Reproductive Biology and Endocrinology

RESEARCH

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Factors affecting biochemical pregnancy loss (BPL) in preimplantation genetic testing for aneuploidy (PGT-A) cycles: machine learning-assisted identification

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Abstract

Purpose To determine the factors influencing the likelihood of biochemical pregnancy loss (BPL) after transfer of a euploid embryo from preimplantation genetic testing for aneuploidy (PGT-A) cycles.

Methods The study employed an observational, retrospective cohort design, encompassing 6020 embryos from 2879 PGT-A cycles conducted between February 2013 and September 2021. Trophectoderm biopsies in day 5 (D5) or day 6 (D6) blastocysts were analyzed by next generation sequencing (NGS). Only single embryo transfers (SET) were considered, totaling 1161 transfers. Of these, 49.9% resulted in positive pregnancy tests, with 18.3% experiencing BPL. To establish a predictive model for BPL, both classical statistical methods and five different supervised classification machine learning algorithms were used. A total of forty-seven factors were incorporated as predictor variables in the machine learning models.

Results Throughout the optimization process for each model, various performance metrics were computed. Random Forest model emerged as the best model, boasting the highest area under the ROC curve (AUC) value of 0.913, alongside an accuracy of 0.830, positive predictive value of 0.857, and negative predictive value of 0.807. For the selected model, SHAP (SHapley Additive exPlanations) values were determined for each of the variables to establish which had the best predictive ability. Notably, variables pertaining to embryo biopsy demonstrated the greatest predictive capacity, followed by factors associated with ovarian stimulation (COS), maternal age, and paternal age.

Conclusions The Random Forest model had a higher predictive power for identifying BPL occurrences in PGT-A cycles. Specifically, variables associated with the embryo biopsy procedure (biopsy day, number of biopsied embryos, and number of biopsied cells) and ovarian stimulation (number of oocytes retrieved and duration of stimulation), exhibited the strongest predictive power.

Keywords Biochemical pregnancy loss (BPL), NGS, PGT-A, Machine learning, Artificial intelligence (AI), SHAP value

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Background

BPL is an early termination of the pregnancy development. In IVF cycles, positive pregnancy test can be detected by measuring chorionic gonadotropin (b-hCG) levels in blood or urine between day 9 and 10 from the embryo transfer day. Implantation is confirmed by visualization of an intrauterine embryo sac by transvaginal ultrasound 7 to 15 days after the positive pregnancy test. If the embryo sac is absent, a BPL has occurred [1–5]. The understanding of BPL in IVF cycles still limited, with the existing literature on associated factors often presenting contradictory data. While embryonic aneuploidy has been suggested as a potential factor linked to BPL, there is a lack of evidence demonstrating genetic aberrations in BPL cases [1]. In addition, it has been observed that in PGT-A cycles, where euploid embryos have been transferred, the BPL rate is the same as that observed in conventional IVF cycles [6, 7]. Furthermore, the transfer of mosaic embryos doesn't seem to alter BPL rates compared to transfers involving euploid embryos [8]. Therefore, the role of chromosomal alterations in BPL rates is questionable [9], suggesting the existence of other contributing factors that warrant further investigation in PGT-A cycles.

Very few studies analyzing the factors for BPL predisposition following euploid embryo transfer after PGT-A have been published. Notably, McQueen's study [10], stands out, finding no discernible differences in maternal age, body mass index (BMI), number of oocytes retrieved, or morphokinetic parameters between the groups of embryos resulting in clinical pregnancy and those leading to BPL. Recently, Muñoz et al. [11] observed no difference in BPL rates between embryos from own oocytes and those from donated oocytes in PGT-A cycles.

Among the most studied factors associated with the BPL rate, are all embryo-related factors. Interestingly, the developmental stage at which embryo transfer occurs doesn't seem to correlate with BPL rates [6]. In contrast, the embryo quality seems to be associated with BPL. Studies carried out by Zanetti [12] and Dai et al. [13] have concluded that poor quality embryos have higher rates of BPL.

On the other hand, the technique used for the oocyte fertilization does not seem to be relevant. The same BPL rates have been observed whether fertilization was performed with conventional IVF or intracytoplasmic sperm injection (ICSI) [14].

Maternal age does not seem to have a significant association with BPL, as multiple studies have discarded its involvement [9, 10, 15, 16]. Moreover, ovarian reserve does not change BPL rates [17–20]. The exception would be women with polycystic ovary disease, in whom a higher rate of BPL has been observed (OR = 1.89, 95% CI 1.48–2.41) although pregnancy and live birth rates per cycle were similar to the control group [21].

The endometrium and its thickness have been assessed as potential variables that could alter the BPL risk. Various studies have proposed different thresholds for endometrial thickness, such as 9 mm [9], 10 mm [22] or 11 mm [12], below which the risk of BPL increases dramatically. Other authors attribute BPL not to endometrial thickness but to inadequate endometrial blood flow [15]. Additionally, the endometrial preparation protocol appears to play a crucial role. BPL rates were notably higher in artificial cycles compared to stimulated cycles (53.2 vs. 29%, respectively; $p = 0.0001$) [23]. However, endometrial scratching did not demonstrate any significant alteration in BPL rates (RR 1.21 (95% CI 0.71–2.07)) [24]. Endometritis increases the risk of BPL, even if it has been cured with antibiotic treatment [25].

The choice of progesterone administration during the luteal phase support may significantly impact the risk of BPL. Subjects receiving only vaginal progesterone showed a doubled risk of BPL compared to those administered intramuscular injections (32.3% vs. 15.6%; $p < 0.001$) [26]. Conversely, no significant differences were observed in BPL rates (OR 0.79, 95% CI 0.31–1.76) after luteal support with intramuscular injection of human chorionic gonadotropin. Furthermore, neither BPL nor clinical miscarriage has been related to estradiol or LH levels on the trigger day [9].

Sperm DNA damage emerges as another significant factor elevating the risk of BPL. Borini et al.'s study suggests that DNA fragmentation could compromise pregnancy progression, leading to miscarriage or BPL [27]. A later meta-analysis confirmed this point. The meta-analysis included 11 studies and a total of 1,549 cycles, resulting in a pooled OR of 2.48 (95% CI 1.52, 4.04; $P < 0.0001$) [28]. In a more recent study, it was found that men from couples who had suffered BPL had higher sperm fragmentation rates (comet assay) than fertile couples: 33.3 vs. 14.9 ($p < 0.001$) [29]. Moreover, low sperm quality, as measured by low sperm count and motility, had also been linked to an increased risk of BPL [12].

