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EMBRYO BIOLOGY

Non-invasive metabolomic profiling of embryo culture media and morphology grading to predict implantation outcome in frozen-thawed embryo transfer cycles

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Abstract

Purpose Assessment of embryo viability is a crucial component of in vitro fertilization and currently relies largely on embryo morphology and cleavage rate. Because morphological assessment remains highly subjective, it can be unreliable in predicting embryo viability. This study investigated the metabolomic profiling of embryo culture media using near-infrared (NIR) spectroscopy for predicting the implantation potential of human embryos in frozen-thawed embryo transfer (FET) cycles.

Methods Spent embryo culture media was collected on day 4 after thawed embryo transfer (n=621) and analysed using NIR spectroscopy. Viability scores were calculated using a predictive multivariate algorithm of fresh embryos with known pregnancy outcomes.

Results The mean viability indices of embryos resulting in clinical pregnancy following FET were significantly higher than those of non-implanted embryos and differed between the 0, 50, and 100 % implantation groups. Notably, the 0 % group index was significantly lower than the 100 % implantation group index (–0.787 ±0.382 vs. 1.064±0.331, P<0.01). To predict implantation outcomes, we examined the area under the ROC curve (AUC_{ROC}), which was significantly higher for the viability than for the morphology score (0.94 vs. 0.55; P<0.01); however, the AUC_{ROC}s for the composite and viability scores did not differ significantly (0.92 vs. 0.94; P>0.05).

Conclusions NIR metabolomic profiling of thawed embryo culture media is independent of morphology and correlates with embryo implantation potential in FET cycles. The viability score alone or in conjunction with morphologic grading is a more objective marker for implantation outcome in FET cycles than morphology alone.

Capsule In this work, we present a new approach to investigate the value of the combination of morphology and viability scores in predicting implantation outcome in FET cycles.

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Keywords Near infrared spectroscopy/NIR · Non-invasive embryo selection · Viability score · Morphologic grading · Frozen-thawed embryo transfer/FET

List of abbreviations

AUC_{ROC}	The area under the ROC curve
FET	Frozen–thawed embryo transfer
IVF	In vitro fertilization
NIR	Near-infrared spectroscopy

SET Single embryo transfer
ARTs Assisted reproductive treatments

Introduction

Embryo cryopreservation has become common in clinical IVF programs. It helps decrease the number of embryos transferred, which decreases the risk of multiple gestations and optimizes the clinical use of supernumerary embryos. The trend of transferring fewer embryos has resulted in more embryos being available for freezing. Although fresh embryo transfer is currently the most commonly utilized IVF method, many studies suggest that pregnancies arising from the transfer of frozen-thawed embryos have better obstetric and perinatal outcomes [1–3]. Thus, frozen-thawed embryo transfer (FET) is being used more in clinical IVF programs.

The success rate and multiple pregnancy rate are two crucial issues in assisted reproductive technology practice [4]. Typically, multiple embryos are transferred to maximize the chances of pregnancy for every IVF cycle. However, this produces a high risk of twin or multi-pregnancy. Therefore, to improve the success rate of individual embryo implantation, it is crucial to select an embryo with optimum reproductive viability [5–7]. Currently, routine embryo selection is primarily based on morphological criteria examined by light microscopy. Many studies have shown that an embryo's morphological appearance and developmental potential are biomarkers of its viability. For example, pronuclear morphology [8–10], the lack of fragmentation [11–14], the lack of multi-nucleated blastomeres [15], and equal-sized blastomeres [16–19] have been shown to be good indicators of embryo viability. Although morphological assessment is very helpful, it is highly subjective and can be unreliable for viability prediction [20–22]. Therefore, objective markers are greatly needed to assist in determining the implantation potential of human embryos. Numerous spectrometric and chromatographic techniques have been suggested as candidates for the measurement of objective biomarkers and represent automated, high-throughput methodologies that provide information-rich profiles of target biological fluids. For example, in recent publications, an optical spectroscopy technique, near-infrared (NIR) spectroscopy was described for the investigation of biological fluids and has been shown to hold promise for future application in clinical settings because of its simple platform and affordability [22].

