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RESEARCH

A machine learning–based score to predict live birth after mosaic embryo transfer

José A. Ortiz[1] · Ruth Morales[1] · Belén Lledó[1,2] · Francisca M. Lozano[1] · Alba M. Cascales[1] · Jorge Ten[2,3] · Juan C. Castillo[4] · Andrea Bernabeu[2,4]

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Abstract

Purpose This study aimed to develop an artificial intelligence–based scoring system to prioritize mosaic embryos according to live birth outcomes.

Methods This multicentre, observational, retrospective study included 264 transferred mosaic embryos from 2583 PGT-A (Preimplantation Genetic Testing for Aneuploidies) cycles performed between January 2017 and January 2023. Trophectoderm (TE) biopsies from day-5 (D5) or day-6 (D6) blastocysts were analysed using Next-Generation Sequencing (NGS) (VeriSeq, Illumina®, San Diego, CA, USA). Biopsied embryos were vitrified and subsequently transferred. Clinical, embryological, and laboratory variables were collected to build predictive machine learning models for live birth. Models excluding cohort-invariant variables were refined to derive the final scoring system.

Results Among mosaic embryos, biochemical, clinical pregnancy, and live birth rates were 50.75%, 41.66%, and 36.36%, respectively. The best-performing model, validated through tenfold cross-validation, identified embryo quality and biopsy day as the most influential predictors (42% and 34% weights, respectively), while mosaicism-related factors such as monosomy/trisomy (18%) and mosaicism degree (6%) had lower influence. The resulting score suggests prioritizing high-quality embryos biopsied on D5, as the type and level of mosaicism play a minor role in gestational potential, except when comparing embryos of similar quality where lower mosaicism levels and absence of monosomy are advantageous.

Conclusion The AI-derived score highlights embryo quality as the primary determinant of success in mosaic embryo transfer, supporting prioritization of high-quality, D5-biopsied embryos to improve live birth outcomes in ART.

Keywords Mosaicism · AI · Machine learning · Live birth

✉ José A. Ortiz
jaortiz@institutobernabeu.com

Ruth Morales
rmorales@institutobernabeu.com

Belén Lledó
blledo@institutobernabeu.com

Francisca M. Lozano
plozano@institutobernabeu.com

Alba M. Cascales
albamariacascales@gmail.com

Jorge Ten
jten@institutobernabeu.com

Juan C. Castillo
jcastillo@institutobernabeu.com

Andrea Bernabeu
abernabeu@institutobernabeu.com

[1] Molecular Biology, Instituto Bernabeu, Alicante, Spain

[2] Cátedra de Medicina Comunitaria y Salud Reproductiva, Miguel Hernandez University, San Juan de Alicante, Spain

[3] Reproductive Biology, Instituto Bernabeu, Alicante, Spain

[4] Reproductive Medicine, Instituto Bernabeu, Alicante, Spain

Introduction

Embryo mosaicism is defined as the presence of two or more chromosomally distinct cell populations within the same embryo, arising from mitotic errors that occur after fertilization [1]. It is a relatively frequent event during preimplantation development, with reported prevalence ranging from 15 to 40% of embryos [2, 3]. Mosaicism typically originates from chromosomal segregation errors, including nondisjunction during cell division, anaphase lag (where a chromatid fails to be correctly incorporated into the daughter nuclei) and DNA replication without cytokinesis, known as endoreplication. Among these mechanisms, anaphase lag is considered the main contributor to mosaicism in preimplantation embryos [4–7].

The implementation of NGS in PGT-A has greatly enhanced the ability to detect mosaicism, as this technology allows the identification of intermediate copy number states, a hallmark of mosaic embryos [8, 9].

The clinical management of mosaic embryos continues to be a subject of debate. Initial guidelines discouraged their transfer, later permitting it only under restricted circumstances, and more recently, professional societies such as the European Society of Human Reproduction and Embryology (ESHRE), the Preimplantation Genetic Diagnosis International Society (PGDIS), and the American Society for Reproductive Medicine (ASRM) have issued joint recommendations [10–12]. These guidelines suggest that transfer may be considered in the absence of euploid embryos, provided that comprehensive genetic counselling is offered. Although the transfer of mosaic embryos may increase the likelihood of achieving pregnancy in in vitro *fertilization* (IVF), it requires follow-up prenatal testing to confirm chromosomal constitution.