In a recent study [30], the authors examined the impact of antiphospholipid antibody detection on BPL and found no increased risk.

To summarize, the literature on the factors affecting BPL is rather confusing. Within this context, we have decided to apply the latest analytical methods, such as artificial intelligence (AI) and, more specifically, machine learning, to identify the variables that can modify the risk of BPL. AI is increasingly used in medicine to analyze clinical data and to make predictions. In a short time, it has proven to be an essential tool in many medical specialties [31–33]. Assisted reproduction techniques are no exception to this process and, different machine learning

and deep learning models are also being applied in the field of human reproduction [34–37]. Numerous examples in the literature show predictive models for diverse aspects including COS [38], embryo developmental potential [39], embryo ploidy [40–45], implantation [41, 46–48], first trimester miscarriage [49], clinical pregnancy [50] and live birth [51, 52].

The objective of our study is to establish a BPL prediction model using machine learning algorithms to identify the best predictors in PGT-A cycles.

Methods

Study design

The study design is observational and retrospective. Clinical outcomes were recorded in a database including additional potential factors (n=47) associated with BPL and related to progenitors, embryos and their biopsy, ovarian stimulation, and adjuvant treatments (Supplementary Table 1).

The study included 6020 embryos biopsied in 2879 PGT-A cycles performed in the clinic's different centers from February 2013 to September 2021. The biopsied embryos were vitrified and transferred in a subsequent cycle. Only single embryo transfers were included in the study (n=1161). The variable to be predicted in the machine learning models were the embryos that, after a positive pregnancy test, had an early fetal loss and no embryo sac was visualized. These embryos were compared in the different models with those in ongoing pregnancies.

PGT-A

PGT-A was indicated for several reasons, including advanced maternal age, altered karyotype or sperm FISH results, history of chromosomal abnormalities in previous offspring, recurrent early pregnancy losses, and recurrent implantation failures. Prior to undergoing the PGT-A procedure, all couples received detailed information and provided their informed consent.

Mature oocytes were fertilized in the laboratory by ICSI following IVF laboratory guidelines. Two or three laser shots at a pulse of 0.536 ms were used to breach the zona pelluzida on Day 3. Blastocysts graded ≥3 [53] with herniating cells were biopsied with the same laser pulses on days 5 (D5) or 6 (D6) of embryo development. A Saturn Active laser from Research Instruments (RI, Germany) was used for this purpose. The biopsied cells were taken from the trophectoderm region and tubed for aneuploidy testing. After lysis of the biopsied cells, embryo genome amplification was performed using the Picoplex kit (Rubicon Genomics®, Ann Arbor, MI, USA) following the manufacturer's instructions. Chromosome analysis of the embryos was carried out by NGS using Illumina's commercial kit Veriseq (San Diego, CA, USA). Embryos with a percentage of cells with chromosomal aberrations equal to or lower than 25% were considered euploid, between 25% and 50% were classified as mosaic and, aneuploid if the percentage was higher than 50%.

Descriptive analysis of variables

The descriptive statistical methods employed in this study varied depending on the type of variable being analyzed. For qualitative variables, descriptive statistics included frequency and percentage distributions. In contrast, quantitative variables were subjected to descriptive analysis using the median and interquartile range.

Data preprocessing

The database was anonymized. Missing values (0.1%) were imputed, and outliers were analyzed and eliminated if necessary. Multicollinearity was assessed to avoid redundancy among highly correlated variables.

Before training the models, class balancing was performed. The database was then randomly split into a training set (80%) and a test set (20%).

Hyperparameter optimization of classification models

Both classical statistical methods like binary logistic regression and five machine learning algorithms for classification were employed, encompassing support vector machines, k-nearest neighbors, random forest, multilayer neural networks, and eXtreme Gradient Boosting (XGBoost).

To ensure data independence and proper model evaluation, we used 5-fold cross-validation, fitting the hyperparameters on the training set.

Final predictive model

The best model was selected based on AUC, which assesses the model's ability to discriminate the dependent variable, BPL. Final metrics, including AUC, sensitivity, specificity, predictive values, accuracy, and the kappa statistic, were derived from the test set (20% of the total database). These results are shown in Fig. 1 and summarized in Table 2.

Important variables: SHAP values

The key predictor variables in the final model were identified using SHAP values, which explain the outcomes of various machine learning models. These values are rooted in game theory [54, 55]. Machine learning algorithms calculate a SHAP value for each predictor in every prediction case, quantifying the variables' impact on the final prediction.

Statistical and machine learning analysis has been carried out using SPSS (v23.0) and R (v. 4.2.0) statistical software.

