploidy, aneuploidy, and mosaicism rates varied across providers (Table 1; Supplementary Table S2). Differences in ploidy rates were also apparent when results were stratified according to SART age groups (Supplementary Table S3). We further evaluated associations between cycle characteristics and diagnoses of euploidy, aneuploidy, and mosaicism (Table 2). As previously established (Franasiak *et al.*, 2014; Demko *et al.*, 2016; Irani *et al.*, 2019), euploidy rates decreased significantly with advancing maternal age (unadjusted OR: 0.35, 95% CI: 0.32–0.39, $P < 0.0001$) (Table 2). Conversely, diagnoses of mosaicism were not associated with maternal age (unadjusted OR: 0.99, 95% CI: 0.98–1.00, $P = 0.064$) (Table 2). Interestingly, however, when considering maternal age as a categorical variable, in our adjusted analysis, Providers G and H presented with higher odds of diagnosing mosaicism for women over the age of 40 years (Supplementary Table S4). We observed lower euploidy rates for blastocysts biopsied on Day 6 (43.9%) and Day 7 (36.3%), compared to those biopsied on Day 5 (47.9%) (Table 2). Similarly, mosaicism rates were slightly higher in Day 6 TE biopsies (6.9%), compared to those obtained on Day 5 (5.0%) (Table 2). Our regression analysis, also demonstrated a positive correlation between the day of biopsy and diagnoses of aneuploidy and mosaicism, confirming previous single-reference laboratory reports (Ai *et al.*, 2022; Walters-Sen *et al.*, 2022) (Table 2). We further confirmed that the number of embryos biopsied per cycle (Ata *et al.*, 2012) was independent from PGT-A results (Table 2). Similarly, when adjusting for maternal age and donor status, we found no association between paternal age and diagnoses of euploidy, aneuploidy or mosaicism, as has been previously suggested (Dviri *et al.*, 2021) (Table 2).

TE biopsies obtained from the four clinics associated with Provider F were all analyzed by the same genetic testing laboratory (Supplementary Tables S1 and S2). After adjusting for confounders, we observed no correlation between clinics referring to Provider F and euploidy, mosaicism, nor aneuploidy rates ($P = 0.108$, $P = 0.109$, $P = 0.109$, respectively) (Supplementary Table S5). We further compared PGT-A results across the six clinics referring to Provider H. Here, we observed a significant difference in adjusted euploidy rates between clinics, with rates varying between 38.3% (95% CI: 37.0–39.7%) and 55.5% (95% CI: 53.2–57.9%) (Supplementary Table S5). Mosaicism rates also varied amongst clinics referring to Provider H, ranging from 7.3% (95% CI: 5.7–8.9%) to 13.7% (95% CI: 9.3–18.1%) (Supplementary Table S5).

Our adjusted analysis demonstrated significant differences between PGT-A results amongst providers (Fig. 2). This is especially relevant when considering diagnoses of euploidy (Fig. 2A) and mosaicism (Fig. 2B), where the greatest differences were observed. Amongst the seven providers that reported mosaicism, rates varied from 3.1% to 25.0% (Table 1). Our adjusted analysis further confirmed these differences ($P < 0.001$) with the likelihood of diagnosing mosaicism amongst providers ranging from 6.5% (95% CI: 5.2–7.4%) for Provider B to 35.6% (95% CI: 32.6–38.7%) for Provider E (Fig. 2B, Supplementary Table S5). Adjusted euploidy rates also varied amongst providers ($P < 0.001$), ranging from 33.7% (95% CI: 28.9–38.5%) to 55.7% (95% CI: 54.1–57.4%) (Fig. 2A; Supplementary Table S6). Notably, euploidy rates were higher for providers that reported the lowest rates of mosaicism, Provider B (adjusted euploidy rate 55.7%, adjusted mosaicism rate 6.5%), Provider H (adjusted euploidy rate 44.5%, adjusted mosaicism rate 9.9%), and Provider D (adjusted euploidy rate 43.8%, adjusted mosaicism rate 11.0%), as well as for Provider A that did not report mosaicism (adjusted euploidy rate 50.0%) (Fig. 2A and D; Supplementary Table S6).

Reporting mosaicism comes at the expense of euploid diagnoses

Among all cycles within our study, 65.3% (n = 4114/11 875) had at least one euploid embryo available for transfer. This chance related inversely with maternal age and directly with the number of embryos biopsied per cycle (Supplementary Table S7). In accordance with euploidy rates, the likelihood of having at least one euploid embryo available for transfer varied significantly amongst providers ($P < 0.001$) (Fig. 2C). This chance was highest for providers that reported the lowest rates of mosaicism, Provider B (86.5%), Provider D (82.1%), and Provider H (79.5%), as well as for Provider A that did not report mosaicism (83.3%) (Fig. 2C and E). When comparing Provider A to all other providers, the chance of having at least one euploid blastocyst available for transfer increased significantly when mosaicism was not diagnosed (OR = 1.30, 95% CI: 1.13–1.50, $P < 0.0001$). If we consider this finding in practical terms, among patients with only one embryo biopsied on Day 5, reporting mosaicism reduced the probability of having a euploid embryo available for transfer by ~6% across all age groups (Supplementary Fig. S1).

Types of aneuploid and mosaic aberrations diagnosed across PGT-A providers

Among the 17 966 aneuploid embryos, 51.2% (n = 9204) presented with a single aneuploidy, 14.1% (n = 2533) were complex aneuploid (three or more abnormalities), while 1.9% (n = 337) carried only sex chromosome abnormalities (without autosomal aneuploidies) (Table 1; Supplementary Table S2). Turner syndrome (monosomy X) was the most prevalent sex chromosome abnormality (47.8%, n = 161/337), with a total prevalence of 0.9% (n = 161/17 966) (Supplementary Table S8). Single whole chromosome aneuploidies were not evenly distributed over the 23 sets of chromosomes. Our analysis confirmed previous reports (Capalbo *et al.*, 2017), revealing that aneuploidies affecting autosomal chromosomes 15, 16, 21, and 22 were significantly more common (Fig. 3A). These results were largely consistent amongst providers (Supplementary Fig. S2). Unlike uniform aneuploidies, segmental aneuploidy rates were independent of maternal age (OR = 0.84, 95% CI: 0.64–1.13, $P = 0.457$). Segmental aberrations were observed across all chromosomes and their frequency per chromosome was correlated with chromosome length (Fig. 3B). Deletions were more prevalent than duplications, with the exception of chromosome 9. These results were largely consistent amongst providers.