Clinical experience has shown that mosaic embryos are capable of resulting in the birth of chromosomally normal children [13–17]. A self-correction mechanism has been proposed to explain this observation, whereby aneuploid cells may be eliminated through apoptosis or confined to the TE, while the inner cell mass (ICM) preserves a euploid constitution [18, 19]. Despite these findings, the implications of low-to-moderate mosaicism remain unclear, particularly with respect to live birth potential and the risk of miscarriage. Although healthy live births from mosaic embryos have been documented, their clinical management remains under debate and underscores the importance of genetic counselling, ensuring that patients are fully informed of the possible risks and outcomes [20, 21].

AI has increasingly contributed to the development of medical applications that support diagnosis and treatment optimization across a wide range of clinical fields. Reproductive medicine is no exception. The large volume of data generated through assisted reproductive technology (ART) provides an ideal foundation for implementing machine learning algorithms capable of optimizing each stage of the treatment process, with the ultimate goal of improving pregnancy outcomes [22–25]. In the specific case of mosaic embryos, several predictive models and scoring systems have been proposed to guide their prioritization for transfer; however, none of these have incorporated AI approaches in their development. In all existing models, the characteristics of mosaicism, such as the number and type of chromosomal abnormalities, the chromosomes involved, and the degree of mosaicism, have played a central role in determining embryo selection for transfer [26, 27].

Medical practice in fertility clinics shows that embryo mosaicism in the context of ART represents a biologically complex and ethically sensitive issue, encompassing its detection, interpretation, and clinical application [28]. The present study evaluated the clinical outcomes of mosaic embryo transfers and, using machine learning methods, developed a prioritization tool (*MosaicScore*) to support clinical decision-making. Unlike previous approaches that emphasized the degree and type of mosaicism, *MosaicScore* primarily relies on embryo quality as the main criterion for selection, while mosaicism-related parameters are considered secondary predictors of implantation potential and the likelihood of live birth.

Material and methods

Study design

This multicentre, observational, retrospective study included a total of 8346 embryos derived from 2583 PGT-A cycles conducted between January 2017 and January 2023. Only mosaic embryos that were subsequently transferred ($n = 264$) were included in the analysis. TE biopsies were performed on blastocysts on days 5, 6, or 7 and analysed using NGS on the Illumina VeriSeq platform (Illumina®, San Diego, CA, USA). Embryos were categorized based on PGT-A results as euploid (< 25% aneuploid cells), mosaic (25–50% aneuploid cells), or aneuploid (> 50% aneuploid cells). Following biopsy, embryos were vitrified and transferred in later cycles.

The main clinical reasons for performing PGT-A included advanced maternal age, abnormal sperm FISH findings, and a history of recurrent miscarriage or implantation failure. Clinical outcomes, as well as detailed information on parental characteristics, IVF cycle parameters, and embryo-specific features such as embryo quality, day of biopsy, and mosaicism-related variables (level of embryo mosaicism, number of chromosomes with mosaicism, number of segmental alterations, number of whole chromosomes alterations and type of mosaicism -monosomies or/and trisomies-), were systematically recorded in a dedicated, prospectively maintained database. Embryo quality was classified according to the ASEBIR criteria [29]. Embryos without an available mosaicism diagnosis were excluded from both the analysis and the predictive model.

Univariate analysis of features

Comparisons between groups defined by live birth outcome (positive vs. negative) were conducted using Pearson's chi-square test for categorical variables. The Shapiro–Wilk test was employed to assess the distribution of continuous variables. For normally distributed variables, differences were analysed with the Student's *t*-test, while non-normally distributed variables were compared using the Mann–Whitney *U* test. The software used to carry out the analysis was R (v. 4.3.1). A *p*-value < 0.05 was considered statistically significant.

Data preprocessing

Prior to analysis, the dataset was anonymized to ensure confidentiality. Only 0.06% of the data were missing and subsequently imputed using the MICE (Multiple Imputation by Chained Equations) algorithm [30]. No outliers were identified. In cases where the variables are highly correlated, only one representative feature was retained in the predictive models, while variables with near-zero variance were excluded due to limited predictive value.

To prepare the data for modelling, class balancing was applied to the target variables. The dataset was then randomly split into a training set (80%) and a test set (20%) prior to model development**.**

Model development and hyperparameter optimization

The original dataset comprised 26 predictor variables, grouped into five categories: maternal, paternal, couple-related, IVF cycle–related, and embryo-specific features, including mosaicism characteristics (Table 1). Variables deemed non-informative or highly correlated were removed.

