

Prediction of Good Quality Blastocyst Formation by Metabolomic Profile of Spent Embryo Culture Media using FTIR Spectroscopy in Women undergoing IVF Cycle : A Cohort Prospective Study

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Abstract

Background: In this study, we evaluated the performance of a multivariate statistical model to predict good quality blastocyst formation by processing chemometric data from FTIR spectral of spent culture media at day 1 cultured.

Methods: This study aimed to determine if metabolomic profile of spent embryo culture media using Fourier-transform infrared spectroscopy (FTIR) could predict good quality blastocyst formation using cohort prospective design. A total of 44 spent culture media from 8 patients were individually collected. Forty-four samples derived from day 1 cultured. All sample was known either form a good blastocyst or no on day 5 cultured. Samples were evaluated using FTIR spectroscopy. The spectra were analyzed using chemometric and multivariate statistical model to make group classification. K fold cross-validation was used, to avoid random correlation. AUC, sensitivity and accuracy of predicting good quality blastocyst were calculated.

Results: FTIR spectroscopy predicted blastocyst formation with Area under the ROC curve (AUC) 0,752 sensitivity 73 % and accuracy 72% from day-1 spent culture media.

Conclusions: Metabolomic profiling of spent embryo culture media using FTIR spectroscopy combined with bioinformatics has the potential to predict blastocyst formation.

Plain English Summary

In controlled ovarian hyper-stimulation on In Vitro Fertilization (IVF), there are many mature oocytes will be obtained, of which produce many embryos, increase the occurrence of multiple pregnancy, and increase the risk to both the mother and fetus. This research is expected to produce a predictive model of the metabolomic profilling of embryo culture medium to predict the blastocyst formation which is a non-invasive and fast way to improve the process of selecting embryos with high implantation potential, without extending embryo culture. A total of 44 spent culture media from 8 patients were individually collected from day 1 cultured. All sample was known either form a good blastocyst or none on day 5 cultured. Samples were evaluated using Fourier-transform infrared spectroscopy (FTIR) spectroscopy. The spectra were analyzed using chemometric and multivariate statistical model to make group classification. K fold cross-validation was used, to avoid random correlation. AUC, sensitivity and accuracy of predicting good quality blastocyst were calculated. This study obtained that FTIR spectroscopy predicted blastocyst formation with Area under the ROC curve (AUC) 0,752 sensitivity 73 % and accuracy 72% from day-1 spent culture media. A rapid analysis of metabolomic patterns of embryo culture medium using FTIR showed an association with blastocyst formation, of which forms a blastocyst associated with the environment in a different way from an embryo that fails to become a good quality blastocyst. Accordingly, metabolomic profiling of spent embryo culture media using FTIR spectroscopy combined with bioinformatics has the potential to predict blastocyst formation.

Background

In vitro fertilization (IVF) has experienced much progress since 1978, and success rates have continued to increase over the past four decades. According to the annual data published by the European Society of Human Reproduction and Embryology in Europe and the Centers for Disease Control and Prevention (CDC) in the USA, the live birth rate for IVF programs ranges from 28–29% between 2011 and 2013. It is estimated that more than 400,000 babies are born from 1.6 million FIV cycles worldwide each year.¹

In controlled ovarian hyperstimulation, many mature oocytes will be obtained, which then produce many embryos. Multiple embryo transfers can increase pregnancy success and multiple pregnancy occurrence. Data from the USA published by the CDC in 2011 found that nearly 46% of FIV infants were twins.² Twin pregnancies increase the risk of both the mother and fetus. These risks include preterm labor, low birth weight, stunted fetal growth, preeclampsia, gestational diabetes, hypertension in pregnancy, placental abruption, and others. There are medical complications in twin pregnancy for mothers and infants, prompting several countries to make rules on the number of embryos allowed to be transferred.

As a consequence of limiting the number of embryos to be transferred, the ability to select embryos with the highest potential to produce healthy births is crucial in the FIV process. Now, several embryo selection methods have been developed. Embryo selection can be done invasively or noninvasively. Invasive methods are used to detect genetic abnormalities. The preimplantation genetic screening procedure requires high and expensive skills. There is a possibility of injury to the embryo; hence, the use of noninvasive methods is preferred.³ Noninvasive methods include selection based on morphology, morphokinetic changes during the initial development phase with time-lapse photography, oxygen consumption, and various biochemical markers in the culture medium or a combination of several methods described above.