Using NIR spectroscopy technology, distinct differences have been found in the composition of the culture media of embryos that resulted in ongoing pregnancies compared with that of embryos that resulted in negative pregnancy outcomes for both fresh [22–29] and frozen-thawed [30, 31] embryo transfer cycles. These differences were observed regardless of the day of transfer and were independent of embryo morphology. In addition, the combination of morphology and viability scores has been shown to be more precise in predicting implantation outcome than has the morphology score alone [28, 32]; furthermore, in fresh embryo transfer cycles, the viability score has been shown to be a better classifier of pregnancy results than was blastocyst morphology [27]. However, reported data on the prediction of implantation outcome in FET cycles are relatively rare [30, 31]. In this work, we present a new approach to investigate the value of the combination of morphology and viability scores in predicting implantation outcome in FET cycles.

Materials and methods

Patient population

From November 2011 to November 2013, spent embryo culture media were collected from 621 frozen-thawed embryos transferred to 323 patients, and pregnancy outcomes were retrospectively analysed. The study was approved by the ethics committee at the Obstetrics and Gynecology Hospital, Fudan University (Shanghai, China).

Ovarian stimulation

In the fresh embryo transfer cycles, ovarian stimulation was performed using long or short protocols with GnRH agonists (Ferring Pharmaceuticals, Saint Prex, Switzerland) and recombinant FSH (Gonal F, Merck-Serono, Geneva, Switzerland). HCG (Profasi, Merck-Serono) was injected when there was at least one follicle of 18 mm in diameter and three or more follicles of 16 mm in diameter. Ultrasonography-directed oocyte retrieval was performed 36 h later. Oocytes were fertilized using either conventional IVF or ICSI and incubated in fertilization media (Vitrolife, Göteborg, Sweden). Normal fertilization was assessed and confirmed by the presence of two pronuclei and a second polar body at 16–18 h after insemination. The embryos were washed and cultured in cleavage medium (Vitrolife) for 48 h before being transferred or frozen. Routine examination of embryo quality included determination of the number of blastomeres, the degree of fragmentation, and blastomere uniformity. Day 3 embryo morphology was scored as follows [12, 27]: grade 1, ≥7 cells, no fragments, and equal blastomeres; grade 2, ≥7 cells, <20 % fragmentation, and equal or unequal blastomeres; grade 3, ≤6 cells, equal or unequal blastomeres, and 20–50 % fragmentation; grade 4, equal or unequal blastomeres, and >50 % fragmentation. Embryos with at least six blastomeres and fragmentation <50 % were frozen on day 3.

Vitrification and warming protocols

The Cryotop method for vitrification was used as described by Kuwayama [33]. Day 3 embryos were equilibrated in equilibration solution [ES; 7.5 % ethylene glycol (EG) and 7.5 % dimethylsulfoxide (DMSO) in HEPES-based medium plus 20 % synthetic serum substitute (SSS)] for 5 min at room temperature. The embryos were then placed in vitrification solution (VS; 15 % EG, 15 % DMSO, and 0.5 M sucrose). After 1 min in VS, the embryos were placed on a Cryotop strip and immediately plunged into liquid nitrogen. For warming, the Cryotop strip was removed from the liquid nitrogen and plunged directly into thawing solution (1.0 M sucrose in HEPES-based medium plus 20 % SSS) for 1 min at 37 °C. Then, the Cryotop strip was sequentially incubated in solutions containing 0.5 M sucrose, 0.25 M sucrose, and sucrose-free HEPES for 3 min each. Finally, the thawed embryos were placed in G2 medium (Vitrolife) containing 20 % SSS and cultured in an incubator at 37 °C with 6 % CO_2 until transfer. Embryos were assessed after thawing by routine morphological criteria and subsequently cultured for 18–20 h in individual 30 μL media drops. Prior to embryo transfer, day 4 embryo morphology was scored as follows [34]: grade A or score 4, more than 75 % of blastomeres undergoing compaction and appearing sphere-shaped with a smooth profile; these embryos had, on average, 8 cells on day 3, with even-sized blastomeres or only slight blastomere size difference and less than 25 % fragmentation. Grade B or score 3, 50–75 % of the blastomeres undergoing compaction or a morula that had a higher percentage of compacted blastomeres but with a slightly irregular-shaped profile. Grade C or score 2, blastomeres with a moderate size difference on day 3, or fragmentation between 25 and 50 %. Grade D or score 1, less than 50 % of the blastomeres undergoing compaction or a higher percentage of blastomeres compacted but severely irregularly shaped. On day 3, these embryos might have had low cell number, or severely uneven-sized blastomeres and multinuclei might be observed; fragmentation was usually over 50 %. Only embryos in the compaction stage or embryos with a blastocoelic cavity were selected for transfer. Assisted hatching was applied to all embryos.

