Predicting IVF outcome
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Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential using morphological scoring

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Chapter 7

ABSTRACT

Selection of embryos based on morphology is still the core of daily laboratory practice in IVF/ICSI. Yet, the selection of embryos is primarily based on experience and local protocols. Since an evidence-based ranking strategy for embryos on day 3 is currently lacking, we constructed a multivariable prediction model, to rank embryos according to their implantation potential. We studied 6,021 fresh embryo transfers between January 2004 and July 2009. We evaluated pronuclear score, early cleavage, number of blastomeres on day 2/3, morphological score on day 2/3 and cleavage rate as potential predictors for ongoing implantation. The outcome measure was ongoing implantation. A model was developed using multivariable logistic regression. This prediction model was externally validated with data from couples treated between August 2009 and September 2011 in the same clinic. Five factors were included in the final prediction model. In the external validation the model showed moderate discriminative capacity (c-statistic 0.70) and calibrated well. The model was able to distinguish embryos with high ongoing implantation potential from embryos with moderate or low ongoing implantation potential. The model can be used by embryologists as an objective tool to rank embryos according to their implantation potential thereby aiding the selection of embryos for transfer.
Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential

INTRODUCTION

In the early days of in vitro fertilization (IVF), pregnancy rates per embryo transfer were very low (1). The only way to increase pregnancy rates at that time was the transfer of large numbers of embryos (2). Over the years implantation rates per embryo improved and the transfer of these large numbers of embryos led to high multiple pregnancy rates (3-5). To reduce these high multiple pregnancy rates, embryo transfer policies restricting the number of embryos to be transferred were introduced. This meant that selection of the embryo(s) with the highest chance of implantation became of paramount importance in IVF, especially since the success rates of cryopreservation of supernumerary embryos were still low at the start of cryopreservation programs (3).

In the last decade, cryopreservation of embryos has become increasingly successful and this has placed embryo selection in a new context (3;6). Optimal selection of embryos can help to minimize the time to pregnancy by transferring the embryo with the highest implantation potential as early as possible. Transferring only one embryo if there is a high chance of implantation could also help to achieve acceptable pregnancy rates while minimizing the chances of multiple pregnancy.

Ever since the start of IVF the selection of embryos has been largely based on morphological characteristics of the embryo. Additional methods for embryo selection, such as selection based on chromosomal status (PGS) or selection based on metabolomic profiles of culture media, have been introduced but upon proper evaluation these methods have been shown to be unable to increase pregnancy rates (7-9). Morphological selection of embryos thus remains the core of daily laboratory practice in IVF/ICSI. Yet, morphological selection of embryos is largely based on clinical experience and local protocols (10;11).

Several authors proposed prediction models to rank embryos according to their implantation potential. Unfortunately, most of these models were developed on small datasets and were not externally validated (11-18). Based on data from a large cohort of consecutively treated IVF/ICSI patients, we constructed a new multivariable prediction model, to rank embryos according to their implantation potential.

MATERIALS AND METHODS

We collected data consecutive IVF/ICSI embryo transfers on day 3 after oocyte retrieval performed between January 2004 and July 2009 in the Center for Reproductive Medicine of the Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, for the development of the model.
For validation of the model we prospectively collected data embryo transfers performed between August 2009 and September 2011 at the same center. Under the legal requirements for clinical research in the Netherlands, this study was exempt from institutional review board (IRB) approval.

Patients
All couples in our study had been trying to conceive for at least 12 months and underwent a basic fertility workup according to the guidelines of the Dutch Society of Obstetrics and Gynecology (19). The indication to start IVF or ICSI treatment was determined according to the Dutch IVF guideline (20). If subfertility was caused by tubal pathology, severe endometriosis, or severe oligozoospermia (post-wash total motile sperm count < 3 million) IVF/ICSI was offered directly (21). In case of one-sided tubal pathology, minimal endometriosis, cervical hostility, mild male oligozoospermia, or unexplained subfertility, at least six intra uterine inseminations (IUI) were applied before IVF/ICSI was offered. In case of ovulation disorders, mainly caused by polycystic ovary syndrome (PCOS), 12 cycles of ovulation induction were applied before IVF/ICSI was offered.