Studies have shown that morphological predictions of embryonic quality cannot accurately show the internal physiological processes that occur in embryos. Around 30–50% of aneuploid embryos have a good morphological appearance, whereas euploid embryos can have a poor morphological appearance.⁴ A study by Seli et al.⁵ found embryos with the same morphological score as having different metabolomic patterns. This shows that other factors affect embryo quality. The metabolic activity of the embryo has been shown to be related to embryo quality, implantation potential, and pregnancy.⁶ The metabolomic analysis of the embryo is done by detecting specific substances that have been consumed around the culture medium or detection of metabolites secreted from the embryo into the culture medium.^{7,8}

Metabolic changes for embryo selection have been extensively studied. Gardner's study using an ultramicrofluorescence assay found that embryos with high pyruvate uptake can form blastocysts.⁹ Hardy et al. and Gott et al. obtained the same result of high pyruvate uptake in embryos reaching blastocysts.¹⁰ However, Conaghan et al. reported a different finding with a decrease in pyruvate uptake in embryos from two to eight cells that had high viability rates (measurement based on clinical pregnancy).¹¹ In a study

measuring amino acids, Brison et al. in 2004 using high-performance liquid chromatography found an association between decreased glycine and leucine and an increase in asparagine in embryo culture medium with an increase in clinical pregnancy and live birth rates.¹² Seli et al.¹³ using proton nuclear magnetic resonance reported an association ($P= 0.002$) between high glutamate levels in embryo culture medium and clinical pregnancy and live birth rates. The above studies use a known substrate target approach.

In addition to the known substrate target measurement approach, metabolomic assessment can also be done by analyzing metabolomic patterns using spectrophotometric devices. The results of examinations using this spectrophotometry can be obtained quickly. Seli et al. in 2007 reported the results of the analysis of the third-day culture medium, which was known to have a pregnancy success outcome. Using a multivariate analysis approach, the pattern of the culture medium spectrum is compared between embryos that produce live births and embryos that fail to implant. In this study, Seli et al. found a significant difference in terms of the average viability score between the two groups (0.67 ± 0.27 vs. 0.29 ± 0.22 ; $p < 0.05$).⁶ Then, the regression algorithm of this study was tested in another study by Scott et al., and they found that the regression algorithm in previous studies could be used to predict pregnancy outcomes in embryos transferred on days 3 or 5.¹⁴ The meta-analysis study by Vergouw et al. in 2014 showed no evidence that selection of embryos by near-infrared spectroscopy (NIR) in addition to morphological assessment can improve embryo selection.¹⁵

The use of metabolomics as an embryo selection method has been widely studied. Several studies have shown an association between embryonic metabolomic profiling and embryonic quality. Other studies that combine morphological and metabolomic examinations have not been shown to be related to the embryo quality. In this study, we conducted further research to assess the relationship of metabolomic profiling of embryo culture medium with the formation of blastocysts. From this research, it is expected to produce an algorithm from the metabolomic profiling of embryo culture medium to predict the blastocyst formation. This metabolomic profiling examination is noninvasive, easy, and fast. If we can predict the potential success of blastocyst formation in a noninvasive and fast way on the second day, then we hope to improve the process of selecting embryos with high implantation potential without extending embryo culture up to the fifth day.

Methods

This research aimed to produce a predictive model of the metabolomic profiling of embryo culture medium to predict the blastocyst formation. This cohort prospective study was used embryo spent culture media as the samples from patients undergoing IVF–intracytoplasmic sperm injection (ICSI) at the Yasmin Clinic, Kencana RSCM, between October and November 2019. All patients declared their willingness to participate in this research by signing an informed consent sheet. The study was conducted under an institutional review board-approved protocol.

The inclusion criteria of this study is the cultured embryo medium of patients undergoing IVF-ICSI cycles because of all infertility etiology. The exclusion criteria of this study is the uncultured embryo medium until day five.

The patients underwent various standard stimulation and monitoring protocol. Oocyte retrieval was performed 35–36 h after oocytes triggering with human chorionic gonadotropin or gonadotropin-releasing hormone agonists. After the oocyte retrieval procedure, the oocytes were cultured in the medium for 4–5 h until they were denuded. A hyaluronidase (Vitrolife) solution of 40 IU/mL was added to accelerate the oocyte denudation process, and then, the oocytes were fertilized by ICSI. The oocytes were individually cultured in 45 µL drops of sequential culture media (Cleav sequential medium; Origio). All individually cultured embryos were incubated in cleav sequential medium until the second day of culture. On days 2 to 4 and days 4 and 5, the embryo was transferred to the sequential blast medium. The morphology of each embryo is recorded and assessed on day 5 after fertilization. In the blastocyst stage, the morphological assessment of the embryo uses Gardner scores based on the degree of expansion, the inner cell mass (ICM), and the Trophectoderm (TE). A good quality blastocyst needs a score of 4 for expansion and A or B for ICM and TE. The spent embryo culture medium was taken on days 2 and stored at –80 °C until analysis.