When considering diagnoses of mosaicism, single whole chromosome mosaic calls were most prevalent (66.0%, n = 1373). Furthermore, 17.2% of blastocysts (n = 357) were diagnosed with two mosaic aberrations, 14.4% (n = 300) were classified as complex mosaic, and 2.4% (n = 49) of blastocysts were found to carry only mosaic abnormalities affecting the sex chromosomes (Table 1; Supplementary Tables S2 and S8). Across all providers, mosaic diagnoses were mostly classified as low level (Table 1). Nevertheless, the frequencies varied across providers depending on the type of single mosaic aberration (Fig. 4). We revealed a relatively similar frequency of mosaic calls across all chromosomes, with the exception of mosaic trisomies associated with chromosome 19, which were significantly more prevalent (Fig. 3C). Diagnoses of mosaic trisomy 19 were predominately classified as low level and were consistent across several providers (Fig. 3C; Supplementary Fig. S3). With the exception of Provider H, six out

Table 2. Association between IVF cycle characteristics and euploidy, aneuploidy, and mosaicism rates.

Variable	Euploid diagnoses	Aneuploid diagnoses	Mosaic diagnoses	Euploidy rate	Aneuploidy rate	Mosaicism rate	Odds ratio (95% confidence interval)*					
							Euploidy		Aneuploidy		Mosaicism	
Cycle characteristics								P-value		P-value		P-value
Maternal age	34.6 (±5.1)	37.9 (±4.5)	34.3 (±5.8)	–	–	–	0.35 (0.32–0.39)	<0.001	1.21 (1.20–1.22)	<0.001	0.99 (0.98–1.00)	0.064
Paternal age	39.5 (±6.4)	40.3 (±6.2)	40.1 (±6.7)	–	–	–	0.98 (0.82–1.16)†	0.796	1.00 (0.99–1.00)†	0.44	1.01 (1.00–1.02)†	0.096
Number of embryos biopsied per cycle	5.2 (±3.6)	4.5 (±3.1)	4.5 (±3.1)	–	–	–	1.01 (1.00–1.01)	0.199	0.99 (0.96–1.02)	0.64	1.02 (1.00–1.04)	0.069
Oocyte source												
Donor	2612/4237	1119/4237	506/4237	61.6%	26.4%	11.9%	1.23 (1.11–1.37)	<0.001	0.65 (0.51–0.83)	0.001	1.04 (0.86–1.26)	0.707
Autologous	13 738/32 158	16 847/32 158	1573/32 158	42.7%	52.4%	4.9%	Reference	–	Reference	–	Reference	–
Day of biopsy												
Day 5	10 330/21 561	10 153/21 561	1078/21 561	47.9%	47.1%	5.0%	Reference	–	Reference	–	Reference	–
Day 6	5734/14 047	7343/14 047	970/14 047	43.9%	52.3%	6.9%	0.71 (0.67–0.74)	<0.001	1.60 (1.40–1.83)	<0.001	1.31 (1.09–1.57)	0.003
Day 7	286/787	470/787	31/787	36.3%	59.7%	3.9%	0.59 (0.50–0.70)	<0.001	2.15 (1.34–3.45)	0.001	1.31 (0.71–2.39)	0.386

* All odds ratios are unadjusted apart from those for paternal age.
† Odds ratios for paternal age were adjusted for maternal age and oocyte source.