Transfer of frozen/thawed embryos at day 4

Natural cycles were performed only in women who had spontaneous ovulation. In women with amenorrhea or irregular menstrual cycles, hormone replacement treatment (HRT) was performed. On day 4 of the replacement cycle, 2 mg per day oestradiol valerate (E_2, Progynova, Schering AG, Berlin, Germany) treatment was initiated, and the dose was increased by 4 mg everyday. When a thickness of the triple endometrial layers of at least 8 mm was observed, 90 mg per day vaginal progesterone (Crinone, Merck-Serono, Switzerland) was administered for 3 days, and then the embryos were thawed and transferred. For natural cycles, ovarian scanning was performed on cycle days 10–11. If there was a leading follicle of 14–15 mm in diameter, plasma E_2, progesterone, and LH levels were measured everyday. Embryo transfer was performed on day 4 after an LH surge. A maximum of two embryos were transferred. All pregnant women continued to take E_2 and progesterone daily until 10 weeks of gestation. The primary end-point of the study was live birth rate. The secondary endpoint was defined as a viable intrauterine pregnancy at 8–10 weeks after embryo transfer. The implantation rate was defined as the percentage of embryos that successfully underwent implantation compared to the number of embryos transferred in a given period.

Analysis of day 4 thawed embryo media samples by NIR spectroscopy

On day 4, thawed embryos were assessed by routine morphological criteria, and the best morphology embryos were selected for transfer. The spent culture media were then tested directly by NIR spectroscopy after removal of the selected embryos. The culture media was analysed directly and did not require freezing in liquid nitrogen and storage at −80 °C. NIR spectroscopy analysis was performed as previously described [28, 35]. Briefly, sample cells were filled with a 10–12 μL aliquot of the media droplet and placed in a holder maintained at 24.3 °C. After at least 4 min in the holder, the sample cell was transferred to the NIR spectrometer (ANTARISII; Thermo Fisher Scientific Inc., New York, NY, USA). NIR spectra data were obtained from each sample. All samples were analysed three times to rule out any measurement failures. The measurement was repeated with the control media to account for any variations between culture conditions and embryos. Control media drops was of the same batch as that used for embryo culture and was kept in an incubator in the same dishes as those containing embryo culture drops under the same conditions.

The validity of the use of an algorithm to predict the viability of frozen-thawed embryos via measurement of metabolites generated by using fresh embryos has been supported by several previous studies incorporating NIR spectroscopy [30] or high-performance liquid chromatography [31]. Our multivariate algorithm for generating viability scores has been developed previously using the NIR spectra of freshly transferred embryos with known foetal cardiac outcome activity. This algorithm was developed using proprietary methodology of Molecular Biometrics and used with modifications as described elsewhere [28, 36–38]. Briefly, to objectively select wavelength regions, a preprocessing method (stability competitive adaptive reweighted sampling, SCARS) based on Haar wavelets was used. The combination of wavelength regions, which most parsimoniously estimated implantation

outcomes, was determined by using partial least square-discriminant analysis (PLS-DA) and genetic algorithm optimization. The spectra generated were assessed blindly by this predictive algorithm, and a viability score was calculated for each individual sample.