After analysis, the samples were thawed at room temperature (25°C) for 30 min. The spectrometer used was Fourier-transform infrared (FTIR) Bruker Tensor 37, which measured the transmission of the reflectant. The collected spectrum comes from waves between 4000 and 400 cm⁻¹, but the resolution used was 4 cm⁻¹. Each sample is represented by a spectrum of thousands of points, and the spectrum represents the transmission of its reflection. We also conducted the analysis of embryo culture medium using liquid chromatography–tandem mass spectrometry (LCMS/MS).

Spectrum data were analyzed by principle component analysis (PCA). The new variables generated are in the form of main components. Then, the number of main components that will be used for further analysis was determined by looking at the total proportion that can be explained by more than 90%.¹⁶ Choosing a variable that can distinguish between the two groups was done using ReliefF, information gain, and gain ratio. ReliefF is a method for selecting variables based on their proximity to other samples in the group and the distance from different classes. It can process data better with less bias and allows interaction between variables. The selection of variables with information gain is based on the coefficient values calculated from each variable, and the variables with the highest coefficient are used. Meanwhile, gain ratio is different from the information gain as it maximizes the variable of information gain but minimizes its value.¹⁷

In this study, four classification methods are used, namely, Decision Tree (Tree), Support Vector Machine (SVM), Logistic Regression, and Random Forest. The Tree is an algorithm where variables are used to create a classification tree. SVM is a classification algorithm that creates a separator field. A good separation was obtained from the fields that had the farthest distance to the training data closest to the two classes. Random Forest makes the classification process by using more than one decision tree.

Logistic regression measures the relationship between the dependent and independent variables.¹⁸ Before the classification process, 20 times cross-validation was performed. The main output is the area under the curve (AUC), sensitivity, and accuracy of the prediction model.

Results

From October to November 2019, 44 embryos were included in the study, coming from eight couples who underwent IVF procedures at the Yasmin Clinic, RSCM Kencana, Jakarta.

Table 1
Distribution of Embryos According to
Morphological Assessments

Variable	(n = 44)	%
Not Blastocyst	13	29,5
<i>Early Blast</i>		
1 AA	3	6,8
1 AB	2	4,5
1 BB	3	6,8
1 CC	1	2,3
<i>Blastocyst</i>		
2 AA	2	4,5
2 CC	1	2,3
3 AA	1	2,3
3 AB	1	2,3
3 BB	1	2,3
<i>Exp Blastocyst</i>		
4 AA	14	31,8
4 AB	2	4,5
Total	44	100

Of the 44 embryos that were the subjects of the study, 29.5% did not become blastocysts. Table 1 shows the distribution of embryos according to their morphology. From 70.5% (31), embryos that became blastocysts found 16 embryos with good quality (4 AA and 4 AB).

Embryos in this study were from eight patients. The mean age of patients was 30.6 ± 4.7 years, with the youngest age at 25 years and oldest at 39 years. The average length of infertility is 4.9 ± 5.9 years, with a minimum of 1 year and a maximum of 19 years. Meanwhile, the most common causes of infertility are

sperm factor ($n=4$), followed by polycystic ovarian syndrome ($n=2$), endometriosis ($n=1$), and unexplained infertility ($n=1$).

Examination by FTIR spectroscopy produces a spectrum of reflectant transmissions derived from molecular vibrations. Figure 1A illustrates the curve between the reflectance values and the wave numbers obtained from 44 samples.

From 44 samples whose spectra are depicted by the colored stripes above, it is found that variations are indeed expected from the embryo metabolism. By calculating the average value of each wave number of groups that are good quality blastocysts and not good quality blastocysts, Fig. 1B is obtained.

Figures 1A and 1B show 44 FTIR spectrum generated from the sample. All spectra show bands almost similar to differences in intensity that are difficult to distinguish visually. There is an absorption peak in several wave numbers, and this is related to various types of functional groups derived from organic compounds in embryo culture medium. Wave number $400\text{--}900\text{ cm}^{-1}$ is the fingerprint region of each compound (not a functional group region)¹⁹; because the culture medium contains many compounds, it is difficult to determine where the compounds are from. At wave number $3000\text{--}3500\text{ cm}^{-1}$, there is a widening of the peak intensity. This area is related to the functional group strain O-H.^{19,20} There is a picture of the absorption peak at wave numbers $1600\text{--}1820\text{ cm}^{-1}$ in both groups. This area is related to the C = O strain of the carboxylate. Wave numbers $3182\text{--}3330\text{ cm}^{-1}$ are uptake of carboxylic acids. To connect the small differences in each sample and obtain more appropriate information, processing data was performed.

The distribution patterns of the sample groups were evaluated using PCA by using FTIR spectrum reflectance data in the wave number range 399 to 4000 cm^{-1} . In this analysis, 12 main components were obtained, and it can provide 98% information of the total variance. The plot obtained from principal component shows the pattern found in the sample. The similarity of the sample is marked by the closeness of one sample to another. Figure 2 is a plot score showing the PC1 plot as the x-axis and PC3 as the y-axis. This plot score illustrates that an embryo has become good quality blastocysts and no, cannot be distinguished, although it is starting to be seen in quadrant 2 where there is no good quality blastocyst.

