Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-024-03364-7

EMBRYO BIOLOGY

Time-lapse analysis of embryos classified as euploid, mosaic, and aneuploid after embryonic trophectoderm biopsy

Ilaria Listorti[1,2] · Giulia Pirastu[1] · Alessandra Ruberti[1] · Marzia Barberi[1] · Andrea Pristerà[1] · Vincenzo Zazzaro[1] · Francesca Spinella[3] · Maria Teresa Varricchio[1] · Manuel Viotti[4] · Ermanno Greco[1,5] · Pierfrancesco Greco[1]

Received: 15 April 2024 / Accepted: 16 December 2024

Abstract

Purpose Analyze morphokinetic, morphology, and KIDscore™Day5 in different PGT-A classes, focusing on putative mosaicism level and type.

Methods The single-center retrospective study analyzed 832 embryoscope-cultured blastocysts from cycles with at least one putative mosaic, conducted from 2020 to 2022. A P-value < 0.05 was considered statistically significant.

Results Putative mosaic embryos were significantly delayed compared to euploid in tPNF, t2, t4, t7, and t8 but significantly faster than aneuploid in tPNF, t2, t3, t4, t5, tSC, tM, tSB, and tB. Regarding the level, low-putative mosaic embryos (< 50%) showed significantly earlier tSC, tM, tSB, and tB compared to aneuploid, whereas high-putative mosaic embryos exhibited significantly earlier tSB and tB. Concerning the type of putative mosaicism, segmental aneuploidies reached significantly earlier t8, tM, and tB than complex aneuploidies. The study also investigated the usefulness of KIDscore™Day5 for embryo selection as an additional tool to PGT-A. A significant decrease in KIDscore™Day5 was observed from euploid to low-putative mosaic, from high-putative mosaic to aneuploid, and between segmental and complex-putative mosaic. The observed differences in KIDscore™Day5 were partially confirmed by transfer results: euploid blastocysts showed the most favorable clinical outcomes compared to low- and high-putative mosaics. Additionally, both euploid and segmental putative mosaic embryos exhibited the best clinical results compared to whole chromosome and complex putative mosaic. Moreover, within the same PGT-A class, embryos with the lowest KIDscore™Day5 values had significantly lower clinical results.

Conclusions The data highlight that morphology, morphokinetics, and chromosome content in trophectoderm biopsy are closely related, and the KIDscore™Day5 algorithm reflects this interplay.

Keywords Mosaicism · Morphokinetic · Next generation sequencing · Time-lapse microscopy · KIDscore™Day5

Introduction

Chromosomal mosaicism, defined as a condition in which there is more than one karyotypically distinct cell population (whole chromosomes or segmental abnormalities) in a single embryo, is a common biological phenomenon in human preimplantation embryos [1].

With the advancements in preimplantation genetic testing (PGT), based on next-generation sequencing (NGS), the sensitivity of intermediate copy number (ICN) detection has improved significantly, providing more accurate quantification of copy number changes in trophectoderm (TE) biopsy samples [2]. Consequently, data interpretation and clinical management of putative mosaic TE-results have become more challenging.

✉ Giulia Pirastu
biologia@icsiroma.it

1 Reproductive Medicine Department, Villa Mafalda, Via Monte Delle Gioie 5, 00199 Rome, Italy

2 Faculty of Bioscience and Agro-Food and Environmental Technologies, University of Teramo, Via Balzarini 1, 64100 Teramo, Italy

3 Eurofins GENOMA Group, Molecular Genetics Laboratories, Via Castel Giubileo 11, 00138 Rome, Italy

4 Zouves Foundation for Reproductive Medicine and Zouves Fertility Center, 1241 East Hillsdale Blvd, Foster City, CA 94404, USA

5 Department of Obstetrics and Gynecology, UniCamillus International University, Via Di Sant'Alessandro 8, 00131 Rome, Italy

Published online: 20 January 2025

Since the first report of healthy live births from embryo transfer with a putative mosaic TE-result [3], several other studies have been conducted in order to investigate the clinical outcomes of this type of embryo [4–7]. Recently, a multicentric study based on data from the International Registry of Mosaic Embryo Transfer (IRMET) on 3000 + putative mosaic embryos confirmed significantly lower ongoing pregnancy rates compared to euploid embryos [8]. In a prospective non-selection trial, the live birth rate, miscarriage rate, and obstetrical outcomes for low-to-medium putative mosaic embryos (< 50%) were reported to be similar to those of euploid embryos. The same study found, after the disaggregation of 20 high-level (50–70%) putative mosaic embryos, a uniform aneuploidy in 65% of the cases [9]. In contrast, there was evidence that even high-level putative mosaic embryos can lead to successful pregnancies and healthy babies [10]. Remarkably, case reports have confirmed the presence of mosaicism in aborted pregnancies or babies born following the transfer of low-level putative mosaic embryos [11, 12]. In summary, there is an imbalance in the published data and further scientific studies are needed to determine whether a putative mosaicism result by TE biopsy actually represents a separate PGT-A class of embryos or whether it is simply the result of different technical laboratory artifacts (ex., technical noise), as claimed by some authors [13]. Furthermore, it should be noted that the presence of mosaicism in a TE biopsy may not reflect the chromosomal constitution of the whole embryo. All these issues complicate the management of patients with only embryos that have putative mosaicism as a TE biopsy result.

Recently, predictive models based on morphokinetic data have been developed to reduce the chance of selecting aneuploid embryos [14, 15]. Embryo abnormalities have been associated with alterations during the early cleavage stages [16] as well as delays in compaction and blastulation [17, 18]. Additionally, it has been observed that blastocyst morphology can be affected by chromosomal abnormalities [19, 20].

While more studies have focused on morphokinetic in euploid and aneuploid embryos, there is limited research on putative mosaic embryos. Some authors have reported morphokinetic delays in NGS-tested high-level putative mosaic embryos compared to euploid ones [21]; other authors have shown a morphokinetic overlap between putative mosaic embryos and euploid and aneuploid ones [22].

In this study, morphokinetic parameters, morphology, and Know Implantation Data Score Day 5 (KIDScore™ Day5, KS-5, Vitrolife) were used to analyze embryos with TE biopsies resulting respectively as euploid, mosaic, or aneuploid. The aim was to investigate whether different amounts of aneuploid cells in TE biopsies could impact embryo development. Morphokinetic assessment may emerge as an additional tool, complementary to genetic counseling, to identify priority criteria for the transfer of embryos with a putative mosaic TE-result.

In the rest of the text, TE biopsy results of euploidy, aneuploidy, and mosaicism are referred to as euploid, aneuploid, and putative mosaic embryos.

Materials and methods

Study design and population

This retrospective cohort study analyzed 832 blastocysts from 212 PGT-A cycles performed on 196 infertile couples from June 2020 to October 2022 at the Villa Mafalda Reproductive Medicine Department (Rome, Italy). Embryos were monitored by means of the TLI and were analyzed by NGS after a TE biopsy. The study group included cycles with at least one mosaic detected. Cycles with only euploid or aneuploid embryos detected were excluded.

PGT-A indications were as follows: female age > 35 years (AMA), repeated implantation failure (RIF), recurrent spontaneous miscarriage (RM), male factor (MF), and other indications (more than one of the previous factors, unexplained infertility, and elective PGT-A). Women with BMI > 35 (kg/cm^2) and males with azoospermia were excluded from the study.

All patients signed an informed consent for the anonymous use of their clinical and biological data. The ethics committee stated that this study, due to its retrospective nature, did not require institutional review board approval.

Ovarian stimulation, insemination technique, and embryo culture

For all the patients, controlled ovarian stimulation (COS) was performed using gonadotropin-releasing hormone antagonist (GnRh-a) short protocol [23]. Individualized recombinant gonadotrophins treatment was started from the 2nd day of the menstrual cycle according to the patient's characteristics. During COS, ovarian response was monitored by routine serum of luteinizing hormone (LH) and estradiol (E2) levels, and ultrasound measurement of follicular size every 2 or 3 days. Doses were adjusted according to patient response. Ganirelix (Orgalutran 0.25 mg/50 ml, MSD, Italy) was administered from the day a follicle larger than 13 mm was identified. When at least one follicle reached a diameter of ≥ 18 mm, recombinant human chorionic gonadotropin (rhCG, Ovitrelle, Merck Serono, Italy) or 0.3 mg of triptorelin acetate (Decapeptyl, Merck, Italy) was administered to trigger ovulation; oocyte retrieval was performed 35–36 h later under sedation.