Ten supervised classification algorithms were applied to the dataset (Table 3). To ensure model independence and robust performance evaluation, tenfold cross-validation was implemented, coupled with systematic hyperparameter tuning. To minimise the risk of overfitting, cross-validation was performed, irrelevant predictor variables were removed, and model performance was continuously monitored during the training, validation, and testing phases.

Model performance was assessed using multiple metrics, including area under the ROC curve (AUC), accuracy, and positive and negative predictive values. The final model was selected based primarily on AUC.

All machine learning analyses were performed using R statistical software (v.4.3.1), with the 'Caret' package [31] employed to implement the various algorithms.

MosaicScore

Predictor selection was performed using the AutoScore [32] framework, which integrates machine learning techniques with the development of clinically interpretable scoring systems. The variables were initially ranked according to their relative importance using a Random Forest model. Predictors were then sequentially incorporated into incremental models following this ranking.

For each incremental model, defined by the number of predictors included, discriminative performance was evaluated using tenfold cross-validation, with the AUC as the primary performance metric. Model performance across different levels of complexity was summarized using a parsimony plot, which depicts the relationship between the number of predictors and the corresponding AUC. The optimal number of predictors was defined as the smallest set achieving the highest AUC, thereby balancing predictive performance and model simplicity. In a second step, the selected predictors were subsequently used in a logistic regression model to construct the score.

The *MosaicScore* was specifically designed for intra-cohort embryo ranking; therefore, only variables varying between embryos within the same cohort were included, and cohort-level predictors were excluded by design.

Declaration AI-assisted text editing

To support the writing process, the author used ChatGPT for suggestions on clarity and readability and to avoid grammatical errors. After this assistance, the authors critically evaluated, revised, and approved all sections of the manuscript, and remains solely responsible for its content. Additionally, our translation and documentation department conducted a final review.

Results

Baseline characteristics

Table 1 presents the baseline parental, clinical, and embryological features of mosaic embryos stratified by live birth outcome. The mean maternal age was 34.95 ± 6.38 years, while paternal age was higher at 39.13 ± 6.74 years. Overall,

Table 1 Characteristics of patients, cycles and mosaic embryo associated with live births and non-live births

		Live births[a]		
Characteristic	Overall[a]	-[a]	+[a]	***p*-value[b]**
	n = 264	*n* = 168	*n* = 96	
Maternal age	34.95 (6.38)	34.72 (6.54)	35.34 (6.10)	0.618
Paternal age	39.13 (6.74)	39.21 (7.11)	38.99 (6.06)	0.371
Cycles with donated oocytes (%)	68.0 (25.8%)	50.0 (29.8%)	18.0 (18.8%)	0.049
Cycles with vitrified oocytes (%)	40.0 (15.2%)	23.0 (13.7%)	17.0 (17.7%)	0.381
Stimulations in luteal phase (%)	19.0 (7.2%)	10.0 (6.0%)	9.0 (9.4%)	0.301
Origin of sperm sample				0.121
Fresh sperm sample	214.0 (81.1%)	129.0 (76.8%)	85.0 (88.5%)	
Criopreserved sperm sample	25.0 (9.5%)	19.0 (11.3%)	6.0 (6.3%)	
Donor sperm sample	22.0 (8.3%)	17.0 (10.1%)	5.0 (5.2%)	
PAAF or Testicular biopsy	3.0 (1.1%)	3.0 (1.8%)	0.0 (0.0%)	
Recovered oocytes	11.25 (6.02)	11.32 (5.78)	11.13 (6.44)	0.561
Recovered mature oocytes	9.04 (4.57)	9.04 (4.36)	9.04 (4.95)	0.796
Sperm count				0.116
Normal	200.0 (75.8%)	133.0 (79.2%)	67.0 (69.8%)	
Oligozoospermia	56.0 (21.2%)	31.0 (18.5%)	25.0 (26.0%)	
Cryptozoospermia	6.0 (2.3%)	2.0 (1.2%)	4.0 (4.2%)	
Azoospermia	2.0 (0.8%)	2.0 (1.2%)	0.0 (0.0%)	
Teratozoospermia	44.0 (16.7%)	29.0 (17.3%)	15.0 (15.6%)	0.731
Asthenozoospermia	55.0 (20.8%)	33.0 (19.6%)	22.0 (22.9%)	0.529
Pathologic sperm DNA fragmentation-TUNEL-(%)	8.0 (3.0%)	5.0 (3.0%)	3.0 (3.1%)	0.905
Altered sperm aneuploidy test -FISH-(%)	23.0 (8.7%)	17.0 (10.1%)	6.0 (6.3%)	0.393
RIF (%)	58.0 (22.0%)	37.0 (22.0%)	21.0 (21.9%)	0.978
RPL (%)	46.0 (17.4%)	28.0 (16.7%)	18.0 (18.8%)	0.668
History of chromosomopathies (%)	55.0 (20.8%)	36.0 (21.4%)	19.0 (19.8%)	0.753
Number of embryos biopsied (%)	3.64 (1.87)	3.65 (1.82)	3.60 (1.96)	0.726
Biopsy day				0.008
D5	162.0 (61.4%)	93.0 (55.4%)	69.0 (71.9%)	
D6	102.0 (38.6%)	75.0 (44.6%)	27.0 (28.1%)	
Embryo quality				0.524
A	118.0 (44.7%)	74.0 (44.0%)	44.0 (45.8%)	
B	129.0 (48.9%)	81.0 (48.2%)	48.0 (50.0%)	
C	17.0 (6.4%)	13.0 (7.7%)	4.0 (4.2%)	
Embryo transfer day				0.361
D5	147.0 (55.7%)	90.0 (53.6%)	57.0 (59.4%)	
D6	117.0 (44.3%)	78.0 (46.4%)	39.0 (40.6%)	
Number of mosaic embryos transferred				0.301
Single embryo transfer (SET)	245.0 (92.8%)	158.0 (94.0%)	87.0 (90.6%)	
Double embryo transfer (DET)	19.0 (7.2%)	10.0 (6.0%)	9.0 (9.4%)	
Level of embryo mosaicism				0.905
Low (25–40%)	161.0 (61.0%)	102.0 (60.7%)	59.0 61.5%)	
Moderate (40–50%)	103.0 (39.0%)	66.0 (39.3%)	37.0 (38.5%)	
Number of chromosomes with mosaicism	1.31 (0.76)	1.38 (0.88)	1.19 (0.44)	0.162
Number of segmental alterations	0.50 (0.57)	0.52 (0.61)	0.45 (0.50)	0.507
Number of whole chromosomes alterations	0.81 (0.94)	0.85 (1.05)	0.74 (0.70)	0.966
Type of mosaicism				0.739
Only monosomies	135.0 (51.1%)	88.0 (52.4%)	47.0 (49.0%)	
Only trisomies	102.0 38.6%)	62.0 (36.9%)	40.0 (41.7%)	
Monosomies and trisomies	27.0 (10.2%)	18.0 (10.7%)	9.0 (9.4%)	