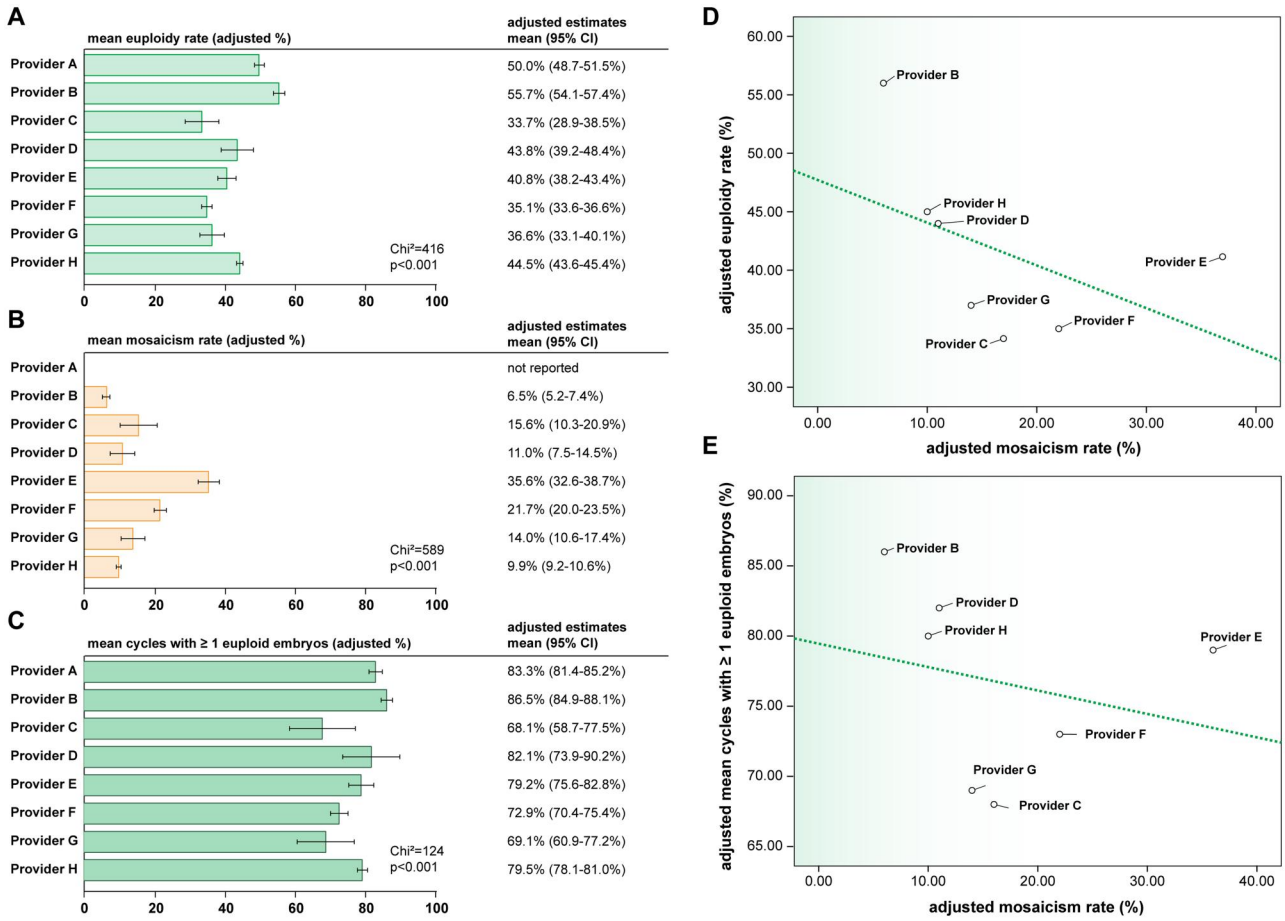

Figure 2. Association between genetic testing provider and preimplantation genetic testing for aneuploidies (PGT-A) results. (**A**) Mean euploidy rates (adjusted estimates) and 95% confidence intervals (CI) across PGT-A providers. (**B**) Mean mosaicism rates (adjusted estimates) and 95% confidence intervals (CI) across PGT-A providers. (**C**) Mean cycles with at least one euploid embryo (adjusted estimates) and 95% confidence intervals across PGT-A providers. (**D**) Correlation between euploidy and mosaicism rates across providers. The analysis was adjusted for maternal age, paternal age, oocyte source, number of embryos biopsied per cycle, and day of biopsy. (**E**) Correlation between adjusted mean cycles with at least on euploid embryo and adjusted mosaicism rates across PGT-A providers. The analysis was adjusted for maternal age, paternal age, oocyte source, number of embryos biopsied per cycle, and day of biopsy.

of the seven providers diagnosed segmental mosaic aberrations (Fig. 3E). In contrast to uniform segmental aberrations, diagnoses of mosaic duplications were more prevalent than mosaic deletions. These were largely diagnosed as high level and predominately affected large chromosomes (Fig. 3D). The proportion of embryos diagnosed with single uniform aneuploidies (trisomies, monosomies, and segmental aberrations) remained consistent amongst providers. However, rates of single mosaic aberrations were highly variable (Fig. 3F). Accordingly, we observed a significantly higher variance amongst providers when comparing the frequency of calling single mosaic abnormalities per individual autosomal chromosome, compared to the incidence of diagnosing single uniform aneuploidies (Fig. 3F).

When considering all PGT-A results, aneuploidy and mosaicism classifications based on abnormality type varied substantially amongst providers (Fig. 5). Compared to the other providers, Provider E had the lowest rates of single, double, and complex aneuploidies and highest rates of single, double, and complex mosaic diagnoses.

Transfer outcomes

We further analyzed clinical outcomes following the transfer of mosaic embryos across 15 clinics (Providers C–H) (Table 3; Supplementary Table S9). Mosaic embryo transfer rates varied amongst clinics, ranging from 0% to 45.5%, with the majority of transfers (83.0%) performed at Clinics F1–F4 (Provider F, Supplementary Table S9). Nevertheless, only 12.7% (245/1932) of all mosaic embryos were ultimately transferred, despite 20.7% (1456/7040) of all patients within this cohort having only a mosaic diagnosis (Table 3; Supplementary Table S9). Moreover, for 4.1% of all patients, mosaic blastocysts were the only available embryos following PGT-A (Table 3, Supplementary Table S9). Markedly, the proportion of patients with only mosaic embryos available varied substantially amongst providers, ranging from 1.1% up to 11.6% (Table 3).

In accordance with previous studies (Popovic *et al.*, 2020; Capalbo *et al.*, 2021; Viotti *et al.*, 2021), the transfer of mosaic embryos resulted in a clinical pregnancy rate of 41.1% and an overall ongoing pregnancy/live birth rate of 35.2% (Table 3; Supplementary Table S9). Clinical miscarriage rates with mosaic embryos were lower compared to previous reports (4.9%) (Capalbo *et al.*, 2021; Viotti *et al.*, 2021). We observed no significant differences in clinical outcomes between blastocysts diagnosed with a mosaic monosomy and those with a mosaic trisomy (Supplementary Fig. S4). Furthermore, outcomes were comparable for embryos diagnosed with mosaicism affecting one (single), two