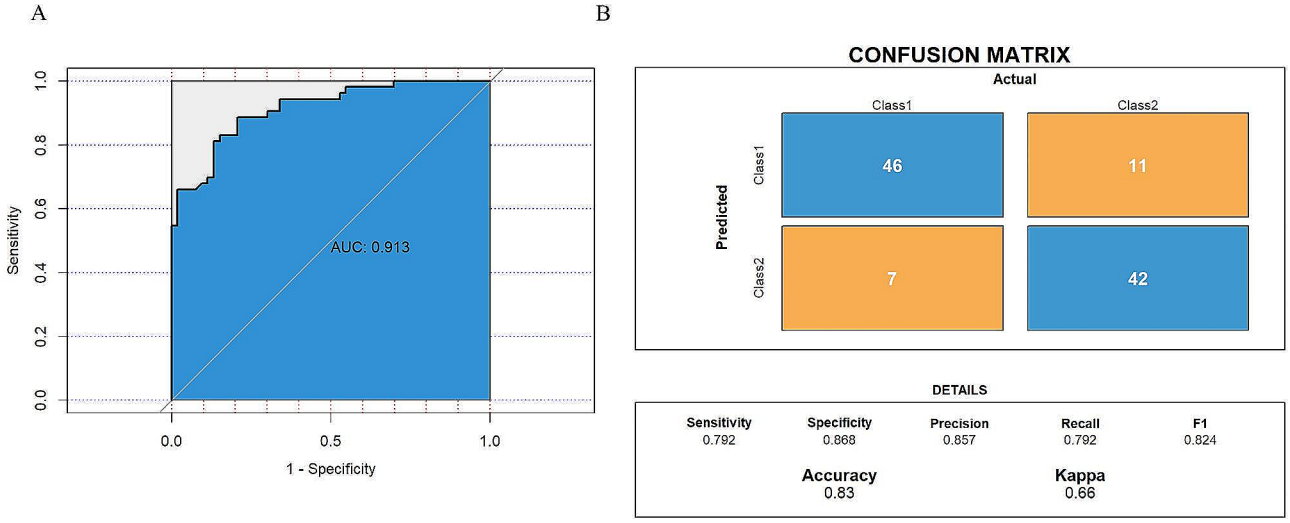

Fig. 1 Performance metrics of Random Forest model. **(A)** ROC curve of Random Forest model. **(B)** Confusion matrix and different performance metrics (sensitivity, specificity, precision, recall, F1, accuracy and kappa) of Random Forest model. All metric parameter values have been obtained from the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

Table 2 Comparison of the different metrics of the final models

model	AUC	Accuracy	Positive predictive value (PPV)	Negative predictive value (PNV)
Binariy Logistic Regression	0.781	0.717	0.735	0.702
Multi-Layer Perceptron	0.500	0.500	-	0.500
Support Vector Machines	0.909	0.821	0.815	0.827
k-Nearest Neighbors	0.786	0.698	0.733	0.672
Random Forest	0.913	0.830	0.857	0.807
eXtreme Gradient Boosting	0.871	0.802	0.833	0.776

Results

A descriptive analysis of the main variables set is shown in Table 2. The medians of maternal and paternal age were: 34 [IQR: 27 to 39] and 39 years [IQR: 35 to 43] respectively. On the other hand, the median of antral follicle count (AFC) was 14 [IQR: 11 to 19]. Notably, 78% of patients exhibited normal sperm count according to WHO's 2010 classification, with only 8% of cycles using sperm donors. Ovarian stimulation yielded a median of 11.0 [IQR: 9.0 to 14.0] oocytes, of which 10.0 [IQR: 8.0 to 12.0] were mature.

Concerning oocyte origin, 39% of cycles involved donated oocytes and 17% utilized vitrified ones. Biopsies predominantly occurred on day 5 (63%), with 60.1% of embryos achieving A quality and 36.4% B quality. Notably, 11.5% of transferred embryos exhibited chromosomal mosaicism. In terms of clinical outcomes, implantation and BPL rates were 40.7% and 18.3%, respectively.

A comprehensive examination of 47 variables spanning maternal, paternal, couple, embryo, and IVF cycle characteristics was conducted to explore factors associated with BPL (Supplementary Table 1).

To identify factors contributing to increased rates of BPL in euploid embryos, we employed a comprehensive approach, using both classical statistical methods and machine learning algorithms (as summarized in Table 1). Each algorithm was optimized to maximize the area under the ROC curve, a widely accepted metric to evaluate a model's predictive performance. Our analysis revealed intriguing results: while the multivariate binary logistic regression model yielded a respectable AUC value of 0.781, the machine learning models outperformed it. Notably, the support vector machines (AUC=0.909), nearest neighbors (AUC=0.786), random forest (AUC=0.913), and XGBoost (AUC=0.871) models displayed superior predictive abilities. Among them, the Random Forest model emerged as the most promising, boasting the highest AUC value (Fig. 1A). This model not only excelled in predictive accuracy but also demonstrated high positive predictive value (0.857), sensitivity (0.792), and specificity (0.868) (Fig. 1B). Furthermore, to provide a comprehensive assessment of model performance, we presented the confusion table for the best final model, indicating an impressive accuracy of 0.830 (Fig. 1B). It is important to note that these performance metrics were derived from the test database, comprising entirely new data that were not used in training the models. This approach ensures the reliability and generalizability of our findings, as the models were evaluated on data they hadn't encountered before, minimizing the risk of overfitting and bias.

The most important prediction variables of the Random Forest model were determined from the SHAP values (Fig. 2). This SHAP value is a measure of the input

Table 1 Descriptive of patients, IVF cycle and PGT-A

Characteristic	Overall, $N = 1,161$[1]
Female age	34 (27, 39)
AFC	14 (11, 19)
Oocyte origin	
Donated	449 (39%)
Own	712 (61%)
Fresh/Vitrified oocyte	
Fresh	969 (83%)
Vitrified	192 (17%)
Number oocyte retrieved	11.0 (9.0, 14.0)
Number MII retrieved	10.0 (8.0, 12.0)
Male age	39 (35, 43)
Sperm count	
Normal	905 (78%)
Oligozoospermia	213 (18%)
Cryptozoospermia	38 (3.3%)
Azoospermia	5 (0.4%)
Teratozoospermia	174 (15%)
Asthenozoospermia	214 (18%)
Semen origin	
Donated	95 (8%)
Own	1066 (92%)
Number embryo biopsied	4.00 (3.00, 5.00)
Number cells biopsied	5.00 (4.00, 6.00)
Number biopsy shots	5.0 (3.0, 9.0)
Biopsy day	
D5	736 (63%)
D6	425 (37%)
Embryo quality[2]	
A	698 (60.1%)
B	423 (36.4%)
C	40 (3.4%)
PGT-A results	
Euploid	1,028 (89%)
Mosaic	133 (11.5%)
Endometrial thickness (mm)	8.60 (7.70, 10.00)
Positive pregnancy test	579 (49.9%)
Biochemical pregnancy loss	106 (18.3%)[3]
Implantation	473 (40.7%)

1 Median (IQR); n (%)

2 Gardner's classification

3 Calculated with respect to positive pregnancy tests

features contribution to the model's final prediction. Figure 2A shows an example of prediction ("Local interpretability"), using the Random Forest model, of an individual embryo where SHAP values have been calculated for each of the features (non-aggregate). As can be seen, the probability of BPL (0.23) is the sum of the SHAP values (some positive and some negative) for each of the predictors.