In the chemometric analysis with this PCA, there was still an overlap between the two groups (Fig. 2). This shows that some typical metabolomic patterns are common in both groups, such as the use of pyruvate by embryos. For this reason, the selection of variables has a role in distinguishing if the blastocysts have a good quality or not between groups. With the ReliefF method, the information gain and gain ratio obtained the following wave numbers: 778, 813–831, 1189, 1899–1901, 2659–2665, 3307, and $3367\text{--}3398\text{ cm}^{-1}$. Wave numbers $700\text{--}1000\text{ cm}^{-1}$ are the vibration of C = C–H bond and Ar–H bending.¹⁹ Meanwhile, wave number 1189 cm^{-1} is related to deoxyribose,²¹ whereas wave numbers $1899\text{--}1901\text{ cm}^{-1}$ are showing strain at C = O (aldehydes, ketones, amides, esters, and anhydrides).¹⁹ Wave numbers $2659\text{--}2665\text{ cm}^{-1}$ represent the strain on the N–H bond associated with amide. Wave

number 3307 cm^{-1} corresponds to amide that is the formation of amino acids, and wave numbers $3367\text{--}3398\text{ cm}^{-1}$ represent the vibrations of O–H, N–H, and C–H.²¹

Taking into account each wave number that has the role of distinguishing between the two groups, classification analysis used several methods, namely, Tree, SVM, Random Forest, and Logistic Regression. Table 2 shows the best precision and AUC, which is using a prediction model with logistic regression.

Table 2
Evaluation Results of Several Prediction Models in Sample Day – 1

Prediction Models	AUC	Accuracy	Precision	Sensitivity
Tree	0,661	0,705	0,696	0,705
SVM	0,737	0,727	0,722	0,727
Random Forest	0,693	0,682	0,670	0,682
Logistic Regression	0,752	0,727	0,755	0,727

AUC, area under the curve; Tree, Decision Tree; SVM, Support Vector Machine.

From the logistic regression prediction model, a receiver operating characteristic (ROC) curve can be obtained as shown in Fig. 3.

Based on the ROC curve, the prediction model has an AUC value of 0.752. This AUC value shows that this prediction model can be used to predict which embryos will become blastocysts or not. Table 3 shows the predictions of the 44 samples of culture medium using the prediction model above.

Table 3
Prediction of good quality blastocyst formation based on FTIR spectrum analysis of day-1 embryo culture medium

Blastocyst	Prediction		Total
	Y	N	
Actual Y	8	8	16
Actual N	1	27	28
Total	9	35	44

Based on Table 3, a positive predictive value (PPV) of 88.9% and negative predictive value (NPV) of 77.1% can be obtained.

Table 4
Results of Examination of Culture Medium
Compounds using LC-MS / MS

Compound name	Apex m/z
N-Acetyl -DL-tryptophan	247,107
Valpromide	144,1381
3-tert-Butyladipic acid	201,1124
Indole-3-acrylic acid	188,0702
Cyclucron	199,1802
DL-Tryptophan	205,0969
Azelaic acid	187,0966S
Valine	118,0862
L-Norleucine	132,1017
Triethyl phosphate	183,0775
N-Acetyl-DL-tryptophan	245,0926
Sebacate (decanedionate)	201,1124
Phenylalanine	166,0859
Beta-hydroxyisovalorey- L carnitine	262,1644
5-Oxoproline	130,0498
N-acetylarginine	218,113
Piperidin	86,09667
5-aminovalerate	118,0862
Proline	116,0706
Sphinganine	302,3049
Inosin	269,0888
Isoleucin	132,1017
N-acetylkynurenine	251, 1022

Based on the LCMS results in Table 4, we can get a picture of the FTIR spectrum of each compound from the spectrum database and then connect it with the wave numbers that play a role in differentiating groups that succeed in becoming good quality blastocysts and not. Then, we found on the first day that

the compound that played a role was N-acetyl-Tryptopan. From the FTIR results obtained, the wave numbers that play a role are 3367–3398 cm⁻¹, which describe the vibrations of the O–H, N–H, and C–H functional groups. From FTIR, only functional groups are obtained. LCMS/MS compounds above N-acetyl-Tryptopan has an FTIR image with a transmission on these waves.

Discussions

The metabolomic pattern of the embryo is obtained from the spectrum analysis resulting from infrared reflection due to vibrations of molecular function groups on embryo culture medium. This culture medium contains the secretion of the embryo. In this study, metabolomic patterns were analyzed using FTIR spectrometers with multivariate analysis. Analysis using a spectrometer makes it possible to get a fingerprint picture pattern of metabolites that can distinguish and classify samples based on their metabolomic patterns without identifying specific metabolites.