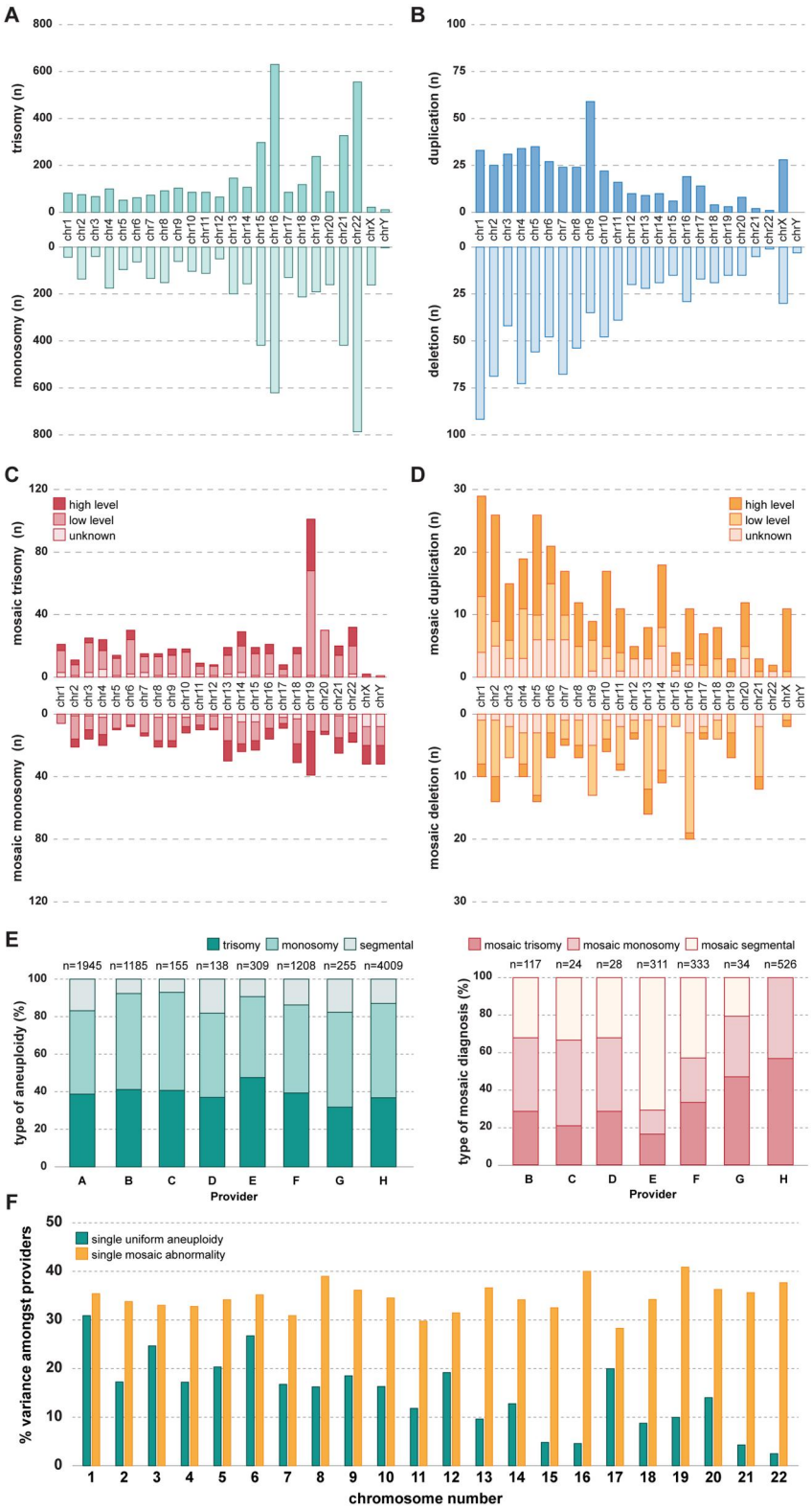

Figure 3. Diagnoses of aneuploidy and mosaicism. (**A**) Frequency of single aneuploidies across individual chromosomes. (**B**) Frequency of single segmental aberrations across individual chromosomes. (**C**) Frequency of single mosaic aberrations across individual chromosomes. (**D**) Frequency of single mosaic segmental aberrations across individual chromosomes. (**E**) Proportion of uniform and mosaic trisomies, monosomies and segmental aberrations per provider. (**F**) Variance across providers per individual autosomal chromosome when diagnosing a single uniform aneuploidy and single mosaic aberration.

(double), or more chromosomes (complex mosaics) (Supplementary Fig. S4 and Table S10). Nevertheless, differentiating complex mosaic diagnoses from non-informative results remains a challenge, as whole genome amplification artifacts may be indistinguishable from mosaic results. Our series largely comprised of low level mosaic transfers (98.8%). Across all clinics, only

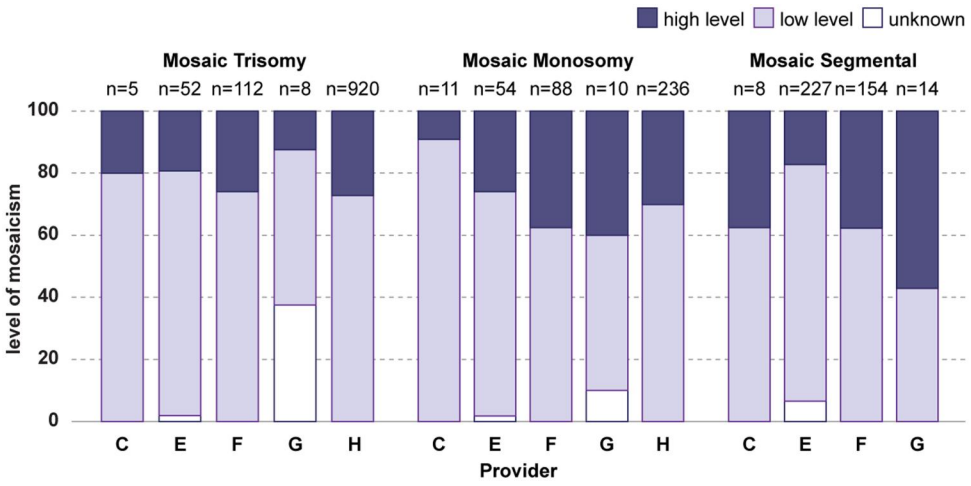

Figure 4. Level of mosaicism diagnosed per PGT-A provider based on the type of single mosaic aberration.

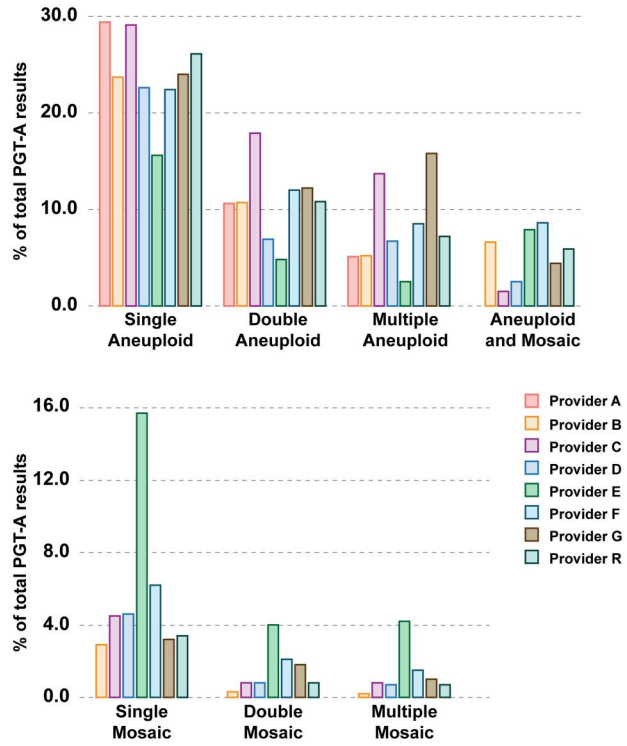

Figure 5. Aneuploidy and mosaicism classifications based on abnormality type per PGT-A provider.

three high level mosaic embryos were transferred, all resulting in a negative pregnancy test (Supplementary Table S10).

Finally, we compared clinical outcomes following the transfer of mosaic (n = 245) and euploid (n = 5721) blastocysts, performed across 10 clinics (Table 4). We observed no significant differences in overall clinical pregnancy ($P = 0.83$), miscarriage ($P = 0.89$), or live birth ($P = 0.98$) rates between uniformly euploid embryos and mosaic embryos (Table 4). The overall live birth rate per euploid embryo transferred was 34.6% compared to 35.1% for mosaic embryos, with a mean risk difference of 0.07 (95% CI = −6.02 to 6.15, Table 4). The confidence intervals were within the predetermined non-inferiority margin of 7.5%, suggesting comparable clinical outcomes for euploid and putatively mosaic embryos. Additionally, we found no evidence that the transfer of a mosaic embryo increased the risk of a clinical miscarriage compared to a euploid embryo transfer (risk difference = −0.19%; 95% CI = −2.95 to 2.57; Table 4 and Fig. 6). Due to the limited number of transfers for some clinics, we were not able to compare outcomes across individual genetic testing providers. However, as the majority of mosaic embryo transfers were performed at Clinics F1–F4 (Provider F), we performed a further sub-analysis comparing mosaic (n = 201) and euploid (n = 1209) embryo transfer outcomes exclusively across these centers (Supplementary Table S11). Notably, these clinics shared a common mosaic embryo transfer policy, and a standardized framework of laboratory techniques, culture conditions, and treatment protocols. This provided a more controlled analysis in which inherent variations amongst different IVF clinics were minimized. Comparable to the overall cohort, we observed similar reproductive outcomes for euploid and mosaic embryos (Fig. 6; Supplementary Table S11).