Figure 2B highlights the ten most significant variables ("Top influencers") in the final Random Forest model, determined by mean SHAP values. Notably, variables associated with embryo biopsy (biopsy day, number of embryos biopsied, laser pulses, and biopsied cells) exhibited the greatest predictive power, followed by variables related to ovarian response and stimulation (number of oocytes retrieved, days of stimulation, total dose, and type of gonadotrophins), as well as maternal and paternal age.

Figure 2C ("Directionality impact") visually represents how identified factors influence BPL using SHAP values. The dot plot illustrates the impact of feature directionality, with the x-axis representing SHAP values and the y-axis featuring features, ordered by their influence on model prediction. Yellow indicates higher feature values, while purple indicates lower values, providing insight into feature impact directionality. For complex feature behavior, Supplementary Fig. 1 offers a two-dimensional representation of these SHAP values ("Feature dependence").

From Fig. 2C and Supplementary Fig. 1, several noteworthy findings emerge:

The variable "biopsy day" exhibited the highest predictive ability, with biopsy at D5 associated with a lower BPL risk compared to D6.

The behavior of the "number of embryos biopsied" variable differed, based on ovarian response, with lower SHAP values for low ovarian responses (<3 oocytes retrieved), and a reversed trend observed for higher responses.

An increase in the "number of laser shots" and "number of biopsied cells" predicted a higher probability of BPL.

Maternal and paternal age positively correlated with BPL risk, with a stronger effect observed for maternal age, though older paternal age (>40 years) was associated with a lower BPL rate.

Variables related to ovarian response and stimulation demonstrated higher BPL risk for low and high ovarian responses, with a decrease in risk observed for normo-responders.

Our analysis revealed significant associations between ovarian stimulation protocols and BPL risk. Slower stimulations correlated with lower SHAP values, suggesting reduced BPL risk. Additionally, longer ovarian stimulation and higher doses of gonadotrophins were linked to decreased BPL risk. Notably, the Random Forest model predicted an increased probability of BPL with the use of recombinant gonadotrophins, either alone or in combination with urinary gonadotrophins (Fig. 2C and Supplementary Fig. 1J and K).

Discussion

Biochemical pregnancy loss (BPL) represents an intriguing yet poorly understood phenomenon in pregnancy development. It occurs when the embryo initially implants but ceases developing before being visualized

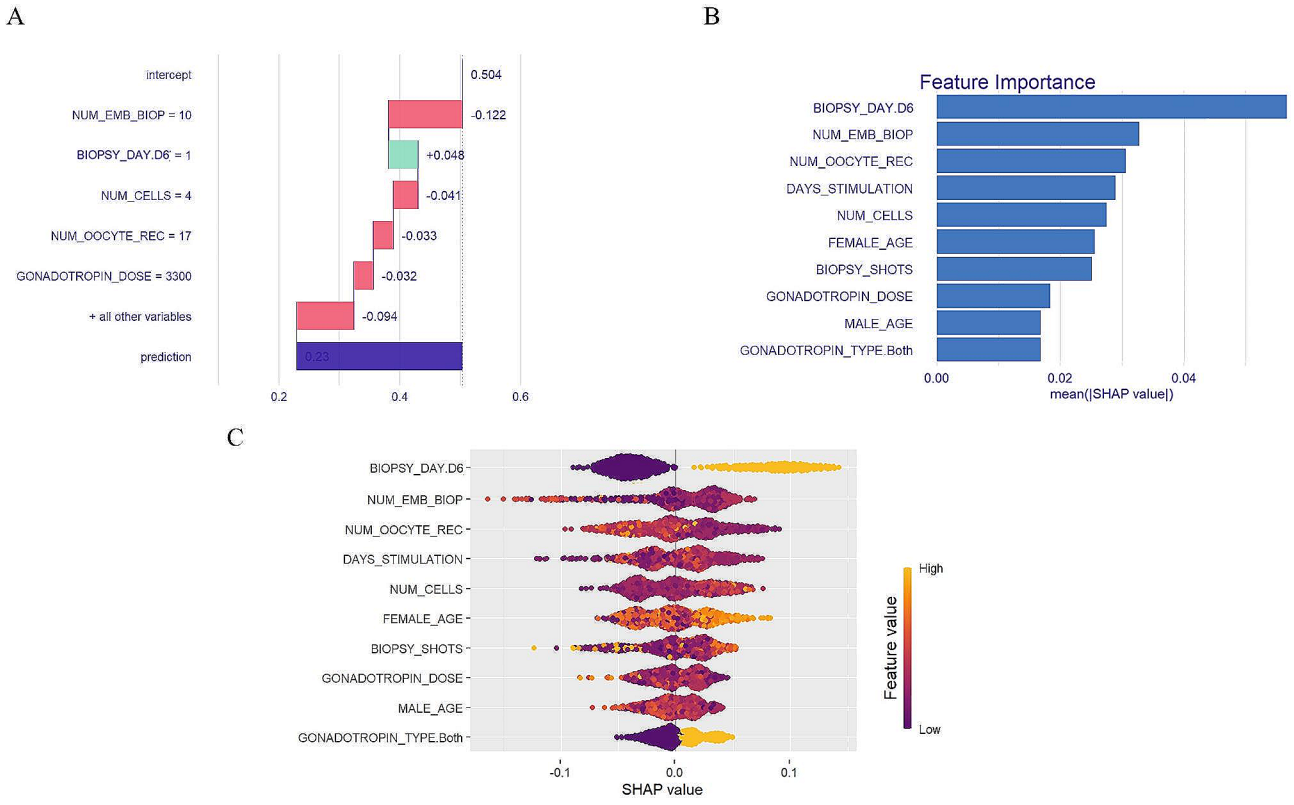

Fig. 2 SHAP plots **(A)** The "Local Interpretability" graph provides insight into an individual embryo's prediction using the Random Forest model. In this graph, SHAP values have been computed for each feature independently (non-aggregate), allowing for a detailed examination of the contribution of each feature to the model's prediction for that specific embryo. **(B)** The "Top Influencers" graph presents the ten most influential predictors in the Random Forest model. These predictors are determined based on the mean of the absolute SHAP values for each feature. **(C)** The "Directionality Impact" graph illustrates the influence of features on the model prediction (Random Forest), with the x-axis representing the SHAP value and the y-axis displaying features ordered by their impact. Each point on the graph corresponds to a SHAP value for a specific prediction and feature. In this graph, yellow indicates the highest values of a feature, while purple represents lower values. The distribution of yellow and purple points provides insight into the directionality impact of the features

via ultrasound. Despite extensive research, the factors contributing to BPL remain elusive, leading to ongoing controversies regarding variable roles [1]. While embryonic chromosomal alterations have been proposed as a potential cause, studies have shown that BPL rates remain unaffected even when transferring euploid embryos in PGT-A cycles. This discrepancy suggests the presence of other contributing factors to this type of miscarriage [6, 7, 9].