Statistical analysis

Viability scores (means±SD) were compared among the 0, 50, and 100 % implantation outcome sample populations. Student's *t* tests were used to compare the mean viability scores among the populations. Univariate analyses were performed using the χ^2 test for dichotomous variables, analysis of variance was used for continuous variables, and the Student-Newman-Keuls test was utilised for pairwise comparisons. A *P* value of <0.05 was considered to indicate a significant difference for all comparisons. All embryos were categorised by one of four morphologic scores: A, B, C, or D, from best to worst. The composite score was a combination of the morphology score and the viability index. Areas under the receiver operating characteristic (ROC) curve (AUC_{ROC}) were estimated to assess the ability of the viability, morphology, and composite scores to predict implantation outcome: the greater the AUC_{ROC}, the better the prediction of the model. Hosmer and Lemeshow [39] proposed thresholds for the interpretation of the AUC_{ROC}: perfect discrimination corresponds with an $AUC_{ROC}=1$ and random prediction corresponds with an $AUC_{ROC}=0.5$. The true positive (TP), true negative (TN), false positive (FP), and false negative (FN) values were calculated by determining the optimal cut-off values based on the ROC curves and then plotting a graph of the sensitivities versus the 1-specificities. The optimal cutoff value was that with the highest efficiency as calculated by sensitivity+specificity for each ROC curve. The percent accuracy of each test was then computed using the equation $\frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100$ with the optimal cutoff value. Pearson's correlation coefficients were also calculated to establish the association between the viability index and the implantation rate and the association between the viability index and the morphology score. All data were analysed using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA).

Results

The baseline characteristics of the 0, 50, and 100 % implantation patients are listed in Table 1. A total of 621 thawed embryos were transferred to 323 patients. There were no significant differences in age, duration of infertility, treatment cycles, infertility cause, frozen-thawed embryo transfer cycles, proportion of IVF/ICSI, or endometrial thickness among the three groups (Tables 1 and 2).

The embryo morphology and viability scores of the 0, 50, and 100 % implantation patients are listed in Table 2. Of the 621 thawed embryos transferred, 249 were compaction-stage embryos, 317 were morula-stage embryos, and 55 were blastocyst-stage embryos. A total of 584 embryos resumed mitosis 18–20 h post-thawing. The live-birth rate per embryo transfer was 29.6 % (184/621), with 182 healthy babies delivered at term and two preterm deliveries at 25 weeks of gestational age because of premature rupture of the membranes. Specifically, 94 singletons and 90 twin infants were born from the FET cycles. Overall, 83 singletons, 90 twin infants, and no triplet were born from the transfer of two embryos. Ninety twin infants arising from the two-embryo transfer pregnancies were tested from fraternal dizygosity (dyzigous, i.e. one infant arising from each of the transferred embryos). The mean viability index was different among these three groups; in particular, the mean viability index of the 0 % implantation group was significantly lower than that of the 100 % implantation group (–0.787±0.382 vs. 1.064±0.331, *P*<0.01). However,

Table 1 Comparison of patients' baseline conditions among the three groups

Characteristic	100 % implantation (*n*=56)	50 % implantation (*n*=83)	0 % implantation (*n*=184)
Age (years)	30.8±4.1	30.6±4.3	31.6±5.2
Infertility duration (years)	4.3±2.6	4.3±2.7	4.3±3.0
Cycles	1.11±0.29	1.10±0.29	1.14±0.43
Infertility indication			
Tube factor (%)	29 (51.8)	42(50.6)	96 (52.2)
Male factor (%)	8 (14.3)	12(14.5)	28 (15.2)
Both factor (%)	15 (26.8)	24(28.9)	50 (27.2)
Unexplained (%)	4 (7.1)	5(6.0)	10 (5.4)
Endometrial thickness (mm)	10.64±2.20	10.54±2.21	10.38±2.18
Proportion of IVF (%)	34 (60.7)	51(61.4)	110 (59.8)
Proportion of ICSI (%)	22 (39.3)	32(38.6)	74 (40.2)

n number of patients

the morphology scores among the groups were not significantly different (3.15±0.51 vs. 3.11±0.46 vs. 3.09±0.47, $P>0.05$).