On routine neonatal examination, the newborns within our cohort were healthy, however, postnatal genotyping of newborns to evaluate the potential persistence of the abnormal cell line after mosaic embryo transfer was not performed. Similarly, we could not perform follow-up analysis of products of conceptions after spontaneous miscarriages and elective prenatal diagnosis procedures.

Discussion

Our results reveal a strong association between the genetic testing provider and PGT-A results. We show that classifying embryos as mosaic, according to current clinical practice, may come at the expense of euploid diagnoses. Technical challenges coupled to inconsistencies in interpreting and reporting intermediate copy number values cast doubt on the clinical utility of current PGT-A practices for accurate embryo selection. Significant differences in mosaicism rates across providers point to technical bias as opposed to true biological variability, raising concerns regarding the accuracy of mosaicism predictions. Despite their apparently normal developmental potential, close to 90% of mosaic blastocysts are not used for IVF treatment. Therefore, we confirm that viable embryos are being inadvertently discarded under the premise of mosaicism (Pagliardini *et al.*, 2020). Our findings highlight the strong need for standardization and quality assurance in the industry and corroborate reservations regarding the clinical value of reporting low-level mosaicism (Paulson and Treff, 2020; Treff and Marin, 2021).

We primarily found that the PGT-A provider has a significant effect on the number of embryos classified as euploid. This is

Table 3. Mosaic diagnoses and clinical outcomes following the transfer of mosaic blastocysts per preimplantation genetic testing provider.

	Provider A	Provider B	Provider C	Provider D	Provider E	Provider F	Provider G	Provider H	Total
Patients, n (%[1])	Not reported	131 (11.4)	20 (17.9)	25 (17.4)	304 (57.7)	431 (28.6)	42 (23.3)	634 (13.9)	1587 (15.8)
Blastocysts, n (%[2])	Not reported	147 (3.1)	32 (6.0)	37 (6.1)	496 (25.0)	551 (10.2)	67 (6.3)	749 (4.9)	2079 (5.7)
Maternal age (years)									
Mean (±SD)	Not applicable	36.6 (5.1)	33.5 (5.2)	33.1 (4.0)	32.6 (6.2)	32.5 (6.6)	36.5 (4.4)	36.2 (4.3)	34.3 (5.8)
Oocyte donation									
Patients, n (%)	Not applicable	25 (2.2)	4 (3.6)	0 (0.0)	113 (21.4)	161 (10.7)	5 (2.8)	49 (1.1)	357 (3.6)
Mosaic embryo only[1]									
Patients, n (%)	Not applicable	29 (2.5)	6 (5.4)	2 (1.4)	61 (11.6)	105 (7.0)	2 (1.1)	129 (2.8)	334 (4.1)
Mean maternal age (±SD)	Not applicable	38.4 (5.0)	38.0 (5.1)	39.0 (0.0)	38.3 (4.3)	35.9 (6.0)	36.5 (4.8)	38.4 (4.0)	37.5 (4.9)
Mosaic embryo transfers[3]									
Patients, n (%)	Not applicable	Data not provided	2 (10.0)	0 (0.0)	24 (7.9)	200 (46.4)	3 (7.1)	12 (1.9)	241 (16.6)*
Oocyte donors, n (%)	Not applicable	Data not provided	0 (0.0)	0 (0.0)	3 (2.7)	60 (37.3)	0 (0.0)	1 (2.0)	64 (17.9)*
Mean maternal age (±SD)	Not applicable	Data not provided	36.5 (6.4)	Not applicable	36.6 (5.3)	34.3 (6.6)	38.3 (2.1)	39.3 (4.0)	34.9 (6.5)*
Blastocysts transferred, n (%)	Not applicable	Data not provided	2 (6.3)	0 (0.0)	25 (5.0)	201 (36.5)	4 (6.0)	13 (1.7)	245 (12.7)*
Mosaic embryo transfer outcomes[4]									
Positive pregnancy test, n (%)	Not applicable	Data not provided	2 (100.0)	0 (0.0)	13 (52.0)	104 (51.7)	1 (25.0)	5 (38.5)	125 (51.2)
Clinical pregnancy, n (%)	Not applicable	Data not provided	2 (100.0)	0 (0.0)	8 (32.0)	83 (41.3)†	1 (25.0)	5 (38.5)	99 (41.1)
Clinical miscarriage, n (%)	Not applicable	Data not provided	0 (0.0)	0 (0.0)	0 (0.0)	11 (5.5)	0 (0.0)	1 (7.7)	12 (4.9)
Live birth, n (%)	Not applicable	Data not provided	2 (100.0)	0 (0.0)	8 (32.0)	71 (35.3)	1 (25.0)	4 (30.8)	86 (35.2)‡

[1] Percent of all patients.
[2] Percent of all diagnoses.
[3] Percent of all mosaic diagnoses.
[4] Percent of all mosaic embryo transfers.
* Total excluding Provider B.
† One clinical pregnancy resulted in fetal demise.
‡ Includes 19 ongoing pregnancies.

Table 4. Clinical outcomes following the transfer of euploid and mosaic blastocysts across 10 clinics associated with five preimplantation genetic testing providers.