All the biological procedures were performed according to our standard laboratory procedures, as described

elsewhere [20]. The total number of oocytes included in the study were inseminated using intracytoplasmic sperm injection (ICSI). Inseminated oocytes were incubated in pre-equilibrated EmbryoSlides (EmbryoSlide®, Vitrolife, Gothenburg, Sweden) with 180 µl of sequential media (G-1^{TM}plus, G-2^{TM}plus, Vitrolife, Gothenburg, Sweden) and 1.6 ml of mineral oil (OVOIL™, Vitrolife, Gothenburg, Sweden) in EmbryoScope + incubator (Vitrolife, Sweden) at 37 °C, 5% O_2, and 6% CO_2, until the blastocyst stage (day 5/6/7). On day 3 a media changeover was performed.

TLI assessment

TLI assessment was performed by two trained senior embryologists to reduce inter-operator variability. All times were noted in hours post insemination (hpi), blinded from the genetic results.

The following morphokinetic parameters were analyzed by strictly observing the annotation protocol [24]: second polar body extrusion (tPB2); fertilization -confirmed by the presence of two pronuclei and two polar bodies-; pronuclear fading (tPNf); division to 2, 3, 4, 5, 6, 7, and 8 cells (t2, t3, t4, t5, t6, t7, t8); time between 2- and 3-cell stage (cc2) and between 3- and 4-cell stage (s2); start of compaction (tSC); timing of morula formation (tM); start of blastulation (tSB); and full blastocyst formation (tB).

The morphology of the blastocysts analyzed was assessed according to Gardner and Schoolcraft criteria [25] and classified for the inner cell mass (ICM) and TE quality into excellent (AA), good (AB/BA), average (BB/AC/CA), and poor quality (CC/BC/CB) [26].

Embryo evaluation was also carried out using the KIDscore™ Day5 version 3.2 (KS-5.3.2) algorithm, a decision support tool integrated within time-lapse systems (EmbryoViewer software; Vitrolife). This algorithm was developed using a multicenter dataset of over 5000 embryos with Known Implantation Data (KID embryos).

The current version (KS-5.3.2) classifies embryos based on: cleavage regularity, developmental timing (t2, t3, t4, t5, tB), and blastocyst quality (ICM/TE quality). Only embryos with a normal fertilization (2PN) were considered from the algorithm. The EmbryoViewer software generates a final linear score, ranging from 1 to 9.9, that reflects the statistical chance of embryo implantation, from low to high. Although KS-5.3.2 is a copyright-protected tool, it has been retrospectively validated in various studies [27, 28].

PGT-A and mosaicism classification

All blastocysts (day 5/6 or 7) underwent PGT-A using the NGS-based platform VeriSeq (Vitrolife, Gothenburg, Sweden). Trophectoderm cell biopsy and blastocyst freezing were performed in accordance with our laboratory's protocol, as reported elsewhere [20]. At the Eurofins Genoma group laboratory, cells were lysed, and genomic DNA was fragmented and randomly amplified using the SurePlex DNA Amplification System (Vitrolife, Gothenburg, Sweden) according to the manufacturer's protocol. The whole-genome amplified DNA product of each sample was processed to prepare a genomic DNA library using VeriSeq PGS workflow (Vitrolife, Gothenburg, Sweden). Purified DNA libraries were normalized using VeriSeq's library normalization protocol. Equal volumes of normalized samples were pooled, denatured, and sequenced. The MiSeq Reagent Kit v.3 (Illumina, San Diego, CA, USA) was used on a miSeq System (Illumina, San Diego, CA, USA). The sequencing data were analyzed using BlueFuse Multi Software (Vitrolife, Gothenburg, Sweden). The NGS platform for mosaic embryo detection was validated at Eurofins Genoma laboratory group using DNA and/or cell mixes as follows: single cells from a euploid (46, XY) cell line were mixed with aneuploid cell lines (47, XX, þ18 and 47, XX, þ21) at different ratio [3, 29].

Embryos showing ICN values for one or more whole chromosomes or sub-chromosomal (segmental) regions were classified as putative mosaic embryos. The ICN range was defined between 1.2 and 1.8 for a mosaic loss, and 2.2 and 2.8 for a mosaic gain (also known as the 20%−80% threshold) [1]. Inside this range, embryos were stratified into two classes: low-level (< 50%) and high-level (≥ 50%) [7]. If more than one abnormality was detected, a higher percentage of mosaicism was considered. Biopsy profile < 20% was annotated as euploid and that > 80% as aneuploid.

The putative mosaic embryos were classified also for the type of chromosomal abnormalities into: segmental aneuploidies (single or double) that involved the gain or loss of chromosomal fragments [4]; whole chromosome aneuploidies (single or double monosomies or trisomies); and complex aneuploidies (more than two chromosomes involved). TE biopsy specimens showing a mosaic with a segmental abnormality and a single whole chromosome aneuploidy were included in the whole chromosome aneuploidy group.

Frozen embryo transfer

Blastocysts selected for single frozen embryo transfer (s-FET) were thawed using the Irvine thawing kit (FujiFilm Irvine, USA). The transfer procedure was performed 2/3 h after thawing. Endometrial preparation was achieved by combining GnRH agonist and estrogen pills or in a spontaneous cycle. A single blastocyst was transferred into the uterus under transabdominal ultrasound guidance using a Wallace catheter (Wallace; Smith Medical, Dublin, Ireland). Blastocyst selection for transfer was primarily based on the PGT-A result and secondarily on morphology, according to Gardner and Schoolcraft criteria [25]. Blastocysts with a PGT-A putative mosaic result

were transferred in the absence of available euploid embryos after appropriate genetic counseling.

Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) or standard error (SE), and categorical variables as number and percentage frequency.

Categorical variables were compared using χ^2 test or Fisher exact test at the 95% confidence level.

To account for multiple data from the same patient, several mixed linear models were used to assess differences in morphokinetic parameters across all groups. The morphokinetic parameters were considered as the dependent variables, groups as the fixed factors, and the patient as a random factor. A logistic mixed effects model was performed to evaluate the difference between groups for the morphological classes. If overall statistical significance was found, pairwise comparisons between groups were conducted and the resulting *P*-values were adjusted according to the Benjamini-Hochberg (BH) method to control the false discovery rate due to multiple comparisons.

Stata 16.1 software and GraphPad Prism 9.0.1 were used. A *P*-value < 0.05 was considered statistically significant.

Sample size calculation

The primary outcome of the study was to evaluate whether low-level putative mosaic embryos, high-level putative mosaic embryos, euploid, and aneuploid embryos differed in KS-5.3.2. The secondary outcomes included an assessment of morphokinetic and morphology of embryos in different PGT-A classes.

Sample size calculation was performed to evaluate whether the sample size of 832 blastocysts was sufficient to correctly assess the primary endpoint, the higher mean KS-5.3.2 in the euploid group and the lower in the aneuploid group.

It was calculated considering a standard deviation of 3 for all 4 groups (euploid, low-level mosaic, high-level mosaic, aneuploid embryos). A total sample size of 416 embryos will be sufficient to evaluate statistical differences between the groups with an alpha level of 0.05 and a power of 0.90 if the embryos are equally distributed across the four groups. In this study, a total of 360 blastocysts were needed, assuming the high-level mosaic group is one-third the size of the others.

Results

Patient characteristics and PGT-A results

A total of 832 blastocysts from 212 PGT-A cycles, performed in a TL incubator, with ≥ 1 putative mosaic were included in the study. The patient and cycle characteristics are described in Table 1. There were 196 patients, and the mean female age per cycle was 35.64 ± 5.56 years. As shown in Fig. 1, the prevalence of mosaicism did not vary among different female age groups ($P = 0.6645$). Conversely, the prevalence of euploid embryos decreased while that of aneuploid embryos increased with female age ($P < 0.0001$). The PGT-A results and stratification of putative mosaic embryos by the level or type are shown in Table 1.

The baseline demographic and cycle characteristics of the embryos divided for ploidy classes (euploid/aneuploid/low-level and high-level putative mosaic) were compared in supplementary table S1. Euploid embryos showed significantly lower maternal age than the other groups ($P < 0.01$), whereas, female BMI, AMH levels, male age, and cycle characteristics were similar among groups.

Association between morphokinetic parameters and chromosomal status

Firstly, the morphokinetic parameters of putative mosaic embryos were compared to euploid and aneuploid embryos (Table 2).