Surprisingly, there is a dearth of studies specifically analyzing BPL in PGT-A cycles, and those that do exist predominantly employ classical statistical methods. [10]. Notably, only one published study, conducted by Zanetti et al., employs non-standard statistical methods such as discriminant analysis to analyze factors associated with BPL [12].

Our study seeks to fill this gap by conducting a comprehensive analysis of factors associated with BPL, using innovative AI methods. Unlike previous endeavors primarily focused on predictive modeling, our objective was to identify the most influential variables and their impact on BPL within PGT-A cycles. By leveraging machine learning techniques, we aimed to uncover relationships and associations between variables that transcend the limitations of classical statistics.

Five distinct machine learning algorithms were employed, encompassing a diverse array of methodologies including neural networks, bagging, boosting, and nearest neighbor techniques. These algorithms included multi-layer perceptron, support vector machines, k-nearest neighbors, random forest, and XGBoost, collectively offering a broad spectrum of analytical approaches. Among these algorithms, Random Forest emerged as the most effective, boasting an impressive AUC of 0.913 alongside other high quality performance metrics.

While machine learning models exhibit remarkable accuracy and predictive power, they often function as "black boxes," obscuring the specific roles of input variables in prediction outcomes. In addressing this limitation, SHAP values have emerged as a valuable tool for illuminating the contribution of individual variables to model predictions. This enhanced understanding of

variable importance is particularly critical in medical applications, where identifying key factors influencing biological processes is paramount. [54, 55]. By making machine learning models more interpretable, SHAP values help mitigate skepticism among clinicians towards AI, thereby facilitating their integration into medical decision-making processes [56].

The SHAP value serves as a crucial metric, quantifying the contribution, whether positive or negative, of each variable to the final prediction of the model. Notably, these SHAP values possess an additive property, enabling the decomposition of the machine learning model's prediction into the sum of the individual SHAP values of each variable. In various fields, such as medical disciplines [57–63], and specifically in reproductive medicine [49], SHAP values have been widely used, providing valuable insights into model interpretation and the importance of variables.

Different graphical representations of SHAP values provide essential information to explain machine learning models. Notably, the "Top Influencers" graph highlights the ten most significant predictors for model prediction. These are calculated based on the absolute SHAP values for each feature. In the context of the Random Forest predictive model utilized in our study, the top ten variables predominantly relate to embryo biopsy, ovarian stimulation, and paternal and maternal age, underscoring their importance in predicting outcomes.

This study makes a significant discovery by explaining how embryo biopsy contributes to the occurrence of BPL in PGT-A cycles. Specifically, our analysis revealed that four of the most influential variables are associated with the biopsy process, wherein cells are extracted from the trophectoderm to ascertain the chromosomal status of the embryo. Remarkably, prior research has not explored the impact of embryo biopsy on BPL risk in PGT-A cycles, making our findings particularly novel and insightful. Our predictive Random Forest model identified several key factors associated with embryo biopsy that greatly influence BPL risk. Foremost among these predictors is the biopsy day, indicating that embryos with delayed development requiring biopsy on Day 6 are at heightened risk of BPL, as predicted by our model.

Furthermore, our analysis unveiled that an increase in the number of biopsied cells correlates with a diminished likelihood of successful implantation and an elevated risk of BPL. Similarly, our machine learning model forecasts a heightened BPL risk with an increase in the number of laser shots during the biopsy procedure. Given harmful impact of excessive laser pulses on the embryo, we recommend using low laser intensity and a minimal number of shots during biopsy to protect the embryo's integrity and improve clinical results [64]. Gentle biopsy procedures are crucial to mitigate negative impacts on embryo implantation and BPL risk. Factors such as heat from laser shots, embryo stress, and reduction in trophectoderm cells may compromise processes essential for establishing a healthy pregnancy. Fortunately, advancements in biopsy technology have significantly reduced these impacts [65, 66].

In our Random Forest model, one of the most pivotal variables is the number of embryos biopsied, exhibiting distinct behaviors in low responders compared to other patients. Women with low ovarian response usually undergo biopsy on a limited number of embryos, leading to reduced chances of IVF success. This subgroup displays a wide dispersion in SHAP values, indicating considerable variability in BPL risk. Conversely, among other patients, a higher number of biopsied embryos correlates with lower SHAP values and decreased BPL rates, likely attributable to enhanced embryo selection and reduced BPL risk.

Interestingly, among existing literature lacks studies exploring the association between ovarian stimulation and BPL risk. However, our comprehensive predictive model identified four variables related to ovarian response and stimulation as significant contributors. Firstly, the number of oocytes retrieved emerges as a crucial factor, with embryos from women exhibiting a normo-response demonstrating lower BPL probabilities. Conversely, both high and low ovarian responses are associated with higher SHAP values and elevated BPL risk.

Moreover, longer stimulations needing higher doses of gonadotrophins are linked to reduced BPL probability. Additionally, the type of gonadotropin administered appears to influence BPL risk, with recombinant gonadotrophins posing an increased risk compared to urinary-derived counterparts. Notably, stimulations utilizing urinary gonadotrophins yield embryos with a lower BPL probability, underscoring the significance of gonadotropin type in predicting BPL outcomes.

While existing literature widely agrees on the negligible impact of maternal age on BPL risk [9, 10, 15, 16], this study's findings diverge significantly. Despite the small sample sizes in previous studies, our model identifies maternal age as a significant predictor, elevating the likelihood of BPL. Similarly, paternal age exhibits a comparable trend, albeit with a milder effect. Notably, our analysis reveals a noteworthy reversal in the risk pattern with advanced paternal age, particularly in older age groups. This intriguing phenomenon suggests that the increasing risk typically associated with older paternal age may be mitigated in cases where patients undergo oocyte donation cycles.