From the results of this study, it was found that the spectrum bands were almost the same in both groups. At wave numbers 3000–3500 cm⁻¹, there is a widening of the peak intensity, and this area is related to the vibration of the O–H functional group. There was an absorption peak at wave numbers 1600–1820 cm⁻¹ in both groups, and this area is related to the C = O strain of the carboxylate. Wave numbers 1062–1085 cm⁻¹ are an area for polysaccharides, whereas wave numbers 3182–3330 cm⁻¹ are for the absorption of carboxylic acids. This is consistent with previous research that states that at the early development, the carboxylic acid metabolism (Krebs cycle) that dominates as the main energy is pyruvate and lactate.²²

In the analysis of embryo culture medium spectrum day 1, several wave numbers were obtained that served to distinguish groups that became good quality blastocysts and not, including wave numbers 778, 813–831, 1189, 1899–1901, 2659–2665, 3307, and 3367–3398 cm⁻¹. Wave numbers 700–1000 cm⁻¹ are a vibration of the C = CH (ethylene) bond associated with ethylene di-amine tetra-acetic acid (EDTA) contained in the culture medium. EDTA is useful as a chelating agent against toxic divalent cations, reducing the formation of reactive oxygen species, as well as a free radical scavenger.²³ Wave number 1189 cm⁻¹ corresponds to deoxyribose.²¹ Ribose is produced from the pentose phosphate pathway for nucleic acid synthesis.²⁴ Wave numbers 1899–1901 cm⁻¹ show the strain at C = O (carbonyl).¹⁹ The composition of culture medium contains many carbonyl groups, and there were sodium bicarbonate, calcium lactate, nonessential amino acids, L. alanil-L-glutamine, calcium pantothenic acid, folic acid, trisodium citrate dehydrate, EDTA, and albumin. Wave numbers 2659–2665 cm⁻¹ represent the strain on the N–H bond associated with amide.²¹ Wave number 3307 cm⁻¹ is also related to amides that form amino acids, and wave numbers 3367–3398 cm⁻¹ represent vibrations from OH, NH, and CH.²¹ These bonds also relate to primary amides that form amino acids.¹⁹ Wave numbers 3367–3398 cm⁻¹ are associated with the compound N-Acetyl-tryptophan, which is an N-acetyl amino acid from tryptophan.²⁵ Previous studies have shown that there is an increase in pyruvate use, lactate production,¹⁰ and glucose use¹³ and a decrease in amino acid exchange and uptake glutamine, arginine, methionine, alanine, and

glycine in culture media.¹² Pyruvate and lactate contain carboxylic acid groups. Amino acids generally consist of the structure of one C atom that binds four groups: an amine group (NH₂), a carboxyl group (COOH), a hydrogen atom (H), and a residual group (R, from a residue) or also called a functional group that distinguishes one amino acid from another amino acid.²⁶ No studies have used FTIR spectrum analyzers on embryo culture medium on the first day.

To differentiate between the two groups, classification analysis was performed using several methods, namely, Tree, SVM, Random Forest, and Logistic Regression. The ability of the prediction model is evaluated using the ROC curve. In a study that used embryo culture medium on the first day, ROC images were obtained from each prediction model. Prediction models using logistic regression produce the highest AUC of 0.752, with a classification accuracy of 0.72 and a sensitivity of 0.727. The 44 samples of this study were then made predictions using prediction models and obtained 88.9% PPV and 77.1% NPV. The study by Sellli et al. in 2007 used analysis with Raman spectrum and NIR spectra on the sample culture medium of the third day and obtained 85.7% sensitivity and 76.5% specificity for Raman spectroscopy and 75% sensitivity and 83.3% specificity for NIR spectroscopy.⁶ A study by Vergouw et al. in 2008, using NIR spectrum analysis, obtained a 53% accuracy of the metabolomic pattern of the third day embryo culture medium, with 36% PPV and 83% NPV.²⁷

Conclusions

From the results of this study, we can find that a rapid analysis of metabolomic patterns of embryo culture medium using FTIR is associated with blastocyst formation. The embryo that forms a blastocyst affects the environment differently from an embryo that fails to become a good quality blastocyst. Although the ability of this prediction model is not very satisfying, it can already distinguish the two groups. This requires a larger sample of research, but this preliminary study shows that FTIR spectroscopy can help predict the formation of blastocysts in an IVF program. Thus, further research is needed on the use of predictive models on samples with different types of culture media and on the determination of whether the direct examination on site is clinically useful.