Putative mosaic embryos were delayed significantly compared to euploid in tPNf ($P = 0.005$), t2 ($P = 0.005$), t4 ($P = 0.021$), t7 ($P = 0.006$), and t8 ($P = 0.033$). Whereas, compared to aneuploid embryos, they showed some significantly earlier cleavage times (tPNf, $P = 0.007$; t2, $P = 0.015$; t3 $P = 0.022$; t4, $P = 0.021$; and t5, $P = 0.039$); moreover, they started compaction, and events to reach full blastocyst significantly earlier (tSC, $P = 0.007$; tM, $P = 0.002$; tSB, $P < 0.001$; tB, $P < 0.001$).

Regarding comparison between euploid and aneuploid embryos, the aneuploid ones were significantly delayed at each time point, except for tPB2. Thus, as expected, among the blastocysts biopsied on day 7 ($n = 33$), 52% ($n = 18$) were categorized by PGT-A as aneuploid, 30% ($n = 10$) as putative mosaic, and 15% ($n = 5$) as euploid ($P < 0.01$).

Morphokinetic parameters in classes of putative mosaic embryos

Secondly, the study analyzed whether the type or level of chromosomal abnormality of putative mosaicism affects the morphokinetic parameters (Table 3).

From pairwise comparisons between putative mosaic embryos divided by type of abnormality, complex aneuploidies showed a significant delay compared to segmental aneuploidies in t8 ($P = 0.048$), tM ($P = 0.042$), and tB ($P = 0.039$) and only in t8 compared to whole chromosome aneuploidies ($P = 0.048$).

From comparison between low- and high-level putative mosaic embryos, no statistical differences were recorded.

Table 1 Patient, cycle characteristics and PGT-A outcomes. Data are presented as *n* (%) for categorical variables or mean and standard deviation for continuous variables. *BMI* body mass index, *AMH* anti-müllerian hormone, *PGT-A* preimplantation genetic testing for aneuploidies, *AMA* advanced maternal age, *RIF* repeated implantation failures, *RM* repeated miscarriages, *MF* male factor, *GnRH* gonadotrophin-releasing hormone. The PGT-A indication "other" includes: more than one of the previous factors, unexplained infertility and elective PGT-A. Abnormal semen samples do not include azoospermic sample. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

Number of PGT-A ICSI cycles	212
Number of patients	196
Mean number of controlled ovarian stimulation cycles per patient	1.1
Female age (years)	35.64 ± 5.56
Male age (years)	40.83 ± 10.08
Female BMI (Kg/m^2)	22.07 ± 3.47
AMH levels (ng/ml)	2.86 ± 2.36
PGT-A indication per cycle	
AMA	104 (49.1%)
RIF	11 (5.2%)
RM	3 (1.4%)
MF	24 (11.3%)
Other	70 (33.0%)
Ovarian stimulation protocol	
GnRH antagonist protocol	212 (100%)
Sperm quality per cycle	
Normal	70 (33.0%)
Abnormal	142 (67.0%)
Retrieved oocytes	2400
Mature oocytes	1860 (77.5%)
Fertilized oocytes (2PN)	1320 (71.0%)
Blastocysts obtained	884 (67.0%)
Blastocyst analyzed for PGT-A	832
Aneuploid blastocysts	232 (27.9%)
Mosaic blastocysts	293 (35.2%)
Euploid blastocysts	307 (36.9%)
Stratification of mosaic blastocysts by level	293
Low-level	216 (73.7%)
High-level	77 (26.3%)
Stratification of mosaic blastocysts by type	293
Segmental aneuploidies	84 (28.7%)
Whole chromosome aneuploidies	79 (26.9%)
Complex aneuploidies	130 (44.4%)

On the contrary, as shown in Table 4, low-level putative mosaic embryos were significantly slower than euploid embryos in tPNf ($P = 0.006$) and t2 ($P = 0.006$); while, they were significantly faster than aneuploid embryos from the onset of embryo compaction until the full blastocyst development (tSC, $P = 0.012$; tM, $P = 0.003$; tSB, $P < 0.0001$; tB, $P < 0.0001$). High-level putative mosaic embryos were significantly delayed compared to euploid ones in t7 ($P = 0.009$) and faster compared with aneuploid embryos in tPNF ($P = 0.032$), t2 ($P = 0.048$), tSB ($P = 0.026$), and tB ($P = 0.004$).

KIDscore^{TM}Day5 in classes of putative mosaic embryos

KS-5.3.2 was evaluated among the different PGT-A classes of embryos (Fig. 2). The KS-5.3.2 decreased from euploid (6.13, 0.14) to low-putative mosaic embryos (5.32, 0.16, $P < 0.001$) and from high- putative mosaic (4.81, 0.23) to aneuploid embryos (4.24, 0.14, $P = 0.0036$). No significant differences were found between low- and high-level putative mosaic embryos ($P = 0.073$).

Complex aneuploid putative mosaic embryos showed a statistically significant lower KS-5.3.2 mean value compared to segmental aneuploidies (4.68, 0.19 vs 5.85, 0.24, $P < 0.001$).

Morphology evaluation and chromosomal status

The study analyzed the blastocyst morphology distribution among euploid, aneuploid, and putative mosaic PGT-A results. As shown in Fig. 3, blastocysts with a putative mosaic PGT-A result exhibited an intermediated rank for each class of morphology. In particular, the putative mosaic class showed fewer excellent and more poor-quality blastocysts compared to the euploid class ($P < 0.001$), and more excellent and fewer poor-quality blastocysts compared to the aneuploid class ($P = 0.001$). The morphology was not evaluated in putative mosaic embryos divided by level or type, as further classification would require a larger study group.

Clinical outcomes

The clinical outcomes of a total of 144 euploid s-FETs and of 82 putative mosaic s-FETs were evaluated.

Firstly, as shown in Table 5, the study analyzed the clinical outcomes of putative mosaic embryos divided by level: the implantation rate (IR) in the low-level ($n = 26$, 40%) and high-level putative mosaic ($n = 4$, 23.5%) groups was significantly lower compared to euploid group ($n = 82$, 56.9%; $P = 0.0257$ and $P = 0.0105$ respectively). These results reflected the corresponding differences in KS-5.3.2 observed in the transferred blastocysts (KS-5.3.2: euploid vs low-level $P = 0.0028$; euploid vs high-level $P = 0.002$). The ongoing pregnancy/live birth rate (OP/LBR) differed only between euploid and high-level putative mosaic groups ($n = 76$, 52.8% vs $n = 3$, 17.6%; $P = 0.0088$).

Secondly, the clinical outcomes in putative mosaic embryos divided by type of abnormality were evaluated: both euploid blastocysts (56.9%; 52.8%) and segmental putative mosaic blastocysts ($n = 17$, 60.7%; $n = 16$, 57.1%)

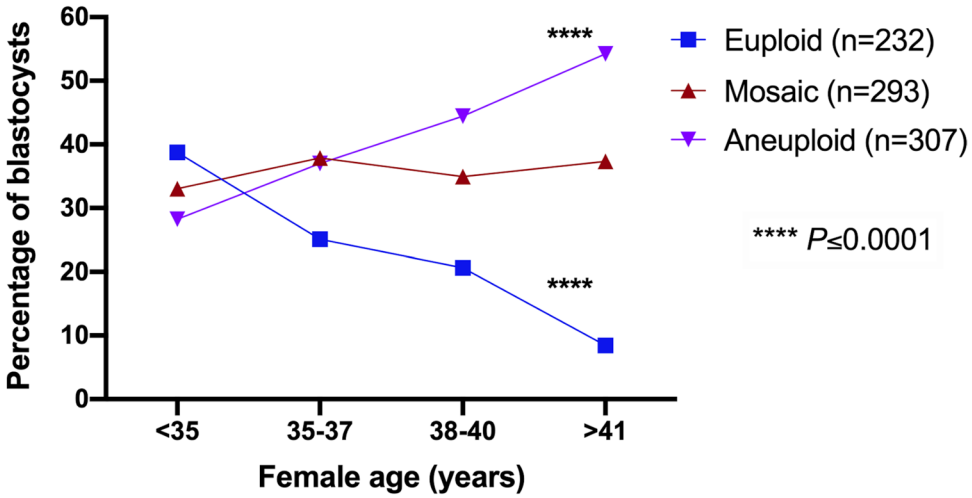

Fig. 1 Ploidy rates stratified by age. Percentage of euploid, putative mosaic, and aneuploid embryos (trophectoderm biopsy results) in different classes of female age (cycles with at least one mosaic embryo). χ^2 test was used at the 95% confidence level. *P*-value < 0.05 was considered statistically significant. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