[a] Mean (SD); *n* (%)

[b] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

semen quality was favourable, with 75.8% of men showing normozoospermia according to WHO criteria [33], and only 8.7% displaying increased sperm aneuploidy. Recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL) were reported in 22.0% and 17.4% of couples, respectively. Donated gametes were used in 25.8% of cycles for oocytes and 8.3% for sperm. A mean of 11.25 ± 6.02 oocytes was retrieved per cycle, of which 9.04 ± 4.57 were mature. Embryo quality was generally high (A: 44.7%, B: 48.9%), and biopsies were performed predominantly on day 5 (55.7%) or day 6 (44.3%).

Table 2 summarizes the clinical outcomes following the transfer of mosaic and euploid embryos. The biochemical, clinical, and live birth pregnancy rates were 50.75% vs. 54.06% ($p = 0.313$), 41.66% vs. 44.95% ($p = 0.269$), and 36.36% vs. 38.50% ($p = 0.504$), respectively. No significant differences in clinical outcomes were observed between mosaic and euploid embryo transfers.

Table 2 Comparation of clinical outcomes: euploid embryos vs mosaic embryos

	Mosaic		
	-	**+**	***p*-value**[d]
	$n = 1831$	$n = 264$	
Positive pregnancy test[a]	990 (54.06%)	134 (50.75%)	0.313
Biochemical pregnancy loss[a, b]	162 (16.36%)	24 (17.91%)	0.651
Clinical pregnancy[a]	823 (44.95%)	110 (41.66%)	0.269
Clinical pregnancy loss[a, c]	118 (14.33%)	14 (12.72%)	0.649
Live birth[a]	705 (38.50%)	96 (36.36%)	0.504

[a] *n* (%)

[b] Calculated with respect to positive pregnancy test

[c] Calculated with respect to positive clinical pregnancy

[d] Pearson's Chi-squared test

Univariate analysis

Univariate analysis revealed few significant differences between live birth and non-live birth groups. Maternal and paternal ages were comparable across groups. However, the use of donated oocytes was significantly less common in cycles resulting in live birth (29.8% vs. 18.8%, $p = 0.049$). Similarly, day of biopsy differed, with a higher frequency of day 6 biopsies among the non-live birth group (44.6% vs. 34.5%, $p = 0.008$). Other variables, including embryo quality, transfer day, number of mosaic embryos transferred, and the degree or type of mosaicism did not vary significantly.