Figure 1 shows the relationship between the implantation results and the metabolomic viability index of the FET cycles. The indices of all successful implantation, and 71 of the 50 % implantation embryos were above 0, while 74 % of the failed implantation embryos had indices below 0. Figure 2 shows that the relative viability index for FET cycles was independent of the morphology grade for day 4 thawed embryo samples. The viability indices of the thawed embryos ($n=621$) are shown for each morphological grade (grade A: $n=66$, grade B: $n=525$, grade C: $n=30$). The Pearson's correlation coefficient for the thawed embryos was −0.060. The null hypothesis of no association was accepted for the day 4 samples ($P=0.132$).

Figure 3 shows the ROC curves for predicting the implantation outcomes in FET cycles by day 4 embryo morphology grading, metabolomic viability score, and composite score. The composite score was a combination of the morphology score and the viability index. The AUC_{ROC} for predicting implantation outcome using the viability score was significantly higher than the AUC_{ROC} for the morphology score (0.94 vs. 0.55, $P<0.01$; Fig. 3), whereas the AUC_{ROC} for the composite score was not significantly different from that of the viability score (0.92 vs. 0.94, $P>0.05$; Fig. 3). The best threshold was 0.365. In addition, the positive-predictive value of metabolomic profiling was 0.86, and the negative-predictive value was 0.91.

Discussion

In this study, we discovered that non-invasive metabolomic profiling of thawed embryo culture media by NIR spectroscopy was capable of predicting pregnancy outcome in women undergoing FET. Furthermore, we are the first to demonstrate that the combination of morphology and viability scores was more precise at assessing reproductive potential in FET cycles than was morphologic grading alone.

We found that the mean viability indices were significantly different among the 0, 50, and 100 % implantation patients, but that the morphology scores were nearly identical between the groups (Table 2). In particular, the mean viability index of the 0 % implantation group was significantly different from that of the 100 % implantation group. These results were in accordance with those of previously reported studies, which indicated that the relative viability scores were statistically significantly different between positive and negative pregnancy outcomes after both fresh [22–29] and frozen-thawed [30, 31] embryo transfer. A positive relationship between implantation outcome and viability index was also observed in this study (Fig. 1). Our results have clinically significant implications: We established that determinants of the embryo media metabolome are quantitatively related to the implantation potential of thawed embryos and that a higher viability index might reflect a higher probability of pregnancy in FET cycles.

Our study also investigated whether metabolomic profiling can provide additional information about embryo viability

Table 2 Embryo morphology and viability scores of the three groups

Characteristic	100 % implantation ($n=56$)	50 % implantation ($n=83$)	0 % implantation ($n=184$)	P value
No. of transfer cycles	56	83	184	
No. of embryo transfer	101	166	354	
Day 4 embryo morphology				
Compaction stage (%)	41 (40.6)	69 (41.6)	139 (39.2)	NS
Morula (%)	49 (48.5)	83 (50.0)	185 (52.3)	NS
Blastocyst (%)	11 (10.9)	14 (8.4)	30 (8.5)	NS
Thawed embryo transfer cycles				
Natural cycles (%)	19 (33.9)	26 (31.3)	61 (33.2)	
HRT cycles (%)	37 (66.1)	57 (68.7)	123 (66.8)	
Resumption of mitosis				
Yes (%)	95 (94.1)	157 (94.6)	332 (93.8)	NS
No (%)	6 (5.9)	9 (5.4)	22 (6.2)	NS
Embryo survival rate				
Full (%)	93 (92.1)	153 (92.2)	319 (90.1)	NS
Partial (%)	8 (7.9)	13 (7.8)	35 (9.9)	NS
Day 4 embryo morphology score	3.15±0.51	3.11±0.46	3.09±0.47	NS
Day 4 embryo viability score	1.064±0.331	0.476±0.483	−0.787±0.382	P<0.01

NS no significant difference, *n* number of patients, *HRT cycles* hormone replacement therapy cycles