Table 2 Morphokinetic parameters in euploid, aneuploid and mosaic embryos (trophectoderm biopsy results). *tPB2,* second polar body extrusion; *tPNf* pronuclear fading; *t2*, *t3*, *t4*, *t5*, *t6*, *t7*, *t8*, time to 2, 3, 4, 5, 6, 7, 8, cells divisions, respectively; *tM* morula formation, *tSB* starting blastulation, *tB* full blastocyst stage; cc2 = t3–t2; s2 = t4–t3. *n* number, *SE* standard error. The values are expressed in hours post insemination. A linear mixed model was performed. *P*-value < 0.05 was considered statistically significant. When the overall *P*-value was not statistically significant, post hoc analysis was not performed. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

Morphokinetic parameters	Euploid embryos $n = 232$	Mosaic embryos $n = 293$	Aneuploid embryos $n = 307$	Overall *P*	Post comparisons *P*		
					Euploid vs aneuploid	Euploid vs mosaic	Mosaic vs aneuploid
	Mean (SE)	Mean (SE)	Mean (SE)				
tPB2	3.85 (0.09)	3.95 (0.080)	4.07 (0.08)	0.119	-	-	-
tPNf	23.82 (0.24)	24.54 (0.21)	25.14 (0.22)	< 0.001	< 0.001	0.005	0.007
t2	26.59 (0.26)	27.41 (0.23)	28.02 (0.24)	< 0.001	< 0.001	0.005	0.015
t3	37.37 (0.35)	37.75 (0.31)	38.57 (0.31)	0.003	0.003	0.309	0.022
t4	38.92 (0.36)	39.90 (0.32)	40.75 (0.32)	< 0.001	< 0.001	0.021	0.021
t5	49.99 (0.54)	50.17 (0.47)	51.46 (0.47)	0.029	0.039	0.771	0.039
t6	52.42 (0.55)	53.72 (0.48)	54.75 (0.49)	0.001	< 0.001	0.056	0.073
t7	55.07 (0.65)	57.27 (0.57)	58.19 (0.58)	< 0.001	< 0.001	0.006	0.185
t8	58.96 (0.80)	61.08 (0.70)	62.69 (0.71)	< 0.001	< 0.001	0.033	0.057
tSC	85.95 (0.79)	86.29 (0.69)	88.46 (0.71)	0.003	0.007	0.691	0.007
tM	93.62 (0.81)	94.54 (0.71)	97.24 (0.73)	< 0.001	< 0.001	0.305	0.002
tSB	103.75 (0.79)	104.23 (0.69)	108.47 (0.7)	< 0.001	< 0.001	0.594	< 0.001
tB	111.80 (0.87)	113.41 (0.75)	119.18 (0.77)	< 0.001	< 0.001	0.112	< 0.001
CC2	10.77 (0.22)	10.34 (0.19)	10.51 (0.19)	0.261	-	-	-
S2	1.56 (0.22)	2.18 (0.20)	2.14 (0.19)	0.061	0.063	0.063	> 0.05

exhibited the best results in term of IR and LB/OPR respect to whole chromosome putative mosaic ($n = 6$, 24%; $n = 5$, 20%, $P < 0.01$) and complex putative mosaic ($n = 7$, 24.1%; $n = 7$, 24.1%, $P < 0.01$), respectively. Pairwise comparisons are shown in Table 6. These results were partially confirmed by KS-5.3.2 of transferred blastocysts.

Because euploid embryos are given first priority for transfer and, in some patients, mosaic embryos were transferred as a second choice, the LB/OPR was also evaluated on the first sFET (both for euploid and putative mosaic subgroups) in order to reduce maternal specific effect. The significant differences reported above between different PGT-A classes in terms of LB/OPR were confirmed, as shown in supplemental Tables 2 and 3.

Table 3 Morphokinetic parameters in different classes of mosaic embryos (trophectoderm biopsy results). *tPB2* second polar body extrusion; *tPNf,* pronuclear fading; *t2*, *t3*, *t4*, *t5*, *t6*, *t7*, *t8*, time to 2, 3, 4, 5, 6, 7, 8, cells divisions, respectively; *tM* morulae formation, *tSB* starting blastulation, *tB* full blastocyst stage, *n* number, *SE* standard error. The values are expressed in hours post insemination. A linear mixed model was performed. [a–b]Different superscript letters indicate a significant difference in post hoc analysis. *P*-value < 0.05 was considered statistically significant. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

	Mosaic embryos type				Mosaic embryos level		
	Complex aneuploid	Segmental aneuploid	Whole chr. aneuploid		Low-level < 50%	High-level ≥ 50%	
Morphokinetic parameters	*n* = 130	*n* = 84	*n* = 79	*Overall P*	*n* = 216	*n* = 77	*Overall P*
	Mean (SE)	Mean (SE)	Mean (SE)		Mean (SE)	Mean (SE)	
tPB2	3.92 (0.10)	3.93 (0.12)	4.04 (0.12)	0.717	3.90 (0.08)	4.12 (0.13)	0.117
tPNf	24.58 (0.30)	24.37 (0.37)	24.61 (0.39)	0.859	24.60 (0.25)	24.31 (0.40)	0.507
t2	27.48 (0.35)	27.23 (0.42)	27.42 (0.44)	0.880	27.50 (0.28)	27.11 (0.44)	0.445
t3	38.03 (0.44)	37.44 (0.52)	37.56 (0.53)	0.610	37.68 (0.35)	37.90 (0.55)	0.705
t4	40.39 (0.46)	39.72 (0.56)	39.32 (0.58)	0.316	39.87 (0.37)	40.00 (0.59)	0.84
t5	50.48 (0.70)	50.07 (0.84)	50.04 (0.87)	0.892	49.92 (0.54)	51.13 (0.88)	0.228
t6	54.69 (0.70)	52.53 (0.84)	53.37 (0.87)	0.112	53.67 (0.55)	53.81 (0.89)	0.889
t7	58.59 (0.87)	56.36 (1.06)	56.18 (1.09)	0.123	56.81 (0.68)	58.63 (1.11)	0.152
t8	63.03 (1.04) [a]	59.66 (1.26) [b]	59.39 (1.30) [b]	0.033	60.62 (0.83)	62.29 (1.34)	0.273
tSC	87.64 (0.98)	84.68 (1.16)	85.41 (1.20)	0.093	85.96 (0.79)	86.76 (1.24)	0.566
tM	96.17 (1.02) [a]	92.47 (1.20) [b]	93.81 (1.23)	0.042	94.14 (0.80)	95.29 (1.29)	0.43
tSB	105.61 (1.00)	102.58 (1.22)	103.62 (1.26)	0.126	103.79 (0.79)	105.37 (1.29)	0.284
tB	115.14 (1.08) [a]	110.98 (1.32) [b]	113.03 (1.36)	0.045	112.89 (0.85)	114.73 (1.40)	0.254

Correlation between clinical outcomes and KS-5.3.2 in the same PGT-A class

To evaluate the clinical efficiency in embryo selection of KS-5.3.2 in the same PGT-A result class, the IR and OP/LBR of a total of 144 euploid s-FETs and 65 low-level putative mosaic s-FETs in different KS-5.3.2 groups were retrospectively evaluated (Fig. 4).

Euploid blastocysts from a lower KS5.3.2 value range ([1.5;5.9], $n = 49$, IR = 40.8%; LB/OPR = 36.7%) displayed a significantly lower IR and OP/LBR than euploid blastocysts from consecutively higher KS-5.3.2 value ranges ([6.0;7.9], $n = 50$, IR = 64%, LBR = 62%, $P < 0.05$; [8.0;9.9], $n = 45$, IR = 64%, LBR = 60%, $P < 0.05$), Fig. 4a.

The same analyses were performed for putative low-level mosaic-transferred blastocysts, significant differences in IR and OP/LBR were found between the group of the lower KS-5.3.2 range of values and the intermediate KS-5.3.2 range of values ([1.5;5.9], $n = 43$, IR = 25.6%, OP/LBR = 23.3% vs; [6.0;7.9], $n = 27$, IR = 51.9%, 48.1%, $P < 0.05$.). No statistical difference was found for the highest KS-5.3.2 range of values [8.0–9.0] (Fig. 4b).