Abbreviations

FTIR : Fourier-transform infrared spectroscopy

AUC : Area under the ROC curve

ESHRE : the European Society of Human Reproduction and Embryology

CDC : the Centers for Disease Control and Prevention

IVF : In Vitro Fertilization

PGS : Preimplantation Genetic Screening

HPLC : High Performance Liquid Chromatography

NIR : Near-infrared spectroscopy

ICSI : intracytoplasmic sperm injection

HCG : Human Chorionic Gonadotropin

GnRHa : Gonadotropin Releasing Hormone Antagonist

ICM : the Inner Cell Mass

TE : the Trophectoderm

LC-MS/MS : Liquid Chromatography-Tandem Mass Spectrometry

PCA : Principle Component Analysis

SVM : Support Vector Machine

PC : Principal Component

EDTA : Ethylene di-amine tetra-acetic acid

ROC : Receiver Operating Characteristics

Declarations

Ethics Approval and Consent to Participate

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental human patients has been conducted with the ethical approval (Ethics Number : KET-1073/UN2.F1/ETHIC/PPM.00.02/2019) of all relevant bodies and that such approvals are acknowledged within the manuscript. Informed consent was applied to each participant before starting this study.

Consent for Publication

Not Applicable

Availability of data and material

Data and material is available under the provision of corresponding author.

Competing Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Author's contribution

Conceived and designed the experiments: BW and ZZ. Performed the experiments: ZZ. Analyzed the data: ZZ, AT, and ISW . Contributed reagents/materials/analysis tools: ZZ, AT, and ISW. Contributed to the writing of the manuscript : BW, ZZ, ISW, and SCZ. Revising the manuscript: ZZ and SCZ. Supervision and analyzed the manuscript: BW, GP, AH, MN, KS, and AKH. All authors read and approved the manuscript.

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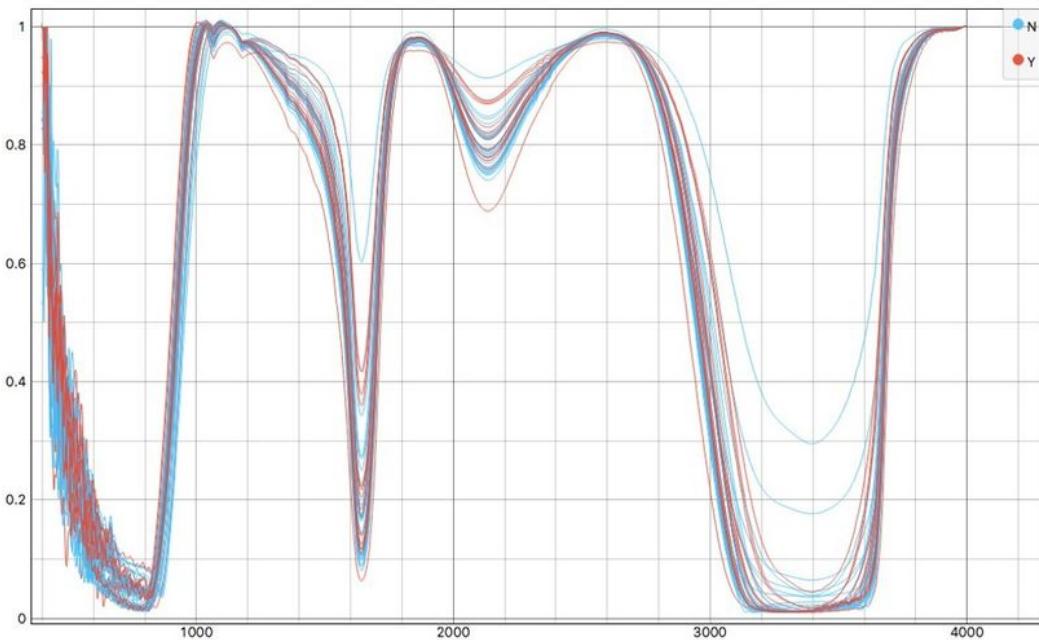
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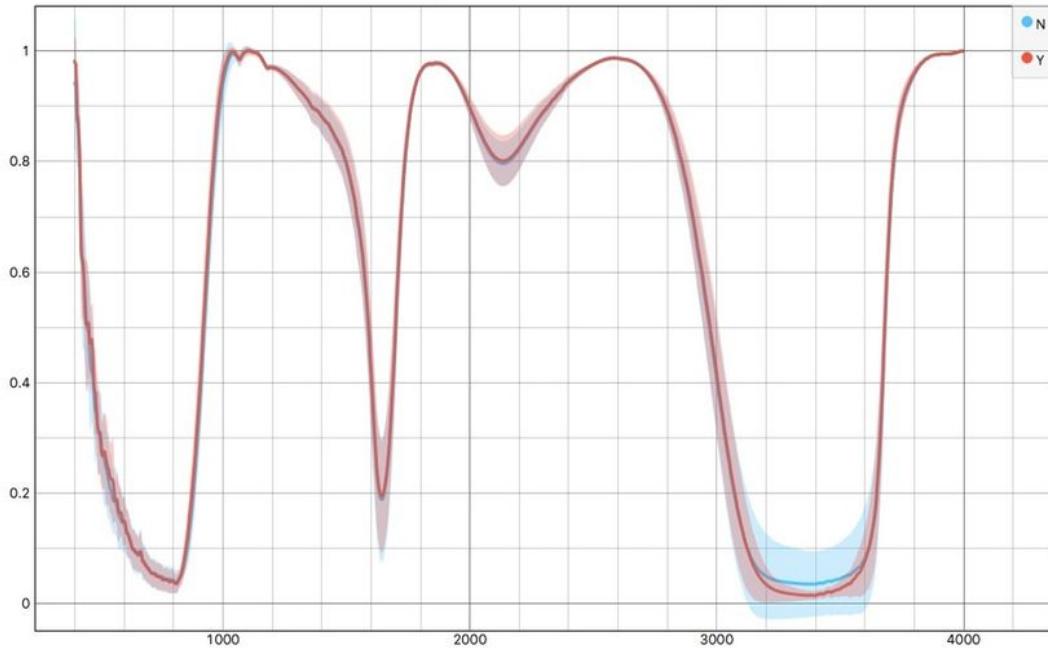
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Figures