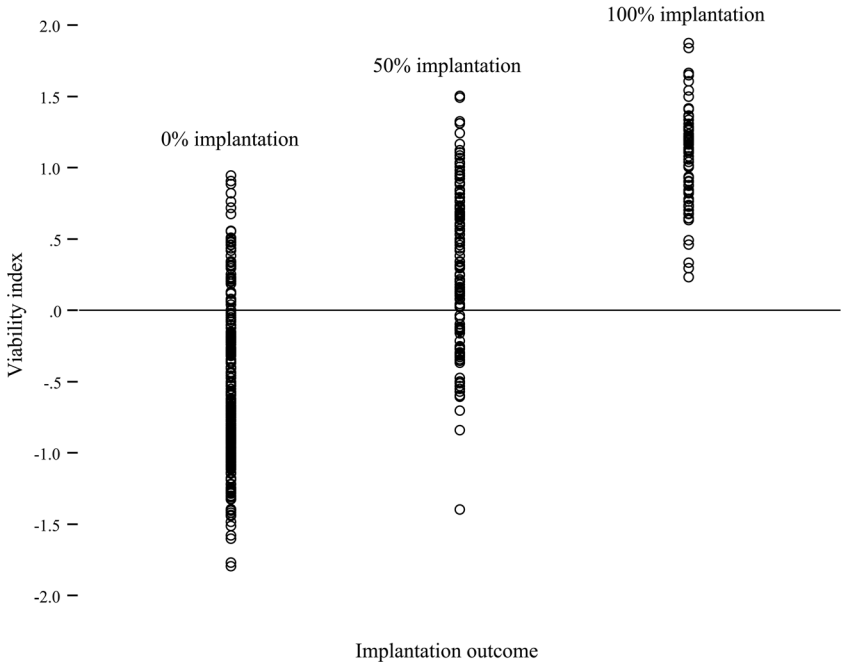

Fig. 1 Relationship between implantation results and metabolomic viability indices in FET cycles determined by blinded analysis of 621 thawed embryos from 323 patients. The data for 354 embryos from 184 patients in the 0 % implantation group are represented on the left side of the figure. The viability indices of 92 embryos were above 0 (26 %) and those of 262 embryos were below 0 (74 %). The data for 166 embryos from 83 patients in the 50 % implantation group are represented on the centre of the figure. The viability indices of 118 embryos were above 0 (71 %) and those of 48 embryos were below 0 (29 %).The data for 101 embryos from 56 patients of the 100 % implantation group are represented on the right side of the figure. All exhibited indices were over 0. *Circles* represent individual patients