IVF/ICSI procedures
Women underwent controlled ovarian hyperstimulation after down-regulation with the GnRH agonist triptorelin (Decapeptyl®) in a long protocol with a midluteal start. Controlled ovarian hyperstimulation was started on cycle day 5 with recombinant FSH or HMG in daily doses ranging from 75 to 450 IU depending on the antral follicle count. Follicular maturation was induced by 10,000 IU human chorionic gonadotropin hormone (hCG) (Pregnyl, Organon). Cumulus-oocyte complexes were recovered by transvaginal ultrasound guided follicle aspiration 36 hours thereafter. Oocytes were inseminated with 10,000 or 15,000 progressively motile spermatozoa (in vitro fertilization) or injected with a single spermatozoon (intracytoplasmic sperm injection) 2-4 hours after follicle aspiration. Embryos were cultured in Human Tubal Fluid (HTF, Cambrex) supplemented with 15% pasteurized plasma protein solution (GPO, Sanquin) or G5-PLUS medium (Vitrolife) containing Human Serum Albumin at 37°C and 5% (HTF) or 6% (G5) CO₂ in air. Embryo transfer was performed on day 3 after oocyte retrieval with a Wallace catheter (Smiths Medical). Luteal phase was supported by progesterone intravaginally two times 200 mg (Utrogestan) per day. An hCG blood test was performed 18 days after oocyte retrieval.

Morphological scoring
Each embryo was cultured individually. Pronuclear scoring was performed 17 to 22 hrs. after insemination/injection and early cleavage was scored 23 to 28 hours after insemination/injection. On day 2 (41 to 46 hrs. after insemination/injection) and day 3 (65 to 70 hrs. after insemination/injection) the number of blastomeres was assessed and each embryo was given a morphological score. For the morphological score the degree of fragmentation of the embryo and the uniformity of the blastomeres were assessed (22). The embryo was given a score of 1 (no fragmentation), 2 (<20% fragmentation), 3 (20-50%
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fragmentation) or 4 (> 50% fragmentation). If the blastomeres of the embryo were non-uniform in size for their developmental stage (ie the 2, 4 or 8 cell stage), the morphological score was augmented with one point with 4 remaining the lowest possible score. If on day 3 the embryo showed signs of compaction, the embryo was scored as a morula and given a grade based on the degree of compaction (score 1: full compaction, score 2: 50 - <100% compaction and score 3: less than 50% compaction).

Number of embryos transferred
Before July 2006 in all women double embryo transfer was performed unless there was a medical indication to limit the number of transferred embryos to one. After July 2006, an individualized transfer policy was adopted. Single embryo transfer (SET) was performed in women under 35 years undergoing their first cycle of IVF/ICSI with at least one top-quality embryo. Double embryo transfer (DET) was performed in women under the age of 35 who did not have a top quality embryo in the first cycle, in women under 35 who failed to get pregnant in their first cycle of IVF/ICSI, and in women between 35 and 38. In women 39 or older three embryos were transferred (TET). These strategies were based on a combination of the Practice Committee of the American Society for Reproductive Medicine guidelines and the Belgian embryo transfer legislation (23;24).

Outcome
The primary outcome was ongoing implantation, defined as the presence of one fetus with cardiac activity at transvaginal ultrasound at a gestational age of at least 11 weeks per embryo transferred.

Predictors
We evaluated pronuclear score, early cleavage, number of blastomeres on day 2 and day 3, morphological score on day 2 and day 3 and the progression of the number of blastomeres from day 2 to day 3 as potential predictors for ongoing implantation. Since we wanted to develop a model to rank embryos, not to calculate the chances of success, we only evaluated embryo parameters, leaving out female and male characteristics. Since within each individual cycle couple and treatment characteristics will be identical for all embryos, they are of no help in ranking embryos according to their implantation potential.

Data analysis
For the development of the model, only cycles with embryos with individual traceability were used. These were cycles with single-, double- or triple embryo transfer resulting in either no implantation or transplantation of all transferred embryos. Monozygotic twins were excluded from the analysis. Embryos on which PGS was performed were also excluded. The embryo was the unit of analysis in model development. This dataset is the development dataset with traceable embryos.
Some of the candidate predictors had missing values. Simple exclusion of couples with missing values on one or more variables commonly causes biased results and decreases statistical efficiency (25). Therefore, missing values in the data were completed by multiple imputation using SPSS (version 18.0). This method uses all available data to impute the missing values based on the correlation between each variable with missing values and all other variables. If a single candidate predictors had more than 25% missing values, they were excluded from the analyses.

We checked the linearity of the associations between the continuous variables number of blastomeres and the categorical variable morphological score and the probability of an ongoing implantation using restricted cubic spline functions in logistic regression and visual inspection. Based on these spline functions, variables were transformed to better approach linearity.

For each candidate predictor, we performed a univariable logistic regression analysis and estimated the corresponding unconditional odds ratio, calculating 95% confidence intervals (CI) and the p-value. All predictors that were significantly associated with ongoing implantation (p<0.3) were entered in a multivariable logistic regression analysis. In deciding between competing expressions of related parameters, we used Akaike’s Information Criterion (AIC) in variable selection (26). The model with the best AIC was selected as the final model. Additionally, we evaluatation all potential predictive factors for interactions using an interaction term.