Discussion

The primary challenge in assisted reproduction is to improve clinical outcomes in order to achieve a live birth from a single embryo transfer. With the implementation of PGT-A using NGS on trophectoderm biopsies, the detection of ICN in chromosomal analysis has become more frequent, making the management of PGT-A results more challenging [30]. During data collection, the prevalence of mosaicism detection was 21%, which aligns with the range of 11.0–25.7% reported by Viotti et al. (2021) in a multi-center study [7]. The high incidence of mosaicism (35.2%) reported here was specific of the study setting: PGT-A cycles with at least one putative mosaic embryo detected were considered. In the "Good practice recommendations for the use of time-lapse technology," the authors annotated maternal age, maternal BMI, ovarian stimulation protocol, and ovarian reserve as possible confounding factors with the use of time-lapse algorithms [24]. Moreover, laboratory factors like the insemination technique, culture medium composition, PH, temperature, osmolality, and O_2 concentration of the medium may influence the embryonic aneuploidy rate [31, 32]. Thus, the

Table 4 Differences in morphokinetic parameters in low- and high-level mosaic embryos compared to euploids and aneuploids (trophectoderm biopsy results). *tPB2* second polar body extrusion; *tPNf* pronuclear fading; *t2*, *t3*, *t4*, *t5*, *t6*, *t7*, *t8*, time to 2, 3, 4, 5, 6, 7, 8 cells divisions, respectively; *tM* morulae formation, *tSB* starting blastulation, *tB* full blastocyst stage, *n* number, *SE* standard error, *E* euploid, *M* mosaic, *A* aneuploid. The values are expressed in hours post insemination. A linear mixed model was performed. When the overall *P*-value was not statistically significant, post hoc analysis was not performed. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

	Euploid embryos	Mosaic embryos level		Aneuploid embryos		Post comparisons *P*					
		< 50	≥ 50								
Morphokinetic parameters	*n* = 232	*n* = 216	*n* = 77	*n* = 307		E vs M < 50	E vs M ≥ 50	E vs A	M < 50 vs M ≥ 50	M < 50 vs A	M ≥ 50 vs A
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Overall *p*						
tPB2	3.85 (0.09)	3.90 (0.08)	4.12 (0.13)	4.07 (0.08)	0.165						
tPNf	23.82 (0.24)	24.60 (0.25)	24.31 (0.40)	25.14 (0.22)	< 0.001	0.006	0.268	< 0.001	0.337	0.057	0.032
t2	26.59 (0.26)	27.50 (0.28)	27.11 (0.44)	28.02 (0.24)	< 0.001	0.006	0.240	< 0.001	0.345	0.095	0.048
t3	37.37 (0.35)	37.68 (0.35)	37.90 (0.55)	38.57 (0.31)	0.009	0.487	0.487	0.006	0.763	0.057	0.402
t4	38.92 (0.36)	39.87 (0.37)	40.00 (0.59)	40.75 (0.32)	< 0.001	0.060	0.152	< 0.001	0.893	0.078	0.152
t5	49.99 (0.54)	49.92 (0.54)	51.13 (0.88)	51.46 (0.47)	0.034	0.848	0.393	0.060	0.393	0.060	0.794
t6	52.42 (0.55)	53.67 (0.55)	53.81 (0.89)	54.75 (0.49)	0.0032	0.144	0.275	0.001	0.920	0.216	0.275
t7	55.07 (0.65)	56.81 (0.68)	58.63 (1.11)	58.19 (0.58)	0.0003	0.056	0.009	0.001	0.222	0.122	0.843
t8	58.96 (0.8)	60.62 (0.83)	62.29 (1.34)	62.69 (0.71)	0.0007	0.114	0.068	0.001	0.408	0.068	0.651
tSC	85.95 (0.79)	85.96 (0.79)	86.76 (1.24)	88.46 (0.71)	0.0075	0.909	0.600	0.012	0.600	0.012	0.430
tM	93.62 (0.81)	94.14 (0.80)	95.29 (1.29)	97.24 (0.73)	0.0003	0.500	0.324	0.001	0.500	0.003	0.294
tSB	103.75 (0.79)	103.79 (0.79)	105.37 (1.29)	108.47 (0.7)	< 0.001	0.882	0.424	0.000	0.424	0.000	0.026
tB	111.8 (0.87)	112.89 (0.85)	114.73 (1.40)	119.18 (0.77)	< 0.001	0.316	0.107	0.000	0.316	0.000	0.004

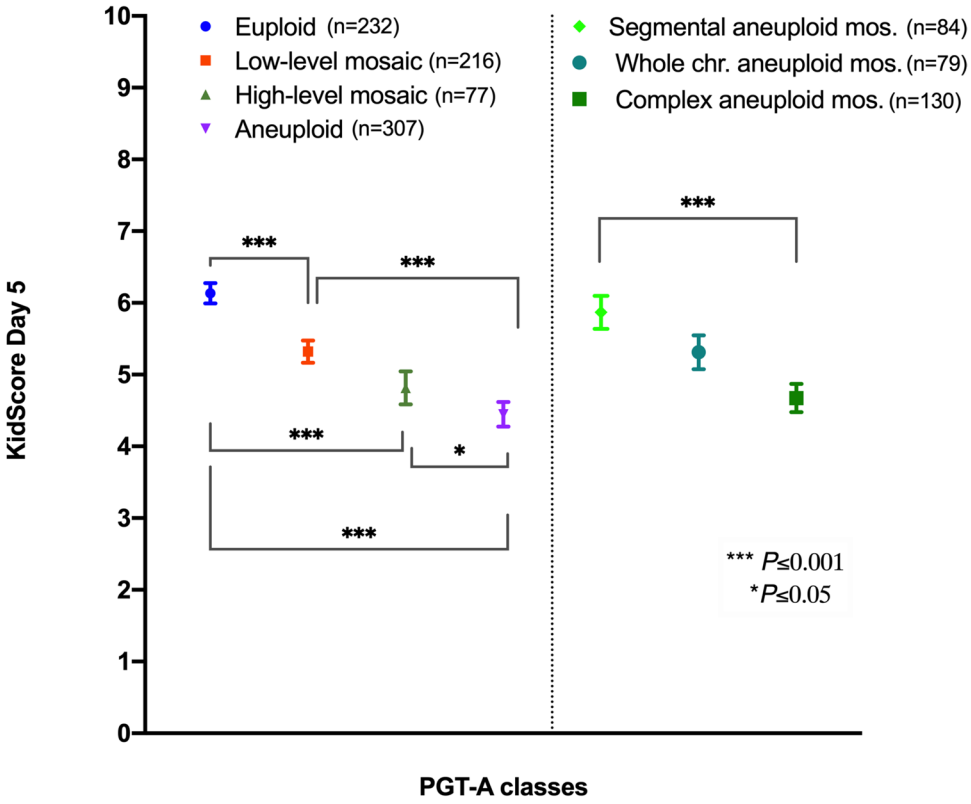

Fig. 2 Distribution of KIDscore™Day5 version 3.2 in euploid/low-level mosaic/high-level mosaic/aneuploid blastocysts and in classes of mosaic blastocysts classified by type (trophectoderm biopsy results). *PGT-A* preimplantation genetic testing for aneuploidy, *chr* chromosome, *mos* mosaic. Data are expressed as mean and 95% confidence interval. Linear mixed model analysis was performed. *P*-value < 0.05 was considered statistically significant. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

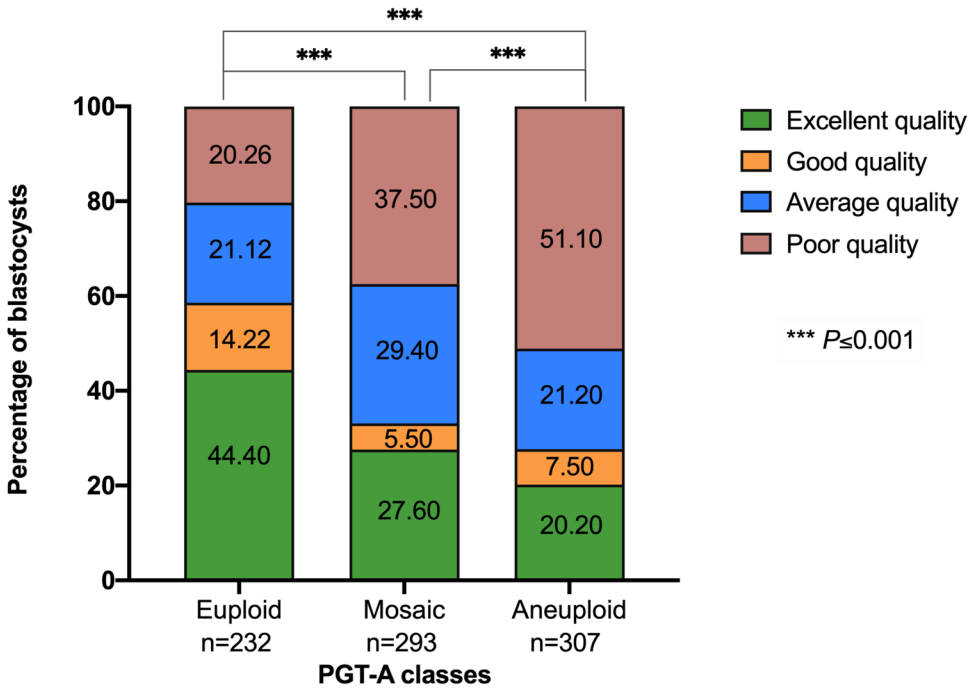

Fig. 3 Distribution of morphology classes in euploid, aneuploid, and mosaic blastocysts (trophectoderm biopsy results). The blastocysts were classified for the ICM and TE quality into excellent (AA), good (AB/BA), average (BB/AC/CA), and poor-quality (CC/BC/CB). *PGT-A* preimplantation genetic testing for aneuploidies. Data are expressed as percentages. A logistic mixed effects model was performed. *P*-value < 0.05 was considered statistically significant. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

approach chosen for the study setting ensured that putative mosaic blastocysts were compared to euploid and aneuploid embryos with overlapping cycle characteristics.