Provider	Clinic		Embryo transfers	Positive pregnancy test, n (%)[1]	Clinical pregnancy, n (%)[1]	Clinical miscarriage, n (%)[1]	Live birth, n (%)[1]
Provider C	**Clinic C1**	Euploid	89	55 (61.8)	46 (51.7)	5 (5.6)	41 (46.1)
		Mosaic	2	2 (100.0)	2 (100.0)	0 (0.0)	2 (100.0)
Provider E	**Clinic E1**	Euploid	407	232 (57.0)	188 (46.2)	11 (2.7)	177 (43.5)
		Mosaic	25	13 (52.0)	8 (32.0)	0 (0.0)	8 (32.0)
Provider F	**Clinic F1**	Euploid	255	112 (43.9)	88 (34.5)	15 (5.9)	73 (28.6)
		Mosaic	37	22 (59.5)	17 (45.9)	5 (13.5)	12 (32.4)
	Clinic F2	Euploid	17	8 (47.1)	6 (35.3)	0 (0.0)	6 (35.3)
		Mosaic	5	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Clinic F3	Euploid	38	16 (42.1)	13 (34.2)	1 (2.6)	12 (31.6)
		Mosaic	5	3 (60.0)	3 (60.0)	0 (0.0)	3 (60.0)
	Clinic F4	Euploid	899	464 (51.6)	386 (42.9)	66 (7.3)	320 (35.6)
		Mosaic	154	78 (50.6)	63 (40.9)	6 (3.9)	56 (36.4)
Provider G	**Clinic G1**	Euploid	185	119 (64.3)	103 (55.7)	15 (8.1)	88 (47.6)
		Mosaic	4	1 (25.0)	1 (25.0)	0 (0.0)	1 (25.0)
Provider H	**Clinic H1**	Euploid	254	90 (35.4)	80 (31.5)	18 (7.1)	62 (24.4)
		Mosaic	1	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
	Clinic H4	Euploid	809	374 (46.2)	320 (39.6)	39 (4.8)	281 (34.7)
		Mosaic	2	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)
	Clinic H5	Euploid	2768	1177 (42.5)	1042 (37.6)	121 (4.4)	921 (33.3)
		Mosaic	10	3 (30.0)	3 (30.0)	0 (0.0)	3 (30.0)
All	**Total**	Euploid	5721	2647 (46.3)	2272 (39.7)	291 (5.1)	1981 (34.6)*
		Mosaic	245	125 (51.0)	99 (40.4)	12 (4.9)	86 (35.1)†
Risk difference, % (95% CI)				4.75 (−1.64 to 11.14)	0.69 (−5.58 to 6.97)	−0.19 (−2.95 to 2.57)	0.07 (−6.02 to 6.15)
P-value				0.145	0.8282	0.8935	0.9828

[1] Percent per embryo transferred.
* Includes 206 ongoing pregnancies.
† Includes 19 ongoing pregnancies.

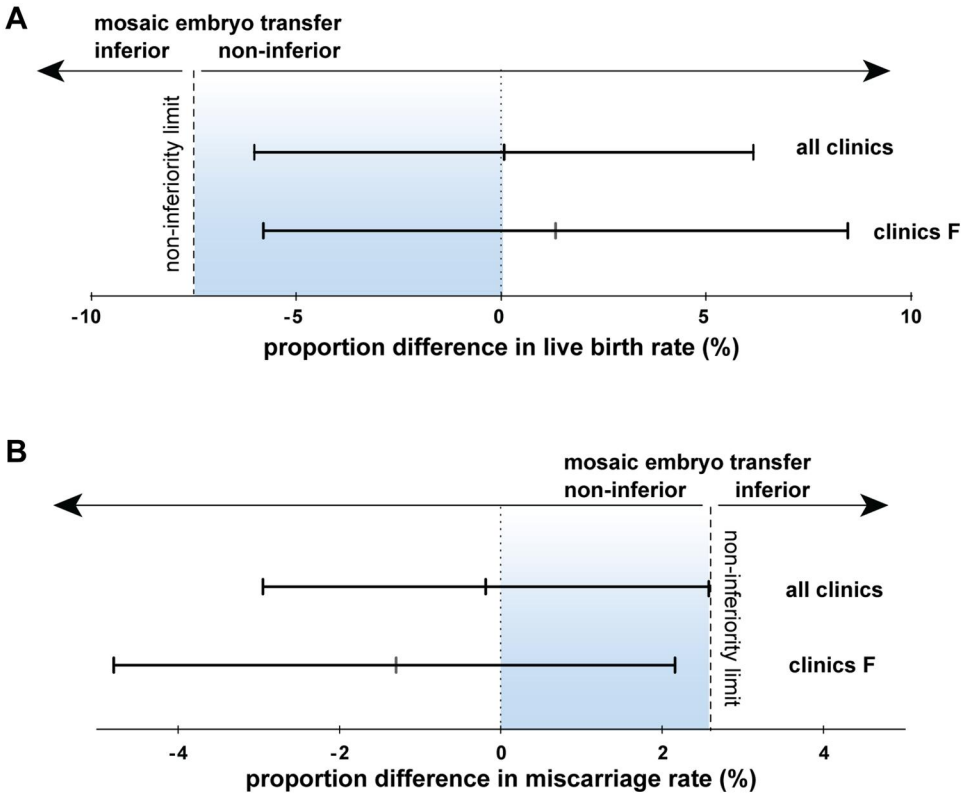

Figure 6. Assessment of non-inferiority. Live birth rates (**A**) and miscarriage rates (**B**) following the transfer of mosaic embryos compared to the transfer of euploid embryos, across all clinics and for Clinics F.

particularly limiting for patients with few or no euploid embryos, such as women of advanced maternal age (Franasiak *et al.*, 2014). Our analysis demonstrates that for patients with one embryo available for biopsy, reporting mosaicism coupled with the clinical decision to not transfer these embryos, reduces the number of transfers by ~6% across all age groups. If we consider that the rate of ongoing pregnancy per euploid embryo transfer is ~50% across all maternal ages (Harton *et al.*, 2013; Munné *et al.*, 2019), this would translate to a 3% reduction in live births per cycle. This probability is calculated based on an overall mosaicism rate of 5.7% across all providers reporting mosaicism in our study. Nevertheless, mosaicism rates varied significantly amongst genetic testing laboratories. Accordingly, higher rates of mosaicism will ultimately lead to a further loss of reproductive potential, with an estimated reduction in live births of up to 12.5% per cycle. These findings highlight the obligation to reconsider indications for PGT-A and specifically, the clinical value of reporting mosaicism. For patients with a limited number of blastocysts available for biopsy, reporting mosaicism remains difficult to justify, as it may ultimately determine the difference between success or failure.

A critical factor, as demonstrated by our study, is that a number of healthy live births have been achieved following the transfer of mosaic blastocysts (Capalbo *et al.*, 2021; Viotti *et al.*, 2021). A recent prospective, non-selection trial showed that the transfer of embryos diagnosed with low (20–30%) or moderate (30–50%) levels of mosaicism, resulted in comparable clinical outcomes and live birth rates compared to the transfer of euploid embryos (Capalbo *et al.*, 2021). We found that predictions of <50% mosaicism constituted around ~70% of all mosaic diagnoses. Previous reports have also suggested that low-level mosaic diagnoses are more frequent, compared to high-level mosaicism (Fragouli *et al.*, 2011). Compared to euploid embryo transfers, we found no evidence of inferior live birth rates or differences in miscarriage rates, following the transfer of mosaic embryos. This suggests that no improvement in embryo selection is conferred by including mosaic diagnoses as a third category of possible classifications, particularly in the case of low level mosaics. Prenatal testing data further confirm this notion. To date, true fetal mosaicism has only been confirmed in ~0.03% of cases in over several thousand putatively mosaic embryos transferred to date (Kahraman *et al.*, 2020; Schlade-Bartusiak *et al.*, 2022; Greco *et al.*, 2023). Mosaic pregnancies have also been reported following euploid embryo transfers (Haddad *et al.*, 2013). Nevertheless, classifying embryos as mosaic has generated a course of action that often has a major impact on the patient, including additional genetic counseling and invasive prenatal diagnosis. Yet, to establish the true risk of fetal mosaicism following the transfer mosaic embryos, longitudinal studies comparing the incidence of mosaicism in pregnancies from embryos diagnosed as euploid or mosaic using the same prenatal testing technologies are required. Therefore, based on current evidence intermediate chromosome copy number values <50% should be considered as a finding of no clinical significance.