No significant differences were detected between the live birth and non-live birth groups regarding mosaicism-related parameters. The proportion of embryos with moderate-level mosaicism (40–50%) was similar across groups (39.3% vs. 38.5%; $p = 0.905$). Likewise, the mean number of chromosomes affected by mosaicism (1.38 ± 0.88 vs. 1.19 ± 0.44; $p = 0.162$) and the incidence of complex mosaicism, involving both monosomies and trisomies (10.7% vs. 9.4%; $p = 0.739$), did not differ significantly. Overall, mosaic embryos leading to live births and those that did not, exhibited comparable chromosomal mosaicism profiles.

Prediction model

We developed several machine learning models with the aim of predicting live birth outcomes. Model performance was primarily evaluated using the AUC, which reflects each model's overall predictive ability. Table 3 summarizes the performance of the top models from each algorithm, including key metrics such as AUC, accuracy, and both positive and negative predictive values. Among the models, the Random Forest algorithm achieved the highest AUC (0.781), followed closely by the C5.0 decision tree (0.769) and the support vector machine (0.741). Random Forest was chosen as the final predictive model because it showed the highest

Table 3 Performance metrics of different predictive models

Model	AUC	Accuracy	Positive predictive Value	Negative predictive value
Generalized Linear Model	0.738	0.654	0.633	0.682
Multi-Layer Perceptron	0.649	0.500	0.500	
Support Vector Machines with Radial Basis Function Kernel	0.741	0.654	0.667	0.643
CART	0.637	0.635	0.621	0.652
C5.0	0.769	0.750	0.760	0.741
Random Forest	0.781	0.635	0.630	0.640
AdaBoost Classification Trees	0.714	0.654	0.643	0.667
Stochastic Gradient Boosting	0.680	0.635	0.652	0.621
Bagged CART	0.639	0.558	0.552	0.565
eXtreme Gradient Boosting	0.732	0.654	0.643	0.667

AUC value, which measures a model's ability to distinguish between positive and negative classes. The corresponding ROC curve, confusion matrix, and additional evaluation metrics for the Random Forest model are presented in Fig. 1.

MosaicScore

Variable prioritisation using the AutoScore framework identified a limited subset of predictors with high discriminative contribution. Incremental model evaluation demonstrated a progressive increase in the AUC as predictors were added sequentially, reaching a maximum with four variables (AUC = 0.61; Fig. 2). This optimal set comprised two embryo-related variables (embryo quality and day of biopsy) and two mosaicism-related variables: type of mosaicism (monosomy, trisomy, or both) and level of mosaicism (low: 25–40%; moderate: 40–50%) (Fig. 2).

Beyond this point, the inclusion of additional predictors did not improve model performance and was associated with minor fluctuations or a decrease in AUC, indicating that most of the predictive information was captured by the first four variables ranked by AutoScore. Accordingly, this subset was selected for the development of the final scoring model, *MosaicScore*. In this model, points are assigned to each variable and category according to their relative predictive contribution, and the total score for each embryo is calculated as the sum of the points across all variables (Table 4). The embryo with the highest total score is prioritised for transfer.