The study aimed to explore the relationship between the morphokinetic and genetic constitution of embryos, particularly focusing on the mosaicism result of TE biopsy. Firstly, we analyzed the morphokinetic and morphology in the main PGT-A categories (euploid, aneuploid, putative mosaic); secondly, given that both the level and type of mosaicism are important criteria in transfer decisions,

Table 5 Effect of mosaicism level on clinical outcomes. Data are presented as *n* (%) for categorical variables or mean and standard error for continuous variables. *n, number, s-FET* single frozen embryo transfer, *KS5.3.2* KIDScore™ Day5 versione 3.2. The ß-hCG value was assessed 10 days after embryo transfer. The implantation rate was defined as the number of fetal heartbeats, detected by transvaginal ultrasound, on the number of blastocysts transferred. The miscarriage rate was the number of spontaneous pregnancy losses before week 20 in which a gestational sac/s or heartbeats was previously observed, per number of pregnancies. The rate of ongoing pregnancy (pregnancy that did not result in an abortion in the first trimester)/live births (delivery with at least one live birth after 22 weeks of gestation) was assessed on transfer cycle. *P*-values were calculated using Kruskal–Wallis tests for continuous variable and $\chi 2$ tests/Fisher exact at the 95% confidence level for pairwise comparisons of proportions. [a–b]Different superscript letters indicate a significant difference (*P*-value < 0.05). Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

	Euploid blastocysts ($n = 144$)	Low-level mosaic blastocysts ($n = 65$)	High-level mosaic blastocysts ($n = 17$)
No. of s-FET cycles	144	65	17
KS5.3.2 of blastocysts transferred	6.56 (0.18) [a]	5.66 (0.27) [b]	4.67 (0.43) [b]
βhCG + test	106 (73.6%) [a]	41 (63.1%)	7 (41.2%) [b]
Implantation rate	82 (56.9%) [a]	26 (40.0%) [b]	4 (23.5%) [b]
Miscarriage rate	6 (7.3%)	1 (3.8%)	1 (25.0%)
Ongoing clinical pregnancy/live birth rate	76 (52. 8%) [a]	25 (38.8%)	3 (17.6%) [b]
Birth weight	3192 (67.74)	3184 (87.56)	3385 (285.0)

Table 6 Effect of mosaicism type on clinical outcomes. Effect of mosaicism level on clinical outcomes. Data are presented as *n* (%) for categorical variables or mean and standard error for continuous variables. *n number, s-FET* single frozen embryo transfer, *KS5.3.2* KIDScore™ Day5 version 3.2. The ß-hCG value was assessed 10 days after embryo transfer. The implantation rate was defined as the number of fetal heartbeats, detected by transvaginal ultrasound, on the number of blastocysts transferred. The miscarriage rate was the number of spontaneous pregnancy losses before week 20 in which a gestational sac/s or heartbeats was previously observed, per number of pregnancies. The rate of ongoing pregnancy (pregnancy that did not result in an abortion in the first trimester)/live births (delivery with at least one live birth after 22 weeks of gestation) was assessed on transfer cycle. *P*-values were calculated using Kruskal–Wallis tests for continuous variable and $\chi 2$ tests/Fisher exact at the 95% confidence level for pairwise comparisons of proportions. [a–b]Different superscript letters indicate a significant difference (*P*-value < 0.05). Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

	Euploid blastocysts ($n = 144$)	Segmental mosaic blastocysts ($n = 28$)	Whole chromosome mosaic blastocysts ($n = 25$)	Complex aneuploid mosaic blastocysts ($n = 29$)
No. of s-FET cycles	144	28	25	29
No. of blastocysts transferred	144	28	25	29
KS5.3.2 of blastocysts transferred	6.56 (0.18) [a]	5.94 (0.45)	5.86 (0.36)	4.71 (0.43) [b]
βhCG + test	106 (73.6%) [a]	20 (71.4%) [a]	17 (68.0%)	12 (41.4%) [b]
Implantation rate	82 (56.9%) [a]	17 (60.7%) [a]	6 (24%) [b]	7 (24.1%) [b]
Miscarriage rate	6 (7.3%)	1 (5.9%)	1 (16.6%)	0 (0%)
Ongoing pregnancy/live birth rate	76 (52.78%) [a]	16 (57.1%) [a]	5 (20.0%) [b]	7 (24.1%) [b]
Birth weight	3192 (67.74)	3119 (124.3)	3233 (203.5)	3346 (110.2)

we repeated the morphokinetic comparison by dividing the putative mosaic category into subclasses and introducing the KS-5 as an additional tool for embryo selection. Finally, to validate the time-lapse data, we evaluated the clinical outcomes of the different ploidy groups.

During the cleavage stages, compared with euploid embryos, putative mosaic embryos were slower in t2, t4, t7, and t8, and compared to aneuploid, they were faster in t2, t3, t4, and t5. Prolonged cell cycles in embryo development are likely to be associated with an activated DNA repair process, incorrect attachment of chromosomes to the spindle, or failure to complete properly the earlier phases of the cell cycle [33]. On the contrary, after embryonic compaction, putative mosaic embryos exhibited no significant differences compared to euploid embryos. This data may suggest an engagement of mosaic embryos in a self-correcting process during development. In contrast, the significant delays in tM, tSB, and tB observed in aneuploid embryos compared to euploid and putative mosaic embryos may indicate an inability to self-correct. A slower development to the blastocysts stage for aneuploid embryos was already reported in a recent study [17]. Concerning the morphology distribution