The exclusion or low prioritization of low-level mosaic embryos for transfer undoubtedly compromises treatment outcomes per cycle. Several studies, including a recent trial, have demonstrated that PGT-A did not improve the frequency of ongoing pregnancy or live birth rates in patients under the age of 35 years (Chang *et al.*, 2016; Kang *et al.*, 2016; Kushnir *et al.*, 2016; Munné *et al.*, 2019). In the trial, mosaic embryos constituted close to 17% of all tested embryos, and were excluded from transfer. Yet, the authors reported vast differences in mosaicism rates, with a trend toward higher euploidy and higher ongoing pregnancy rates in the genetic testing laboratories that did not report mosaicism (Munné *et al.*, 2019). Our results support and extend these findings, providing evidence that classifying embryos as mosaic reduces the number of euploid embryos that are ultimately transferred. Similarly, a large randomized trial performed in women considered to have a good prognosis for success, recently demonstrated that PGT-A results in lower cumulative live birth rates compared to IVF without PGT-A (Yan *et al.*, 2021). In this trial, embryos diagnosed as mosaic (constituting close to 12% of all PGT-A results) were considered clinically unsuitable and not transferred. Yet, as exemplified by our data, diagnoses of mosaicism and subsequent exclusion of mosaic embryos for transfer may account for the failure to demonstrate improved outcomes with the use of PGT-A. PGT-A may endeavor to maximize live birth rates per transfer, however, current practices limit its clinical utility per IVF cycle by excluding potentially viable embryos.

Diagnosing and reporting mosaicism remains the limiting factor in the interpretation of PGT-A results. Whole genome amplification protocols, NGS platforms, software thresholds, provider specific guidelines, and other technical and interpretative factors may all play a role (American Society for Reproductive Medicine, 2020). For instance, we observed high rates of low-level mosaic trisomies affecting chromosome 19 across several providers. This result points to technical bias, particularly as chromosome 19 has many unique characteristics, including the highest GC content of any chromosome (Grimwood *et al.*, 2004), making it difficult to sequence. Moreover, studies have shown that chromosome 19 exhibits very low mis-segregation rates during mitosis (Dumont *et al.*, 2020). While technical challenges persist, providers must place greater focus on quality assurance. It is crucial to emphasize that the landscape of mosaic embryo classification encompasses a wealth of complexities, some of which may not even be well defined. Moreover, each PGT-A platform comes with its distinct characteristics, including differences in accuracy, sensitivity, and specificity. As a result, attempting a direct comparison between providers employing different platforms may be confounded by inherent disparities in the technologies employed (Bardos *et al.*, 2023). Consequently, the comparison of mosaic embryo outcomes in our study also becomes complex due to the impact of such platform-related variations. We acknowledge that these differences represent an inherent limitation in our study. However, it is precisely these variations that underscore the real-world diversity among PGT-A laboratories. By acknowledging these challenges, we aim to present a comprehensive view of current practices and highlight the complexities involved in mosaic embryo classification. To minimize potential biases in embryo classification, the performance characteristics of each PGT-A platform must be thoroughly evaluated prior to clinical implementation. As such, we hope that this study contributes to advancing the field by promoting transparent discussions about the technical challenges and quality assurance measures required in the clinical implementation of PGT-A.

Our study further revealed that clinic-specific differences including patient factors and subtleties in case management may influence embryo aneuploidy (Munné *et al.*, 2017a). However, in line with previous findings (Coll *et al.*, 2022a), we show that, in our cohort, embryology laboratory practices and techniques, such as the TE biopsy, sample handling and embryo culture did not considerably increase the rate of mosaic diagnoses. Nevertheless, as data regarding blastocyst morphology and other clinic-specific factors was not available, this finding warrants

cautious interpretation. Although poor quality blastocysts were not included in the clinical workflow, blastocyst morphology may inherently influence calls of putative mosaicism and should be considered in further analyses. Similarly, differences in culture conditions or other center-specific ART treatment practices may also affect ploidy rates (Munné et al., 2017a). A further limitation of our study is that several providers served only one clinic. Due to the varied composition of providers in the study, we cannot entirely exclude the correlation between varying clinical practices and diagnoses of mosaicism. Nevertheless, our study clearly illustrates that variability can be introduced at different levels when diagnosing and reporting chromosomal mosaicism, raising concerns regarding the accuracy and relevance of such predictions. Due to the interplay of all of these factors, achieving a precise diagnosis may ultimately be unattainable.

At present, careful consideration is recommended in cases in which mosaic embryos are considered for transfer (Cram et al., 2019; Leigh et al., 2022). Yet, clinics adopt different approaches and guidelines when considering the transfer of mosaic embryos. As a retrospective analysis, we recognize that our study has inherent limitations in directly controlling for individual mosaic embryo transfer policies across clinics. Nevertheless, our subanalysis of mosaic embryo transfer outcomes across Clinics F1–F4 provides a valuable perspective. It allowed us to explore outcomes within a more controlled context where inherent variations amongst different providers were minimized. Even within this more homogenous setting, mosaic embryos exhibited outcomes comparable to their euploid counterparts. However, the lack of consensus on standardized transfer criteria for mosaic embryos highlights the complexities and challenges faced by both patients and clinicians in making informed decisions. We wish to stress the importance of addressing this diversity to optimize the clinical utilization of mosaic embryos. All current guidelines prioritize uniformly euploid embryos for transfer, yet such rankings may diverge from morphological evaluations. It is unclear whether good quality blastocysts diagnosed as mosaic will perform better compared to uniformly euploid embryos of poor morphology. While euploid blastocysts may have similar potential regardless of morphology (Shear et al., 2020), some studies suggest that poor quality euploid blastocysts entail higher rates of miscarriage (Irani et al., 2017). There is currently little evidence to ascertain which embryos with mosaic results have the best chance of resulting in a successful pregnancy, or the lowest risk of an undesirable outcome (American Society for Reproductive Medicine, 2020). These discrepancies inherently complicate clinical management and may ultimately limit the utility of PGT-A. Barad and colleagues recently demonstrated that embryos diagnosed as unsuitable for transfer by some providers and clinics may in fact result in live births (Barad et al., 2022). The lack of standardization in proprietary algorithms used to perform PGT-A analysis across providers heightens the challenges of interpreting PGT-A results by individual clinics. Yet, in the study of Barad et al. (2022) almost all embryos diagnosed with uniform aneuploidies resulted in implantation failure or miscarriage. As shown in our study, variation amongst providers was minimized when reporting uniform whole chromosome aberrations. Several studies have confirmed the clinical accuracy of PGT-A for diagnosing uniform aneuploidies (Popovic et al., 2019; Tiegs et al., 2021; Ata et al., 2022; Capalbo et al., 2022). Thus to achieve greater standardization, the main objective of PGT-A should be centered on the diagnosis of meiotic aberrations. Accordingly, rather than embryo selection, PGT-A should serve as a deselection tool, aiming to minimize adverse clinical outcomes.