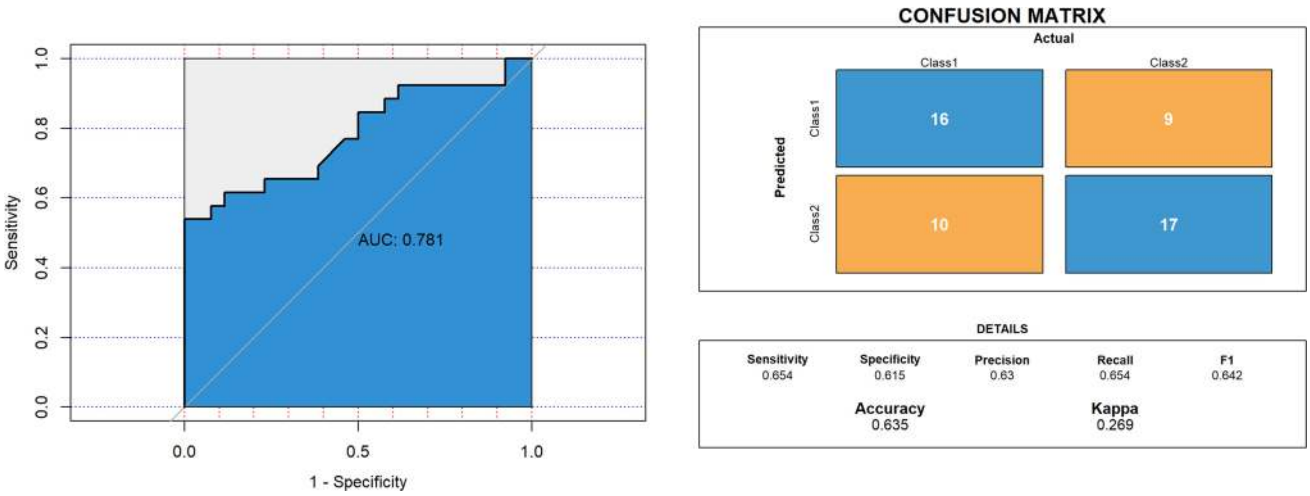

Fig. 1 Performance metrics of Random Forest model. **A** ROC curve of Random Forest model. **B** Confusion matrix and different performance metrics (sensitivity, specificity, precision, recall, F1, accuracy and kappa) of Random Forest model. All metric parameter values have been obtained from the test database (20% of the original dataset)

Fig. 2 Final Parsimony plot based on tenfold cross validation. The figure illustrates the relationship between model complexity and discriminative performance. The *x*-axis represents the number of predictors sequentially included according to their importance ranking derived from AutoScore, while the *y*-axis shows the mean area under the receiver operating characteristic curve (AUC) obtained from tenfold cross-validation. The maximum AUC is achieved with four predictors, identifying this point as the optimal balance between predictive performance and model parsimony

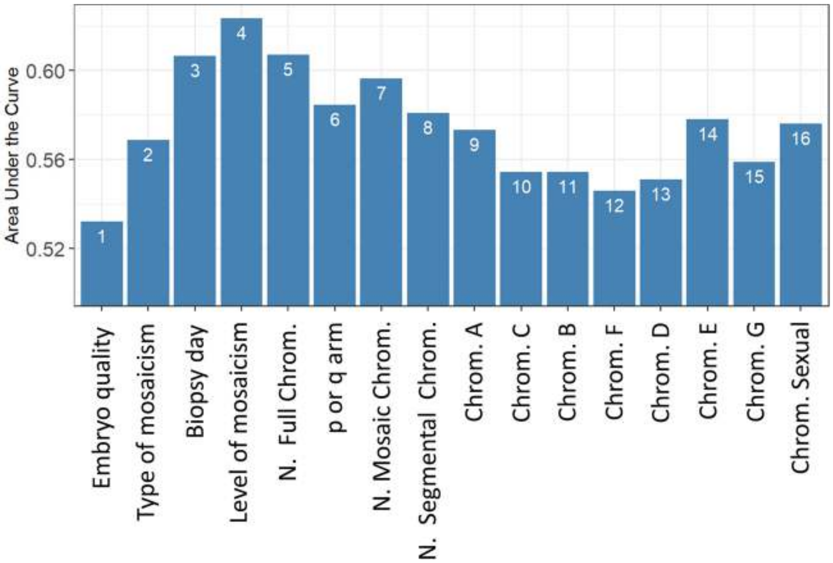

Table 4 Scoring system to prioritise the transfer of mosaic embryos

Characteristic	Max. score contribution (%)	Value	Partial score
Embryo quality	42	*A*	42
		B	39
		C	8
Biopsy day		*D5*	34
	34	*D6*	0
Type of mosaicism		*Trisomy*	18
		Monosomy	3
	18	*Monosomy and trisomy*	0
Level of mosaicism		*Low (25–40%)*	6
	6	*Moderate (40–50%)*	0

Embryo quality accounted for the largest proportion of the total score (42%), followed by day of biopsy (34%). Mosaicism-related factors contributed to a lesser extent, with the presence of monosomy and/or trisomy accounting for 18% and the level of mosaicism for 6% of the total score (Table 4).

Discussion

The clinical management of embryos diagnosed as mosaic continues to be highly controversial [12]. The appearance of NGS has enabled the identification of embryos in which only a proportion of biopsied cells are aneuploid, creating a new and complex diagnostic category [1]. Reported rates of mosaicism vary widely among fertility centres. In our centre, approximately 15% of embryos are classified as mosaic, which is lower than the figures reported by other groups, where rates of up to 40% have been described [2, 3]. These discrepancies may reflect differences in laboratory protocols, bioinformatic pipelines, or threshold criteria for defining mosaicism, highlighting the need for greater standardization.