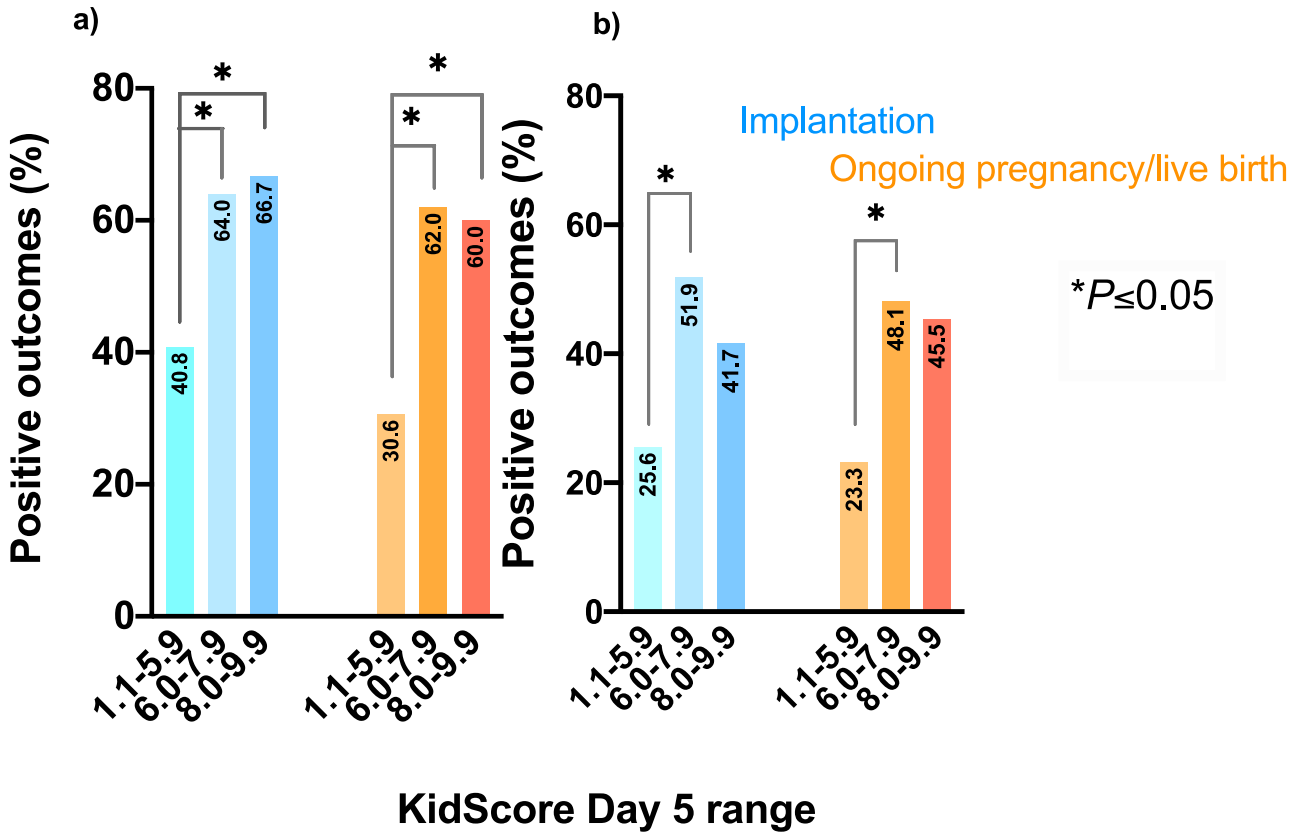

Fig. 4 Implantation and ongoing pregnancy/live birth rate in different KidScore Day 5 range (**a**) of euploid embryos [1.5–5.9], $n=49$; [6.0–7.9], $n=50$; [8.0–9.6], $n=45$; (**b**) of low-level putative mosaic embryos [1.5–5.9] $n=43$; [6.0–7.9] $n=27$; [8.0–9.6], $n=12$. Embryos with chromosomally mosaic results after TE biopsy will be labeled as "mosaic"

in the main ploidy classes, in line with previous research, a significantly higher rate of excellent blastocysts and a lower rate of poor-quality blastocysts were reported in the euploid compared to the mosaic group [7, 22]. Additionally, in this study, poor-quality blastocysts were more common in the aneuploid than in the mosaic categories, suggesting, as described previously, a correlation between ICM and TE quality and chromosomal euploid/aneuploid status [5].

As mentioned earlier in the text, the study investigated whether the aneuploidy cell percentage in TE biopsies (mosaicism level) impacts on the morphokinetic. Data showed differences between euploid embryos and both low- (tPNf and t2) and high-level (t7) putative mosaic embryos.

This partially confirmed Lee's previous work, which reported differences only between high-level mosaic embryos and euploid embryos (t5, t8, s2) [21]. Although these differences were observed at different time points, a susceptibility of putative mosaic embryos to mitotic errors emerged.

Regarding the comparison with aneuploid embryos, the development to blastocyst stage of euploid embryos, as well as that of both low- and high-level putative mosaic embryos, proceeded faster. Specifically, euploid embryos differed from aneuploid ones in all considered timings; low-level putative mosaics differed from compaction onwards, and high-level putative mosaics differed from the start of blastulation onwards. This highlights that as the number of aneuploid cells in embryos increases, the gap with aneuploid embryos narrows. On the contrary, Martin et al. (2021) observed that aneuploid blastocysts exhibited a significant delay in blastulation compared only to euploid blastocysts [22]. The differences in kinetic parameters reported in our study could be attributed to NGS analysis, laboratory conditions, or variations in the patient cohort. Notably, our study differs from that of Martin et al. (2021) in some important aspects: they used a 30–70% range to classify putative mosaic embryos, and blastocysts with segmental and/or sex chromosome mosaicism were not analyzed [22].

To the best of our knowledge, this is the first time the morphokinetic behavior of putative mosaic embryos has been delineated by analyzing the types of chromosomal abnormalities involved in putative mosaicism (segmental aneuploidy, whole chromosome aneuploidy, and complex aneuploidy). As the complexity of the mosaicism detected in the TE biopsy samples increases, there was a delay in the blastocyst development. Notably, significant differences

were observed in morulation and full blastocyst development between segmental and complex putative mosaics.

Time-lapse technology has enabled the development of algorithms capable of predicting the probability of embryo implantation based on kinetic and morphological parameters. Although various researchers have previously investigated the correlation between KS-5 scores and ploidy rates [34, 35], this study is, as far as we know, the first to test the KS-5's capability to discriminate between putative mosaicism subclasses (level and type). A significant decrease in the KS-5.3.2 score was observed from the euploid to the low-mosaic class and from the high-putative mosaic to the aneuploid class. Additionally, complex putative mosaic aneuploidies showed a lower value compared to putative mosaic segmental aneuploidies. However, the differences observed in KS-5.3.2 did not always correlate with clinical outcomes. Significant differences were observed in OP/LBR between euploid and high-level putative mosaic embryos, as well as between euploid/segmental putative mosaic embryos and whole chromosome/complex aneuploid putative mosaic embryos. These clinical outcomes partially overlapped with the results reported by Viotti et al. (2021), which showed a progressive worsening with the increasing severity of mosaic aneuploidy in TE biopsy [7]. The differences between the two studies (specifically, Viotti reported differences between low-level and high-level; and whole chromosome vs complex) can be attributed to the varied study designs and sample sizes. Finally, it is important to note that, as revealed by this study, even though high-level putative mosaic embryos have shown very poor clinical outcomes (leading several IVF centers to exclude them from embryo transfer), they can still result in live births.

This study also investigated the correlation between KS-5.3.2 and clinical outcomes within the same PGT-A result subclass. A correlation between IR, OP/LBR, and KS-5.3.2 in euploid s-FET was found, reinforcing the significant difference in implantation rate previously reported by Gazzo et al. between the highest and lowest KS-5 classes [34]. From the KS-5.3.2 analysis in the putative low-level mosaic class, it emerged that embryos with the lowest KS-5.3.2 values should be considered second choices for transfer compared to those with intermediate KS-5.3.2 values. The embryos with the highest KS-5.3.2 values did not show differences compared to the previous categories; this result may be explained by the impaired number of embryos in each KS-5.3.2 range. In the future, it may be interesting to enlarge the sample size to study the correlation between KS-5.3.2 and live birth rate in the other PGT-A mosaicism subclasses.

Limitations

Limitations in our study can be observed. First the retrospective design. Second the limited sample size; embryo development is a dynamic process affected by both extrinsic and intrinsic factors, making it difficult to generalize findings from a single-center study. Third, as reported in the literature, there are dissimilarities in the diagnosis and interpretation of mosaic results among PGT-A laboratories and different ranges to classify an embryo as mosaic [36], making it difficult to compare our results with that of other studies. Furthermore, PGT-A from a single TE biopsy may not reflect the content of the entire embryo [37]. Fourth, the study setting (cycles with only euploids or aneuploids were not considered) could underestimate the true differences between euploid and aneuploid embryos. However, the purpose of the study is to evaluate the outcomes in cycles where mosaics were obtained.

Conclusion

The intricate correlation between cellular processes and genetic factors continues to be an area of active research in reproductive medicine. Data highlight that morphology, morphokinetic, and chromosome content in TE biopsy are closely related to each other and the KS-5 algorithm reflects this interplay. Although data confirmed, as noted in literature, that PGT-A remains the gold standard for embryo selection [38], KS-5 could be considered an additional tool for selecting embryos with the same PGT-A result. Focusing on putative mosaic TE-biopsy results, the study revealed that increasing the number of aneuploid cells or the complexity of chromosome abnormalities influences the morphokinetic behavior of the embryos. This supports the hypothesis of a distinct developmental identity for the putative mosaic category.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10815-024-03364-7.

Declarations

Competing interests The authors declare no competing interests.