In our study, high level mosaicism was reported much less frequently compared to low grade diagnoses. Yet, the level of mosaicism remains an important consideration. Embryos classified as high level mosaics have been associated with significantly lower ongoing pregnancy rates compared to low level mosaic embryos and euploid blastocysts (Viotti et al., 2021). In our study, maternal age was largely independent of mosaicism rates, however, two providers (Provider G and Provider H) presented with higher odds of diagnosing mosaicism for women over the age of 40 years. Notably, these providers also showed a higher frequency of high level mosaic diagnoses. This suggests that in such cases, uniformly aneuploid embryos, which are indeed associated with maternal age, may occasionally be misclassified as mosaic (Treff and Marin, 2021). Among all providers that reported mosaicism, Provider E presented with the highest incidence of single mosaic aberrations, mirrored by the lowest incidence of single aneuploidies. These findings similarly indicate that some embryos diagnosed with mosaic aberrations may in fact just be aneuploid. The transfer of such embryos under the premise of mosaicism would ultimately cause patients harm, as the reproductive potential of uniformly aneuploid embryos is close to 0% (Scott et al., 2012; Munné et al., 2020; Tiegs et al., 2021). This would also account for the higher miscarriage rates observed following the transfer of a cohort of mosaic embryos compared to the transfer of euploid embryos (Popovic et al., 2020). Recent studies suggest that only ~2% of embryos display evidence of aneuploidies of mitotic origin (Popovic et al., 2023; Rana et al., 2023).

While full elucidation of all possible confounders accounting for variability is impossible, we provide substantial evidence regarding the impact of provider reporting policies on PGT-A results. Ultimately, we still lack the genetic tools to definitively assess mosaicism. To achieve high-quality care, greater standardization of PGT-A classification schemes and proprietary algorithms used to interpret intermediate copy number values is paramount. In light of these complexities and the ongoing debate about the clinical significance of intermediate copy number values, it is essential to carefully consider the utility of reporting mosaicism. Until a consensus is reached on standardized and evidence-based guidelines for mosaic embryo classification, the potential negative impact of reporting mosaicism on patient outcomes remains significant. As our study highlights, reporting mosaicism may potentially result in the exclusion of clinically viable embryos, likely due to the prevailing high risk perception associated with such diagnoses. This situation raises considerable concerns, as patients are ultimately not being given the best possible chances of a successful pregnancy. Considering our research findings and the existing body of evidence, the utility of reporting embryos with <50% mosaicism requires careful reconsideration. Using a dual classification strategy may be a more prudent approach. Accordingly, embryos with low mosaicism (<50%) would be classified as euploid and those with high mosaicism (>50%) would be classified as aneuploid. As shown in our study, diagnosing mosaicism using tertiary (euploid, mosaic, and aneuploid) instead of binary (euploid and aneuploid) classification methods ultimately modifies the relative number of euploid embryos available for transfer. However, it will remain crucial for each laboratory to undertake meticulous validation procedures. This step is paramount for minimizing potential sources of variability and ensuring that classification outcomes are accurate and consistent across different settings.

We show that the proportion of patients with only mosaic embryos available for transfer varied considerably amongst providers, ranging from 1.1% to 11.6%. While we cannot establish the precise number of these patients who decided not to undergo a mosaic embryo transfer, we show that ultimately only 12.7% of all mosaic embryos are transferred. Mosaic diagnoses have thus inherently complicated outcome interpretation and embryo transfer policies. Coll *et al.* recently showed that many patients do not end up using their stored mosaic embryos primarily due to a high risk perception associated with such diagnoses (Coll *et al.*, 2022b). Interestingly, in their study, neither reproductive history nor information on mosaic embryo characteristics received through counseling played a significant role in the clinical decision about whether to transfer a mosaic embryo. While many patients may decide to keep their mosaic embryos stored, few end up using these embryos for clinical treatment.

By limiting the availability of embryos for transfer, reporting mosaicism may reduce the chance of success of an IVF treatment with PGT-A. Therefore in the current landscape of diverse practices and technologies, the decision to report mosaicism must be approached cautiously. Our study shines a light on the complexities at play and underscores the potential drawbacks of reporting low-level mosaicism. As the field navigates these challenges, and until further clinical data are available, we advocate for a meticulous consideration of these insights and encourage continued efforts toward standardization to enhance embryo utilization and patient outcomes. Straightforward PGT-A diagnoses will alleviate current risk perceptions surrounding mosaicism and ensure that potentially viable embryos are not being discarded or left unused.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

M.P. conceived and designed the study, collected and interpreted clinical data, performed data analysis, and wrote the manuscript; L.B. collected clinical data and performed data analysis; A.R.L., A.L.R.d.C.L., D.S., B.L., R.M., J.A.O., N.P.P., M.P., F.A., M.G., A.P., B.M., L.D., F.V.M., D.S., M.R., E.P.d.l.B., and A.R. collected clinical data and contributed to data interpretation; R.V. contributed to the study design and data interpretation, and critically reviewed the manuscript. All authors reviewed the manuscript.

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Conflict of interest

M.P., L.B., A.R.L., A.L.R.d.C.L., N.P.P., M.P., D.S., F.A., A.P., B.M., L.D., F.V.M., D.S., M.R., E.P.d.l.B., A.R., and R.V. have no competing interests to declare. B.L., R.M., and J.A.O. are full time employees of IB Biotech, the genetics company of the Instituto Bernabeu group, which performs preimplantation genetic testing. M.G. is a full time employee of Novagen, the genetics company of Cegyr, which performs preimplantation genetic testing.

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