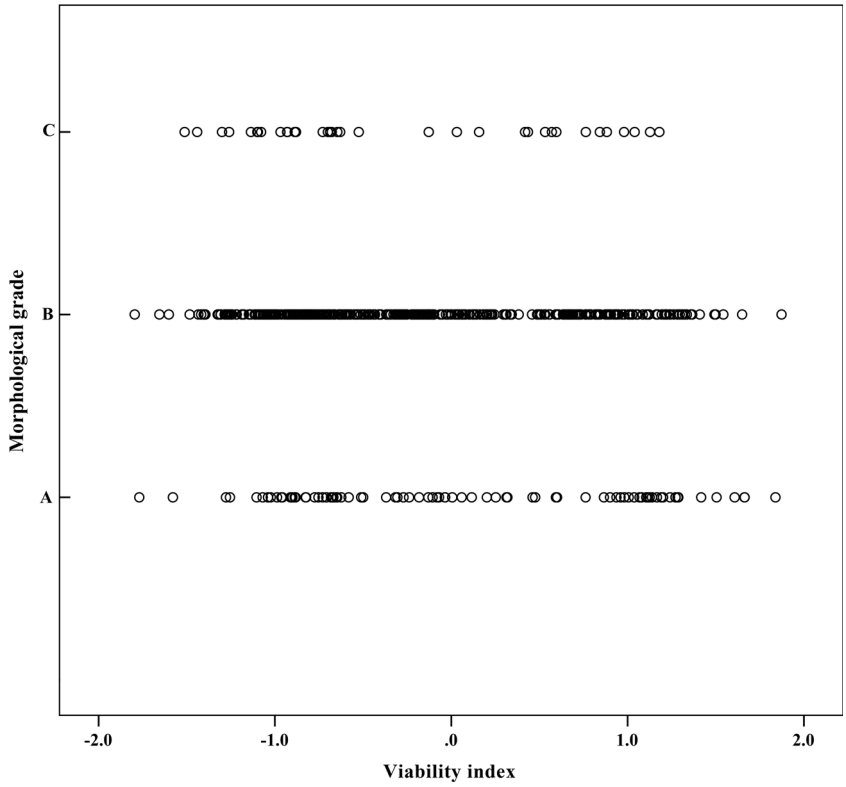

Fig. 2 Relationship between relative viability index and morphological grade for day 4 thawed embryos in FET cycles. Viability indices of thawed embryos (n=621) are shown for each morphological grade (grade A: n=66; grade B: n=525; grade C: n=30). The Pearson correlation coefficient was −0.060. The null hypothesis of no association is accepted for day 4 (P=0.132) samples. *Circles* represent individual patients

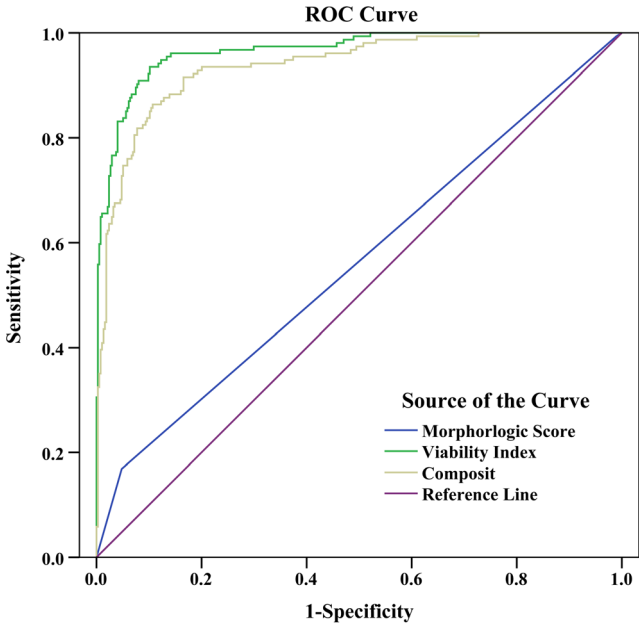

Fig. 3 ROC curves of composite score (*yellow*), viability index (*green*), and morphology score (*blue*) for day 4 thawed embryo culture medium samples in FET cycles. The optimal cutoff value was 0.365. AUC_{ROC} for composite score was 0.92, AUC_{ROC} for viability score was 0.94, and AUC_{ROC} for morphological grade was 0.55. $P<0.01$, composite score vs. morphology score

independent of morphological grading. Figure 2 shows that the viability indices calculated by the metabolomic profiling of spent culture media are not correlated with the morphological grading of day 4 thawed embryos in FET cycles. This result was consistent with that of a previous study, which showed that viability indices are not correlated with morphological grades of day 2 or day 3 fresh embryos in FET cycles [25]. Vergouw et al. [22] also determined using spent culture media that the viability index is not correlated with the number of blastomeres or the percent fragmentation.

In the present study, despite 93 % of the thawed embryos being of good quality, the implantation rate after day 4 FET was only 29.6 %. Although other factors impact implantation results, our results indicate that not all embryos that exhibit good quality morphologically will implant. This is consistent with a previous study that showed that morphology alone cannot distinguish between sibling embryos [22, 40]. In this study, we investigated whether spent culture media analysed by NIR spectroscopy can be used as an adjunct to morphology when selecting the most viable embryo to transfer in FET cycles. In our study, based on morphologic grading alone, embryos with viability indices >0.365 were more likely to result in pregnancy compared with those with viability indices <.365 (Figs. 1 and 3). These findings demonstrate that metabolomic profiling is a non-invasive and objective technology that allows for greater discrimination of thawed embryos of similar morphology.