The proportion of aneuploid cells within a mosaic embryo varies widely, and the absence of consensus on diagnostic thresholds remains a major obstacle for both clinicians and patients. In our study, we classified embryos with < 25% aneuploid cells as euploid, 25–50% as mosaic, and > 50% as aneuploid. However, other centres adopt markedly different criteria, in some cases labelling embryos with up to 70% aneuploid cells as mosaic [3]. This heterogeneity illustrates the lack of standardization in the field, undermining the comparability of published results and complicating the development of universally applicable clinical strategies.

Initially, mosaic embryos were systematically excluded from transfer due to concerns regarding their developmental competence and potential health risks. In light of scientific evidence demonstrating live births without chromosomal anomalies, the practice of transferring these embryos gained wider acceptance [14, 15]. Nevertheless, published data continued to highlight inferior clinical outcomes, notably an increased incidence of clinical miscarriage when compared with transfers of euploid embryos [27, 34, 35].

None of the live births derived from mosaic embryos included in this study (Table 1) exhibited chromosomal abnormalities, and their perinatal outcomes were comparable to those observed with euploid embryos [13]. In addition, although not a primary objective of this study, clinical outcomes following mosaic embryo transfer were also comparable to those obtained with euploid embryos (Table 2), in contrast to earlier reports [27, 34, 35]. These findings are consistent with more recent evidence suggesting that clinical outcomes do not differ significantly between euploid embryos and those with mosaicism levels below 50% [36, 37]. Nevertheless, given that this analysis was not specifically designed to address this question, further studies incorporating appropriate adjustment for potential confounding variables are warranted to confirm these observations.

To provide a clinically meaningful tool, we developed a predictive model focused on the most relevant outcome, live birth, rather than intermediate endpoints such as implantation or clinical pregnancy. The model was initially built using 26 predictors, including parental, cycle, and embryonic variables, and tested across multiple machine-learning algorithms. Although the Random Forest method achieved the highest predictive performance (AUC = 0.781), variables shared by all embryos within a cohort (e.g., maternal age, oocyte source) were excluded in a second model, as they do not discriminate between embryos within the same transfer decision.

The second model, developed to construct the scoring system, followed a sequential variable inclusion strategy in which predictors were added one at a time and the corresponding AUC was estimated. The model achieving the highest AUC was selected as the final configuration, and the variables included were subsequently used to derive the score (Fig. 2).

The maximum AUC was reached with a model including four predictors. Two were embryo-related variables—embryo quality and day of biopsy (day 5 vs. day 6)—and two described mosaicism characteristics: type of mosaicism (monosomy, trisomy, or both) and the percentage of aneuploid cells (25–40% vs. 40–50%) (Fig. 2). Predictors were initially ranked according to their importance using a Random Forest algorithm; however, higher-ranking variables did not necessarily receive higher weights in the final score, which was derived using multivariate logistic regression.

Parsimony analysis showed that the four-variable model provided the most favourable balance between discriminative performance and model simplicity. The absence of performance improvement after the inclusion of additional predictors suggests informational redundancy, a common feature of clinical datasets with correlated variables. Adopting a parsimonious model enhances interpretability, reduces the risk of overfitting, and facilitates clinical implementation, which is particularly relevant for scoring systems intended to support decision-making in assisted reproduction.

The contribution of each variable to the final score was heterogeneous and predominantly driven by embryo-related factors: embryo quality accounted for 42% of the total score and day of biopsy for 34%, whereas mosaicism-related variables contributed less (type of mosaicism, 18%; level of mosaicism, 6%). Notably, one of the most influential predictors, day of biopsy, was also among the variables showing statistically significant differences in univariate analysis between embryos that resulted in a live birth and those that did not (day 5: 71.9% vs. 28.1%; Table 1). Overall, the scoring system highlights the predominance of embryo-related characteristics over mosaicism-related features in predicting live birth following mosaic embryo transfer (76% vs. 24%).

The lower discriminative performance observed for the *MosaicScore* (AUC $\approx$ 0.61) compared with the initial Random Forest model (AUC $\approx$ 0.78) was anticipated and reflects key methodological and conceptual differences between both approaches. A central distinction lies between global prediction of live birth probability and the development of a tool for intra-cohort embryo prioritization, as well as between a full predictive model and a parsimonious, interpretable scoring system. The reduction in AUC observed in the *MosaicScore* is primarily a consequence of both the exclusion of cohort-level predictors and the use of a parsimonious modeling strategy. While such variables contribute substantially to global prediction, they do not provide discriminatory information for embryo selection within a given cohort and were therefore intentionally excluded. Importantly, the *MosaicScore* is intended as a ranking tool rather than a probability estimator, and its performance should be interpreted within this specific clinical context.