A



B

Figure 1

1A. Fourier-transform infrared spectrum analysis of 44 embryo culture on medium day 1. 1B. Fourier-transform infrared spectrum analysis (Fig. 1A) calculated the mean value of the groups that became good quality blastocysts (red) and not (blue)

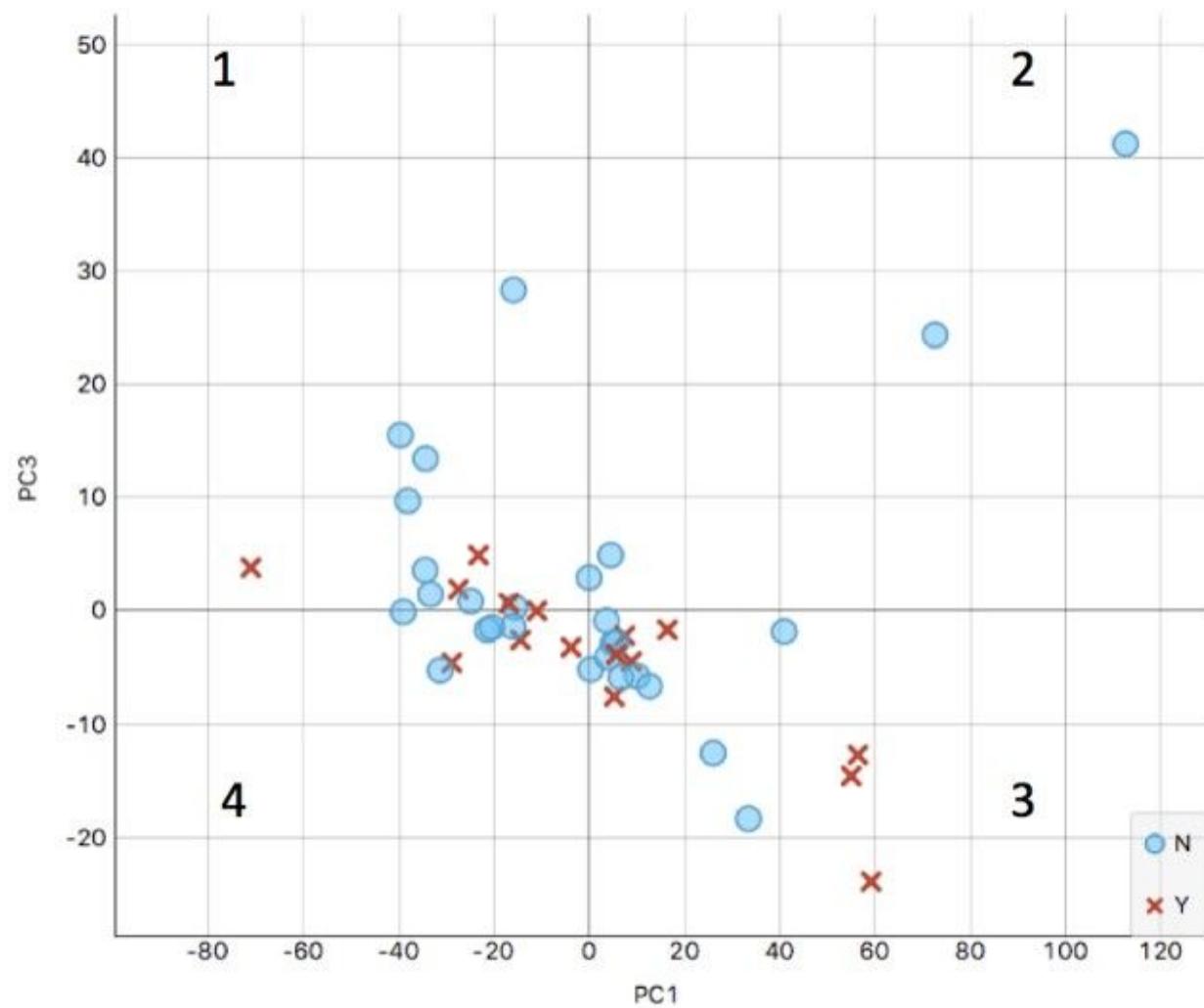


Figure 2