To prevent overfitting and a too optimistic impression of model performance, a linear shrinkage factor was estimated based on model fit and the number of parameters (26). Coefficients in the model were then corrected by this shrinkage factor.

**Performance**
Performance of the final model was first evaluated by assessing the ability of the model to distinguish between embryos that achieved an ongoing implantation and those that did not. To evaluate discrimination of the model, the area under the receiver operating characteristic curve (AUC), also known as the c-statistic, was calculated. The c-statistic expresses the probability that in any pair of embryos, in which one implanted and the other did not, the embryo that implanted actually had a higher score.

To extrapolate the implantation rates from the dataset with individual embryo traceability to the total dataset a correction factor was used. The correction factor was calculated as the ratio of the overall implantation rate – the number of implantations relative to the number of transferred embryos in the total dataset – versus the implantation rate in the individual traceability dataset: the number of implantations relative to the number of transferred embryos in the individual traceability dataset.
Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential

We calculated for each traceable embryo in development dataset and in the validation dataset the implantation probability. Ideally, these probabilities should show a wide range, making it easier to rank embryos based on their implantation potential.

To evaluate agreement between calculated probabilities of an ongoing implantation and observed proportions of ongoing implantation, we calculated the Hosmer-Lemeshow goodness-of-fit test statistic.

In addition we compared the average calculated probabilities of ongoing implantation in disjoint subgroups defined by quintiles with the observed ongoing implantation rate in the corresponding groups. The predicted proportion and the observed proportion of ongoing implantations (for traceable embryos) were compared by plotting the observed ongoing implantation rate versus the average probability in each of the groups, as calculated from the model.

To evaluate any miscalibration, we also fitted a calibration model using logistic regression, with the linear combination of variables in the prediction model as the only variable (26;27).

External validation

We performed an external, temporal validation (26). We evaluated model performance in more recent embryo transfers on day 3 after oocyte retrieval, performed between August 2009 and September 2011 in the Centre for Reproductive Medicine of the Academic Medical Centre, the Netherlands. We performed the validation on the validation dataset with traceable embryos. We also performed a validation on the complete development set and validation set (on a transfer level) containing all transferred embryos. The probability of success in transfer was calculated based on the calculated probabilities of the embryos transferred. The predicted proportion and the observed proportion of success (for all transfers) were compared by plotting the observed ongoing implantation rate versus the average probability in each of the groups, as calculated from the model.

Updating the model

After the external validation, we updated our model based on all available data through re-calibration (28;29). We fitted the linear combination of the variables in our model as the only variable in a logistic regression model, using all traceable embryos in the development set and the validation set. Based on the estimated slope and intercept of that model we adjusted the intercept and coefficients of our prediction model, to create a final, updated model.
RESULTS

Between January 2004 and July 2009, 3,143 embryo transfers had been performed, transferring a total of 6,021 embryos (average 1.9 embryos per transfer). Of these, 848 implanted: a total ongoing implantation rate of 14.1%. The transfers led to a viable intra-uterine pregnancy of at least 11 weeks in 713 cases (23%); in 247 transfers all embryos transferred implanted, in 466 transfers fewer embryos implanted than transferred, and in 2,430 embryo transfers no embryos implanted (Suppl Fig 1). A total of 5,028 embryos had exact traceability and were used further for model development.

Between August 2009 and September 2011, 1,666 additional embryo transfers were performed, transferring a total of 3,060 embryos (average 1.8 embryos per transfer). The ongoing pregnancy rate in this validation set was 21% (351/1,666). In 152 transfers all embryos transferred implanted, in 199 transfers less embryos implanted than transferred, and in 1315 embryo transfers no embryos implanted (Suppl Fig 1).

The baseline characteristics of all embryo transfers and the datasets with traceable embryos are summarized in Suppl Table I, both for the development set and for the validation set.

Analysis with spline functions demonstrated a nonlinear association between the number of blastomeres on day 2 and day 3 after oocyte retrieval and ongoing implantation. We transformed both variables to better fit the data. The number of blastomeres on day 2 was recoded as the absolute value of the deviation from 4; an embryo with six blastomeres was recoded to a score two (6 minus 4) and an embryo with three blastomeres was recoded to a score of 1 (4 minus 3). Similarly, the number of blastomeres on day 3 was recoded as the absolute value of the deviation from 8. All embryo morphology scores could adequately be described by linear functions (Suppl Fig 2).