In this study, for predicting pregnancy, the AUC_{ROC} of the viability score was significantly higher than that of the morphology score (0.94 vs. 0.55, $P<0.005$; Fig. 3), whereas the AUC_{ROC} of the composite score was not significantly different from that of the viability score (0.92 vs. 0.94, $P>0.05$; Fig. 3). The viability index and the combination of the viability index and morphological grading were more accurate in predicting outcome than was morphological grading alone. Therefore, the viability score alone or in conjunction with morphologic grading has more precise predictive value than does morphologic grading alone in women undergoing FET. Previous studies using NIR spectroscopy technology have also demonstrated that the viability score alone and in combination with morphologic grading are potentially better indicators of pregnancy outcome than is morphology alone in women undergoing single embryo transfer (SET) on day 5 [32] or day 3 [28] after fresh embryo transfer. However, before metabolomic assessment methods in conjunction with morphology assessment can be more broadly accepted for the selection of embryos for transfer, additional research is required to determine the advantages and limitations of these methods, preferably by randomized controlled trials (RCTs).

We note, however, that the conclusion reached by our study differed from the findings of previously published RCTs [41, 42]. In those studies, no beneficial effect on pregnancy and live birth rates of embryo selection by NIR combined with morphology was found when compared with the rates obtained with embryo selection by morphology alone. However, the RCT by Sfontouris et al. [43] did show significantly altered implantation rates in favour of the morphology plus viability score group compared with the control group for day 5 transfers (but not for days 2 or 3 transfers), although the pregnancy and live birth rates were similar between the two study groups. A significant difference between the previous RCTs and our studies was the multivariate algorithm. To objectively choose wavelength regions, a different preprocessing method (stability competitive adaptive reweighted sampling, SCARS) based on Haar wavelets was used in our multivariate algorithm. This probably reduces the susceptibility of the utilised algorithms to noise which improves the accuracy and repeatability of the viability indices [28, 36–38]. In addition, day 4 frozen-thawed human embryos were transferred in our trials, whereas days 2, 3, or 5 fresh embryos were transferred in the cited RCTs [42–44].

In our study, we were able to analyse each testing sample in approximately 1 min using 10 μL of media. Furthermore, the NIR spectroscopy instrument is small and convenient to operate. These benefits make this procedure valuable for both research and clinical application, potentially improving the efficacy of elective SET in FET cycles with wide application. Currently, morphology scores are generally utilised to forecast the implantation potential of thawed embryos. However, the limitations of selection based on morphology are well known,

although most pregnancies do result from high-grade embryos. On one hand, there is no precise morphological scoring system that is considered to be optimal for embryo selection; on the other hand, the morphological scoring system is subjective and does not provide a robust test of implantation potential. Therefore, the morphology score does not constitute an ideal sensitive and precise observation index of embryo function. The embryos that have high morphology scores might also have genetic or epigenetic shortcomings that could hinder their implantation and development. Fortunately, metabolomics provides a distinct opportunity to investigate the associations between the genotype of an organism and its resulting phenotype, as the metabolome represents the end product of gene expression. Simultaneously, metabolomics also reflects the association between organism physiology and environmental conditions [28, 45]. Together, these factors might explain why the metabolomic profiling of spent thawed embryo culture media was found to be a more objective marker for implantation outcome in FET cycles than morphology grading.

In conclusion, our findings indicate that metabolomic models alone or in combination with morphology assessment improve the accuracy of the prediction of embryo viability in FET cycles compared with morphology assessment alone. Thus, metabolomic technology might be used in addition to morphology assessment for the assessment of embryo viability prior to embryo transfer. As pregnancies that result from FET cycles might have better obstetric and perinatal outcomes than those that occur after fresh embryo transfer [1–3], FET has become more predominant within assisted reproductive treatments (ARTs). An improved understanding of embryo viability will aid in determining which thawed embryo(s) should be transferred in FET cycles, especially in women undergoing SET. Overall, metabolomic profiling by NIR spectroscopy is a rapid, objective, and non-invasive embryo assessment technique that might offer additional information about the implantation potential of thawed embryos and allow for more precise decisions regarding the number of embryos to be transferred. In this manner, we can potentially decrease the probability of multiple gestations in FET cycles while maintaining or even improving pregnancy rates.

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Conflict of interest The authors declare that they have no conflict of interest.

Author contributions Conceived and designed the experiments: XL, SL, and XS; Performed the experiments: XL, YX, and JF; Analysed the data: XL and WZ; Wrote the manuscript: XL; Revised the manuscript: SL and XS; All authors approved the revisions to the manuscript.

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