Interestingly, the Random Forest model does not assign significant importance to variables associated with the male factor, except for paternal age as previously

mentioned. Despite extensive research linking male factors to BPL risk, particularly sperm DNA damage [27–29] our model indicates low predictive capacity for this variable, rendering it excluded from the top 10 most influential variables. Likewise, sperm quality fails to emerge as a significant contributor to BPL risk in our predictive model, contrary to assertions made by some researchers [12].

Interestingly enough, our top-performing machine learning model does not identify endometrial thickness or the endometrial preparation protocol as significant predictors, contradicting findings from previous studies. Endometrial thickness has been extensively studied, with lower values often associated with increased BPL risk For instance, Dickey et al. highlighted the role of endometrial thickness, demonstrating that thickness less than 9 mm on the hCG administration day correlated with a higher BPL incidence, while thickness equal to or greater than 9 mm was associated with lower BPL rates [9]. Subsequent research established varying cutoff points, such as 10 mm [22] or 11 mm [12], showcasing a substantial reduction in BPL rates with thicker endometrium. A limitation of our study concerning the endometrium is that we did not include various methods of luteal phase support among the predictors, which could potentially influence BPL rates.

Furthermore, adjunctive treatments like scratching [24] and others such as filgrastrim, intralipids, heparin, aspirin, or hCG, as published, do not emerge as significant variables in predicting BPL risk.

Conclusions

This study explores biochemical pregnancy loss (BPL) in IVF cycles, especially those involving PGT-A, an area historically underexplored in IVF research despite its clinical significance. The study has been limited to biochemical pregnancy loss (BPL) and has not been extended to other clinical parameters of PGT-A cycles. Using advanced machine learning techniques, we identified key factors influencing BPL rates in PGT-A cycles, notably variables related to embryo biopsy and ovarian stimulation such as biopsy timing, number of embryos and cells biopsied, oocyte count, and stimulation duration. Understanding these factors is crucial for developing targeted interventions to reduce BPL rates and improve overall IVF success.

While our findings are promising, further validation through prospective studies is necessary to enhance reliability and applicability, leading to more effective clinical interventions and personalized treatment strategies in assisted reproductive technology.

This research aims to advance our understanding of BPL mechanisms and risk factors, ultimately improving IVF outcomes for individuals and couples seeking fertility treatment.

Abbreviations

PGT-A	Preimplantation Genetic Testing for Aneuploidy
BPL	Biochemical Pregnancy Loss
AI	Artificial intelligence
D5	Day 5
D6	Day 6
NGS	Next Generation Sequencing
SHAP	SHapley Additive exPlanations
COS	Controlled Ovarian Stimulation
IVF	In Vitro Fertilization
ICSI	IntraCytoplasmic Sperm Injection
SET	Single Embryo Transfer
BMI	Body Mass Index
b-hCG	Chorionic Gonadotropin
AFC	Antral Follicle Count
AUC	Area Under the ROC curve
XGBoost	eXtreme Gradient Boosting

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-024-01271-1.

Supplementary Fig. 1 Feature dependence plot. The two-dimensional representation of SHAP values depicts the interaction between features for the ten most crucial predictors identified by the Random Forest model. This figure serves as a supplement to Fig. 2C, providing additional insight into the SHAP value patterns of variables with more intricate relationships.

Supplementary Table 1

Acknowledgements

We would like to thank Arancha Estañ and Anna Pitas PhD for their valuable assistance in the English manuscript preparation and review.

Author contributions

A.M., A.C. and A.R-A.: collection of data and critical review of article. BL and RM: study conception, design, interpretation of the data and critical review of article. AB and RB: recruitment of patients and critical review of article. JCC: recruitment of patients and critical review and writing of article. J.A.O.: study conception, design, interpretation, data analysis and writing article.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability

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

Declarations

Ethical approval

The data included in this study were within the framework of routine clinical activity. All the study was carried out with the formal approval of the Institutional Review Board of our clinic and following the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 30 May 2024 / Accepted: 29 July 2024
Published online: 08 August 2024