Plot score analysis of the main components of embryos that formed good quality blastocysts (Y) and do not formed good quality blastocysts (N)

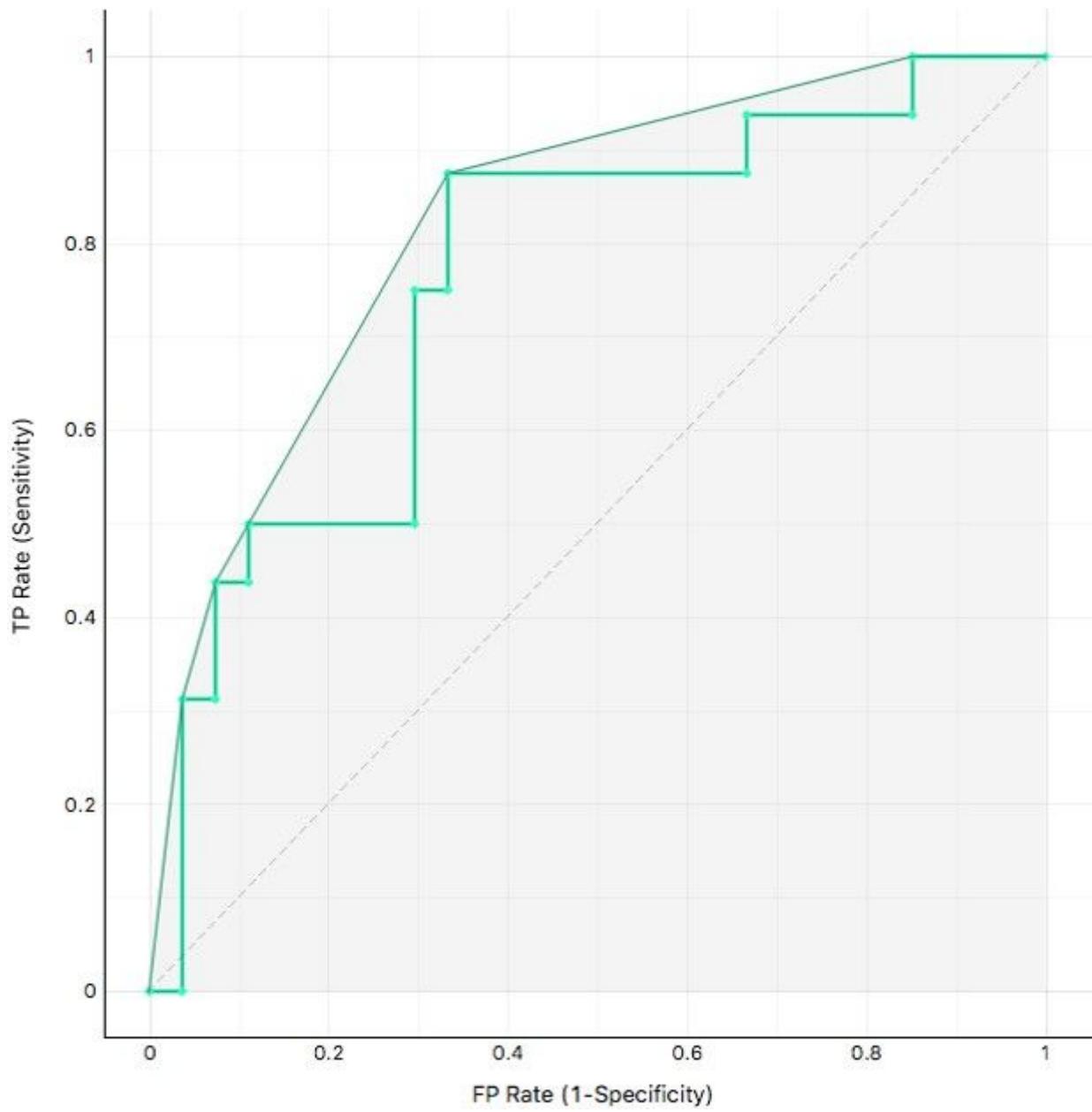


Figure 3

Receiver operating characteristic curve prediction model for logistic regression in sample day 1

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