Although calibration is an important component of predictive model evaluation, it was not formally assessed in this study due to the limited sample size and the focus on discrimination for embryo prioritisation rather than absolute risk estimation.

Machine learning algorithms are increasingly used in medicine to support clinical decision-making by providing objective and reproducible predictive frameworks. In reproductive medicine, this is particularly relevant given the high-dimensional nature of data generated in assisted reproductive technology (ART) [23, 38]. Although embryo quality and developmental timing remain the main established predictors of outcome, the added value of AI-based approaches lies in their ability to integrate multiple variables and translate them into a standardized and consistent prioritization framework. In this study, the AI model is not intended to replace conventional embryological assessment, but to support the objective ranking of multiple mosaic embryos within the same cohort, reducing subjectivity in clinical decision-making.

The embryo selection criteria defined by *MosaicScore* for mosaic embryo transfer differ substantially from those previously proposed by Viotti et al. [27] and Grati et al. [26]. First, *MosaicScore* was developed exclusively using embryos with a mosaicism level below 50%. By contrast, the approaches proposed by Viotti et al. and Grati et al. include embryos with higher degrees of mosaicism, in which mosaicism-related variables are considered to be central to embryo prioritisation. In addition, the criteria established by Grati et al. incorporate the specific chromosomes involved, prioritising alterations not associated with viable aneuploidies, spontaneous miscarriage, or uniparental disomy. By contrast, *MosaicScore* proposes a prioritisation strategy comparable to that traditionally applied to euploid embryos, an approach that aligns with the hypothesis put forward by Capalbo et al. [36], who suggest that mosaic embryos have reproductive potential equivalent to that of chromosomally normal embryos and should therefore be prioritised according to standard embryo quality criteria. Nevertheless, we recommend that, in the event of pregnancy, prenatal testing be performed to definitively exclude chromosomal abnormalities.

Integrating our results with current ESHRE, ASRM and PGDIS guidelines, we propose the following practical approach in IVF with PGT-A: transfer euploid embryos first, and when mosaic embryos are considered, apply the *MosaicScore* to prioritize those of highest quality and biopsied on day 5. Mosaicism features should guide transfer only when embryos are otherwise equivalent, with preference given to embryos showing low-level mosaicism and monosomy rather than trisomy. As recommended, prenatal diagnostic testing should follow any pregnancy achieved after mosaic embryo transfer.

An analysis of PGT-A cycles revealed the proportion of cycles in which embryos with mosaicism were diagnosed. Specifically, among the 2583 cycles analysed, 25.39% produced at least one mosaic embryo and of these, 19.35% produced more than one mosaic embryo, a scenario in which prioritisation tools are particularly relevant. Although this represents a minority of all PGT-A cycles, the clinical impact may be significant in complex cases in which euploid embryos are unavailable, as *MosaicScore* may support more informed decision-making and potentially improve live birth outcomes through optimised selection of mosaic embryos.

The main limitations of this study are the relatively small sample size of mosaic embryo transfers and its retrospective design, which may limit the generalisability of the findings. Consequently, prospective multicentre studies with larger and more diverse cohorts are required to externally validate the *MosaicScore* and to robustly assess its predictive performance across different clinical settings. Such studies would also enable further evaluation of the model and, if necessary, its refinement to enhance clinical applicability.

In summary, our findings indicate that, within the limitations of this retrospective analysis, mosaic embryos prioritised according to embryo quality and developmental timing can be associated with live births. The *MosaicScore* is intended as a structured and transparent decision-support tool to assist clinicians and patients in situations where no euploid embryos are available, rather than as a definitive predictor of outcome. Given the lack of consensus regarding diagnostic thresholds and the absence of prospective validation, these results should be interpreted with caution. Overall, mosaic embryos may represent a potential additional option in selected clinical scenarios, and the *MosaicScore* could provide support in the prioritisation process, although further validation is required before routine clinical implementation.

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Data availability Data will be made available on request to the authors.

Declarations

Ethics approval The data included in this study were within the framework of routine clinical activity. All work was conducted with formal approval of the Institutional Review Board, and it follows the principles of the Declaration of Helsinki.

Competing interests The authors declare no competing interests.

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