References

1. Carp HJA, Biochemical Pregnancies. How Should They Be Interpreted? Gynecological and Reproductive Endocrinology & Metabolism [Internet]. [cited 2022 Oct 3]; https://gremjournal.com/journal/01-2020/biochemical-pregnancies-how-should-they-be-interpreted/
2. Annan JJK, Gudi A, Bhide P, Shah A, Homburg R. Biochemical pregnancy during assisted conception: a little bit pregnant. J Clin Med Res. 2013;5:269–74.
3. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, Goddijn M, et al. Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. Hum Reprod. 2015;30:495–8.
4. Alves C, Jenkins SM, Rapp A. Early Pregnancy Loss (Spontaneous Abortion). StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Jun 30]. http://www.ncbi.nlm.nih.gov/books/NBK560521/
5. Chaudhry K, Tafti D, Siccardi MA. Anembryonic Pregnancy. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [cited 2022 Sep 29]. http://www.ncbi.nlm.nih.gov/books/NBK499938/
6. Vaiarelli A, Cimadomo D, Patrizio P, Venturella R, Orlando G, Soscia D, et al. Biochemical pregnancy loss after frozen embryo transfer seems independent of embryo developmental stage and chromosomal status. Reprod Biomed Online. 2018;37:349–57.
7. Sato T, Sugiura-Ogasawara M, Ozawa F, Yamamoto T, Kato T, Kurahashi H, et al. Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. Hum Reprod. 2019;34:2340–8.
8. Zhang L, Wei D, Zhu Y, Gao Y, Yan J, Chen Z-J. Rates of live birth after mosaic embryo transfer compared with euploid embryo transfer. J Assist Reprod Genet. 2019;36:165–72.
9. Dickey RP, Olar TT, Taylor SN, Curole DN, Harrigill K. Relationship of biochemical pregnancy to pre-ovulatory endometrial thickness and pattern in patients undergoing ovulation induction. Hum Reprod. 1993;8:327–30.
10. McQueen DB, Mazur J, Kimelman D, Confino R, Robins JC, Bernardi LA, et al. Can embryo morphokinetic parameters predict euploid pregnancy loss? Fertil Steril. 2021;115:382–8.
11. Munoz E, Taboas E, Alvarez M, Gil E, Perez A, Portela S et al. Is biochemical pregnancy loss associated with embryo or endometrium? A retrospective cohort study in frozen single embryo transfer of own and donated oocytes. Hum Reprod. 2024;deae106.
12. Zanetti BF, Braga DP, de Setti AF, Iaconelli AS, Borges A. Predictive factors for biochemical pregnancy in intracytoplasmic sperm injection cycles. Reprod Biol. 2019;19:55–60.
13. Dai X, Gao T, Xia X, Cao F, Yu C, Li T, et al. Analysis of biochemical and clinical pregnancy loss between frozen-thawed embryo transfer of blastocysts and day 3 cleavage embryos in Young women: a Comprehensive comparison. Front Endocrinol (Lausanne). 2021;12:785658.
14. Buckett WM, Chian R-C, Dean NL, Sylvestre C, Holzer HEG, Tan SL. Pregnancy loss in pregnancies conceived after in vitro oocyte maturation, conventional in vitro fertilization, and intracytoplasmic sperm injection. Fertil Steril. 2008;90:546–50.
15. Zhang X, Guo F, Wang Q, Bai W, Zhao A. Low-dose aspirin improves blood perfusion of endometrium of unexplained recurrent biochemical pregnancy loss. Int J Gynaecol Obstet. 2022;157:418–23.
16. Dahan MH, Zeadna A, Dahan D, Son W-Y, Steiner N. The biochemical pregnancy loss rate remains stable up irrespective of age and differs in pattern from clinical miscarriages. Gynecol Endocrinol. 2021;37:61–4.
17. Richardson A, Mascarenhas M, Balen A. Is a woman's chronological age or ovarian age more important in determining perinatal outcome after assisted reproductive treatment? Hum Fertil (Camb). 2021;24:144–50.
18. Hu S, Xu B, Jin L. Perinatal outcome in young patients with diminished ovarian reserve undergoing assisted reproductive technology. Fertil Steril. 2020;114:118–e1241.
19. Tiegs AW, Sun L, Scott RT, Goodman LR. Comparison of pregnancy outcomes following intrauterine insemination in young women with decreased versus normal ovarian reserve. Fertil Steril. 2020;113:788–e7964.
20. Bishop LA, Richter KS, Patounakis G, Andriani L, Moon K, Devine K. Diminished ovarian reserve as measured by means of baseline follicle-stimulating hormone and antral follicle count is not associated with pregnancy loss in younger in vitro fertilization patients. Fertil Steril. 2017;108:980–7.
21. Tang K, Wu L, Luo Y, Gong B. In vitro fertilization outcomes in women with polycystic ovary syndrome: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2021;259:146–52.
22. Gallos ID, Khairy M, Chu J, Rajkhowa M, Tobias A, Campbell A, et al. Optimal endometrial thickness to maximize live births and minimize pregnancy losses: analysis of 25,767 fresh embryo transfers. Reprod Biomed Online. 2018;37:542–8.
23. Hatoum I, Bellon L, Swierkowski N, Ouazana M, Bouba S, Fathallah K, et al. Disparities in reproductive outcomes according to the endometrial preparation protocol in frozen embryo transfer: the risk of early pregnancy loss in frozen embryo transfer cycles. J Assist Reprod Genet. 2018;35:425–9.
24. van Hoogenhuijze NE, Mol F, Laven JSE, Groenewoud ER, Traas MaF, Janssen C et al. a. H,. Endometrial scratching in women with one failed IVF/ICSI cycle-outcomes of a randomised controlled trial (SCRaTCH). Hum Reprod. 2021;36:87–98.
25. Zhang Q, Yang G, Tan J, Xiong Y, Xu Y, Xu Y, et al. Antibiotic cured chronic endometritis remains a risk factor for early pregnancy loss in the subsequent frozen euploid embryo transfer. Reprod Biomed Online. 2024;48:103611.
26. Devine K, Richter KS, Jahandideh S, Widra EA, McKeeby JL. Intramuscular progesterone optimizes live birth from programmed frozen embryo transfer: a randomized clinical trial. Fertil Steril. 2021;116:633–43.
27. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod. 2006;21:2876–81.
28. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod. 2008;23:2663–8.
29. Haddock L, Gordon S, Lewis SEM, Larsen P, Shehata A, Shehata H. Sperm DNA fragmentation is a novel biomarker for early pregnancy loss. Reprod Biomed Online. 2021;42:175–84.
30. Mu F, Wang M, Zeng X, Liu L, Wang F. Preconception non-criteria antiphospholipid antibodies and risk of subsequent early pregnancy loss: a retrospective study. Reprod Sci. 2024;31:746–53.
31. Jiang F, Jiang Y, Zhi H, Dong Y, Li H, Ma S et al. Artificial intelligence in healthcare: past, present and future. Stroke Vasc Neurol [Internet]. 2017 [cited 2022 Oct 4];2. https://svn.bmj.com/content/2/4/230
32. Bajwa J, Munir U, Nori A, Williams B. Artificial intelligence in healthcare: transforming the practice of medicine. Future Healthc J. 2021;8:e188–94.
33. Rajkomar A, Dean J, Kohane I. Machine learning in Medicine. N Engl J Med. 2019;380:1347–58.
34. Raef B, Ferdousi R. A review of machine learning approaches in assisted Reproductive technologies. Acta Inf Med. 2019;27:205–11.
35. Swain J, VerMilyea MT, Meseguer M, Ezcurra D, Fertility AI. Forum Group. AI in the treatment of fertility: key considerations. J Assist Reprod Genet. 2020;37:2817–24.
36. Wang R, Pan W, Jin L, Li Y, Geng Y, Gao C, et al. Artificial intelligence in reproductive medicine. Reproduction. 2019;158:R139–54.
37. Dimitriadis I, Zaninovic N, Badiola AC, Bormann CL. Artificial intelligence in the embryology laboratory: a review. Reprod Biomed Online. 2022;44:435–48.
38. Correa N, Cerquides J, Arcos JL, Vassena R. Supporting first FSH dosage for ovarian stimulation with machine learning. Reprod Biomed Online. 2022;S1472–6483(22):00420–5.
39. Erlich I, Ben-Meir A, Har-Vardi I, Grifo J, Wang F, Mccaffrey C, et al. Pseudo contrastive labeling for predicting IVF embryo developmental potential. Sci Rep. 2022;12:2488.
40. Barnes J, Brendel M, Gao VR, Rajendran S, Kim J, Li Q, et al. A non-invasive artificial intelligence approach for the prediction of human blastocyst ploidy: a retrospective model development and validation study. Lancet Digit Health. 2023;5:e28–40.
41. Chavez-Badiola A, Flores-Saiffe-Farías A, Mendizabal-Ruiz G, Drakeley AJ, Cohen J. Embryo Ranking Intelligent classification algorithm (ERICA): artificial intelligence clinical assistant predicting embryo ploidy and implantation. Reprod Biomed Online. 2020;41:585–93.
42. Chen L, Li W, Liu Y, Peng Z, Cai L, Zhang N, et al. Non-invasive embryo selection strategy for clinical IVF to avoid wastage of potentially competent embryos. Reprod Biomed Online. 2022;45:26–34.
43. De Gheselle S, Jacques C, Chambost J, Blank C, Declerck K, De Croo I, et al. Machine learning for prediction of euploidy in human embryos: in search of the best-performing model and predictive features. Fertil Steril. 2022;117:738–46.
44. Diakiw SM, Hall JMM, VerMilyea MD, Amin J, Aizpurua J, Giardini L, et al. Development of an artificial intelligence model for predicting the likelihood of human embryo euploidy based on blastocyst images from multiple imaging systems during IVF. Hum Reprod. 2022;37:1746–59.