References

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017;108:393–406.
2. Munné S, Wells D. Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing. Fertil Steril. 2017;107:1085–91. https://doi.org/10.1016/j.fertnstert.2017.03.024.
3. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med. 2015;373:2089–90. https://doi.org/10.1056/NEJMc1500421.
4. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, et al. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid–aneuploid

blastocysts. Hum Genet. 2017;136:805–19. https://doi.org/10.1007/s00439-017-1797-4.
5. Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertil Steril. 2019;111:280–93. https://doi.org/10.1016/j.fertnstert.2018.10.019.
6. Munné S, Spinella F, Grifo J, Zhang J, Beltran MP, Fragouli E, et al. Clinical outcomes after the transfer of blastocysts characterized as mosaic by high resolution Next Generation Sequencing-further insights. Eur J Med Genet. 2020;63:103741. https://doi.org/10.1016/j.ejmg.2019.103741.
7. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril. 2021;115:1212–24. https://doi.org/10.1016/j.fertnstert.2020.11.041.
8. Viotti M. O-47. Assessing the risk-benefit of mosaic embryo transfers using 3,500+ clinical outcome data points. Fertil Steril. 2023;120:e20–1. https://doi.org/10.1016/j.fertnstert.2023.08.724.
9. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet. 2021;108:2238–47. https://doi.org/10.1016/j.ajhg.2021.11.002.
10. De Rycke M, Capalbo A, Coonen E, Coticchio G, Fiorentino F, Goossens V, et al. ESHRE survey results and good practice recommendations on managing chromosomal mosaicism. Hum Reprod Open. 2022;2022:hoac044. https://doi.org/10.1093/hropen/hoac044.
11. Greco E, Yakovlev P, Kornilov N, Vyatkina S, Bogdanova D, Ermakova M, et al. Two clinical case reports of embryonic mosaicism identified with PGT-A persisting during pregnancy as true fetal mosaicism. Hum Reprod. 2023;38:315–23. https://doi.org/10.1093/humrep/deac263.
12. Kahraman S, Cetinkaya M, Yuksel B, Yesil M, Cetinkaya CP. The birth of a baby with mosaicism resulting from a known mosaic embryo transfer: a case report. Hum Reprod. 2020;35:727–33.
13. Treff NR, Marin D. The, "mosaic" embryo: misconceptions and misinterpretations in preimplantation genetic testing for aneuploidy. Fertil Steril. 2021;116:1205–11. https://doi.org/10.1016/j.fertnstert.2021.06.027.
14. Nogales MDC, Bronet F, Basile N, María Martínez E, Liñán A, Rodrigo L, et al. Type of chromosome abnormality affects embryo morphology dynamics. Fertil Steril. 2017;107:229–235.e2. https://doi.org/10.1016/j.fertnstert.2016.09.019.
15. Basile N, Nogales MDC, Bronet F, Florensa M, Riqueiros M, Rodrigo L, et al. Increasing the probability of selecting chromosomally normal embryos by time-lapse morphokinetics analysis. Fertil Steril. 2014;101:699–704. https://doi.org/10.1016/j.fertnstert.2013.12.005.
16. Chavez SL, Loewke KE, Han J, Moussavi F, Colls P, Munne S, et al. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. Nat Commun. 2012;3:1251. https://doi.org/10.1038/ncomms2249.
17. Serrano-Novillo C, Uroz L, Màrquez C. Novel time-lapse parameters correlate with embryo ploidy and suggest an improvement in non-invasive embryo selection. J Clin Med. 2023;12:2983. https://doi.org/10.3390/jcm12082983.
18. Campbell A, Fishel S, Bowman N, Duffy S, Sedler M, Fontes C, et al. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. Reprod Biomed Online. 2013;26:477–85. https://doi.org/10.1016/j.rbmo.2013.02.006.
19. Alfarawati S, Fragouli E, Colls P, Stevens J, GutiErrez-Mateo C, Schoolcraft WB, et al. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertil Steril. 2011;95:520–4. https://doi.org/10.1016/j.fertnstert.2010.04.003.
20. Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F, et al. Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study. Hum Reprod. 2016;31:2245–54. https://doi.org/10.1093/humrep/dew183.
21. Lee CI, Chen CH, Huang CC, Cheng EH, Chen HH, Ho ST, et al. Embryo morphokinetics is potentially associated with clinical outcomes of single-embryo transfers in preimplantation genetic testing for aneuploidy cycles. Reprod Biomed Online. 2019;39:569–79. https://doi.org/10.1016/j.rbmo.2019.05.020.
22. Martín Á, Rodrigo L, Beltrán D, Meseguer M, Rubio C, Mercader A, et al. The morphokinetic signature of mosaic embryos: evidence in support of their own genetic identity. Fertil Steril. 2021;116:165–73. https://doi.org/10.1016/j.fertnstert.2020.12.031.
23. Diedrich K, Diedrich C, Santos E, Zoll C, Al-hasani S, Reissmann T, et al. Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. Hum Reprod. 1994;9:788–91. https://doi.org/10.1093/oxfordjournals.humrep.a138597.
24 Apter S, Ebner T, Freour T, Guns Y, Kovacic B, Le Clef N, et al. Good practice recommendations for the use of time-lapse technology†. Hum Reprod Open. 2020;2020:Hoaa008. https://doi.org/10.1093/hropen/hoaa008.
25. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. Toward reproductive certainty: fertility and genetics beyond. London: Parthenon Publishing; 1999. pp. 378–88.
26. Casciani V, Cimadomo D, Trio S, Chiappetta V, Innocenti F, Iussig B, et al. Association between iDAScore v1.0, senior embryologists' grading and euploidy in 546 blastocysts obtained during 189 PGT-A cycles. Hum Reprod. 2022;37:i280. https://doi.org/10.1093/humrep/deac107.182.
27. Kato K, Ueno S, Berntsen J, Ito M, Shimazaki K, Uchiyama K, Okimura T. Comparing prediction of ongoing pregnancy and live birth outcomes in patients with advanced and younger maternal age patients using KIDScore™ day 5: a large-cohort retrospective study with single vitrified-warmed blastocyst transfer. Reprod Biol Endocrinol. 2021;19:98. https://doi.org/10.1186/s12958-021-00767-427.
28. Bori L, Meseguer F, Valera MA, Galan A, Remohi J, Meseguer M. The higher the score, the better the clinical outcome: retrospective evaluation of automatic embryo grading as a support tool for embryo selection in IVF laboratories. Hum Reprod. 2022;37:1148–60. https://doi.org/10.1093/humrep/deac066.
29. Spinella F, Fiorentino F, Biricik A, Bono S, Ruberti A, Cotroneo E, et al. Extent of chromosomal mosaicism influences the clinical outcome of in vitro fertilization treatments. Fertil Steril. 2018;109:77–83. https://doi.org/10.1016/j.fertnstert.2017.09.025.
30. Viotti M, Greco E, Grifo JA, Madjunkov M, Librach C, Cetinkaya M, et al. Chromosomal, gestational, and neonatal outcomes of embryos classified as mosaic by preimplantation genetic testing for aneuploidy. Fertil Steril. 2023;120:957–66. https://doi.org/10.1016/j.fertnstert.2023.07.022.
31. Swain JE. Controversies in ART: can the IVF laboratory influence preimplantation embryo aneuploidy? Reprod Biomed Online. 2019;39:599–607. https://doi.org/10.1016/j.rbmo.2019.06.009.
32. Desai N, Yao M, Richards EG, Goldberg JM. Randomized study of G-TL and global media for blastocyst culture in the EmbryoScope: morphokinetics, pregnancy, and live births after single-embryo transfer. Fertil Steril. 2020;114:1207–15. https://doi.org/10.1016/j.fertnstert.2020.06.049.
33. Lagalla C, Tarozzi N, Sciajno R, Wells D, Santo D, Nadalini M, et al. Embryos with morphokinetic abnormalities may develop into

euploid blastocysts. Reprod Biomed Online. 2016;1822:1913–20. https://doi.org/10.1016/j.rbmo.2016.11.008.
34. Gazzo E, Peña F, Valdéz F, Chung A, Bonomini C, Ascenzo M, et al. The Kidscore™ D5 algorithm as an additional tool to morphological assessment and PGT-A in embryo selection: a time-lapse study. JBRA Assist Reprod. 2020;24:55–60. https://doi.org/10.5935/1518-0557.20190054.
35. Zou Y, Sui Y, Fu J, Ge N, Sun X, Sun Y. The morphokinetic signature of human blastocysts with mosaicism and the clinical outcomes following transfer of embryos with low-level mosaicism. J Ovarian Res. 2024;17:10. https://doi.org/10.1186/s13048-023-01324-w.
36. Leigh D, Cram DS, Rechitsky S, Handyside A, Wells D, Munne S, et al. PGDIS position statement on the transfer of mosaic embryos 2021. Reprod Biomed Online. 2022;45:19–25. https://doi.org/10.1016/j.rbmo.2022.03.013.
37. Kim J, Tao X, Cheng M, Steward A, Guo V, Zhan Y, et al. The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing platform for preimplantation genetic testing-aneuploidy. Fertil Steril. 2022;117:315–23. https://doi.org/10.1016/j.fertnstert.2021.10.011.
38. Rienzi L, Capalbo A, Stoppa M, Romano S, Maggiulli R, Albricci L, et al. No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poor-prognosis patients: a longitudinal cohort study. Reprod Biomed Online. 2015;30:57–66. https://doi.org/10.1016/j.rbmo.2014.09.012.

Attestation statement: Data regarding any of the subjects in the study has not been previously published. Data will be made available to the editors of the journal for review or query upon request.