45. Ortiz JA, Morales R, Lledó B, Vicente JA, González J, García-Hernández EM, et al. Application of machine learning to predict aneuploidy and mosaicism in embryos from in vitro fertilization cycles. AJOG Global Rep. 2022;2:100103.
46. Cheredath A, Uppangala S, Jijo CSA, R A, Kumar VL. P, Combining machine learning with Metabolomic and embryologic data improves embryo implantation prediction. Reprod Sci. 2022.
47. Raef B, Maleki M, Ferdousi R. Computational prediction of implantation outcome after embryo transfer. Health Inf J. 2020;26:1810–26.
48. Bori L, Paya E, Alegre L, Viloria TA, Remohi JA, Naranjo V, et al. Novel and conventional embryo parameters as input data for artificial neural networks: an artificial intelligence model applied for prediction of the implantation potential. Fertil Steril. 2020;114:1232–41.
49. Amitai T, Kan-Tor Y, Or Y, Shoham Z, Shofaro Y, Richter D et al. Embryo classification beyond pregnancy: early prediction of first trimester miscarriage using machine learning. J Assist Reprod Genet. 2022.
50. Yland JJ, Wang T, Zad Z, Willis SK, Wang TR, Wesselink AK, et al. Predictive models of pregnancy based on data from a preconception cohort study. Hum Reprod. 2022;37:565–76.
51. Bardet L, Excoffier J-B, Salaun-Penquer N, Ortala M, Pasquier M, Mathieu d'Argent E, et al. Comparison of predictive models for cumulative live birth rate after treatment with ART. Reprod Biomed Online. 2022;45:246–55.
52. Bori L, Dominguez F, Fernandez EI, Del Gallego R, Alegre L, Hickman C, et al. An artificial intelligence model based on the proteomic profile of euploid embryos and blastocyst morphology: a preliminary study. Reprod Biomed Online. 2021;42:340–50.
53. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000;73:1155–8.
54. Arrow KJ, Barankin EW, Blackwell D, Bott R, Dalkey N, Dresher M et al. Contributions to the Theory of Games (AM-28), Volume II [Internet]. Princeton University Press; 1953 [cited 2022 Oct 4]. https://www.jstor.org/stable/j.ctt1b9x1zv
55. Štrumbelj E, Kononenko I. Explaining prediction models and individual predictions with feature contributions. Knowl Inf Syst. 2014;41:647–65.
56. Afnan MAM, Liu Y, Conitzer V, Rudin C, Mishra A, Savulescu J, et al. Interpretable, not black-box, artificial intelligence should be used for embryo selection. Hum Reprod Open. 2021;2021:hoab040.
57. Bloch L, Friedrich CM. Data analysis with Shapley values for automatic subject selection in Alzheimer's disease data sets using interpretable machine learning. Alzheimers Res Ther. 2021;13:155.
58. Lundberg SM, Nair B, Vavilala MS, Horibe M, Eisses MJ, Adams T, et al. Explainable machine-learning predictions for the prevention of hypoxaemia during surgery. Nat Biomed Eng. 2018;2:749–60.
59. Wojtuch A, Jankowski R, Podlewska S. How can SHAP values help to shape metabolic stability of chemical compounds? J Cheminform. 2021;13:74.
60. Wang K, Tian J, Zheng C, Yang H, Ren J, Liu Y, et al. Interpretable prediction of 3-year all-cause mortality in patients with heart failure caused by coronary heart disease based on machine learning and SHAP. Comput Biol Med. 2021;137:104813.
61. Li Y, Brendel M, Wu N, Ge W, Zhang H, Rietschel P, et al. Machine learning models for identifying predictors of clinical outcomes with first-line immune checkpoint inhibitor therapy in advanced non-small cell lung cancer. Sci Rep. 2022;12:17670.
62. Zihni E, Madai VI, Livne M, Galinovic I, Khalil AA, Fiebach JB, et al. Opening the black box of artificial intelligence for clinical decision support: a study predicting stroke outcome. PLoS ONE. 2020;15:e0231166.
63. Rodríguez-Pérez R, Bajorath J. Interpretation of machine learning models using shapley values: application to compound potency and multi-target activity predictions. J Comput Aided Mol Des. 2020;34:1013–26.
64. Aoyama N, Kato K. Trophectoderm biopsy for preimplantation genetic test and technical tips: a review. Reprod Med Biol. 2020;19:222–31.
65. Guzman L, Nuñez D, López R, Inoue N, Portella J, Vizcarra F, et al. The number of biopsied trophectoderm cells may affect pregnancy outcomes. J Assist Reprod Genet. 2019;36:145–51.
66. Xiong S, Liu W, Wang J, Liu J, Gao Y, Wu L, et al. Trophectoderm biopsy protocols may impact the rate of mosaic blastocysts in cycles with pre-implantation genetic testing for aneuploidy. J Assist Reprod Genet. 2021;38:1153–62.

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