In univariable analysis, early cleavage, number of blastomeres on day 2 and 3, morphological score on day 2 and day 3, and progression from 4 blastomeres on day 2 to 8 blastomeres on day 3 were found to be significantly associated with ongoing implantation. The pronuclear score (p=0.26) and the presence of morulae on day 3 (p=0.2) were moderately associated with ongoing implantation (Suppl Table 2).

In multivariable analysis five factors were found to be significantly associated with ongoing implantation: early cleavage, number of blastomeres on day 2, number of blastomeres on day 3, the morphological score on day 3 and presence of morulae on day 3. These factors were included in the final multivariable logistic regression model (Table I). None of the evaluation interactions between these terms was statistically significant; no interaction terms were included in the final model.

We compared the goodness-of-fit of the final model to that of two other models: a model with only day 3 blastomeres and a model with day 3 blastomeres and the morphological
Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential score on day 3. Our final model fitted the data significantly better than the other two models (likelihood ratio test; p < 0.001)

Figure 1 depicts the spread of predicted probabilities in the development set and validation set with traceable embryos. The calculated probabilities for both datasets ranged from 0.00 to 0.39, with a mean of 0.14, which corresponds to the overall implantation rate in this selected set of embryos.

**Figure 1** Distribution of the calculated probabilities in the development set and validation set with traceable embryos

The model had a moderate discriminative capacity in the development set with traceable embryos. The c-statistic was 0.73 (95% CI: 0.70 to 0.75). There was good calibration; the goodness-of-fit test (Hosmer-Lemeshow) showed no significant miscalibration (p=0.27), the calibration slope was 1.02 (95% CI: 0.87 to 1.18) and the calibration intercept was 0.05 (95% CI: -0.31 to 0.42). The calibration plot showed that the model calibrated well (Figure 2a).

Discriminative capacity in the validation set with traceable embryos was similar to that in the development set, with a c-statistic of 0.70 (95% CI: 0.67 to 0.74). In the validation set the model also calibrated well (Figure 2b). The slope of the linear predictor (calibration slope) was 0.89 (95% CI: 0.69 to 1.09); the calibration intercept was -0.26 (95% CI: -0.74 to 0.24).

We also evaluated performance of the model for all embryo transfers. The model calibrated well both in the complete development set and validation set (Figure 2c and d).
Figure 2 | Calibration plots, showing the association between the calculated and observed embryo ongoing implantation rates

- **a. Development set with traceable embryos**
- **b. Validation set with traceable embryos**

The updated final model and a simplified embryo score are presented in Table I. The total score can be calculated with the following formula:

\[
\text{Total score} = 103 + (2 \times \text{early cleavage (yes=1, no=0)}) + (-3 \times \text{number of blastomeres on day 2 deviating from 4}) + (-3 \times \text{number of blastomeres on day 3 deviating from 8 (morula=0)}) + (-5 \times \text{morphological score on day 3 (morula=0)}) + (-11 \times \text{morula (yes=1, no=0)})
\]
Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Updated model</th>
<th>Embryo Score</th>
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<tr>
<td>Intercept</td>
<td>-1.0579</td>
<td>103</td>
</tr>
<tr>
<td>Early cleavage</td>
<td>0.2492</td>
<td>2</td>
</tr>
<tr>
<td>Number of blastomeres on day 2 calculated as: value of the deviation from 4 ¹</td>
<td>-0.3324</td>
<td>-3</td>
</tr>
<tr>
<td>Number of blastomeres on day 3 calculated as: value of the deviation from 8 ²</td>
<td>-0.3128</td>
<td>-3</td>
</tr>
<tr>
<td>Morphological score day 3 ³</td>
<td>-0.5305</td>
<td>-5</td>
</tr>
<tr>
<td>Morula on day 3 ⁴</td>
<td>-1.1940</td>
<td>-11</td>
</tr>
</tbody>
</table>

¹ Number of blastomeres = absolute value (number of blastomeres - 4)
² Number of blastomeres = absolute value (number of blastomeres - 8), morula = 0
³ Morula = 0
⁴ Presence of morula=1, no morula = 0.

The higher the total score, the higher the ongoing implantation potential of the embryo.

Table II depicts a hypothetical case of a couple that has 10 embryos after an IVF/ICSI cycle. Their embryos are ranked according to their implantation potential based on our model. For example, an embryo with early cleavage, 4-blastomeres on day 2, 8-blastomeres on day 3 with a morphological score of 1 on day 3 would have a total score of 100 (103 + (2*1) + (-3*0) + (-3*0) + (-5*1) + (-11*0)) (Table II, embryo 10). The embryo with the highest total score has the highest chance of implantation compared to the other 9 embryos.

<table>
<thead>
<tr>
<th></th>
<th>Early cleavage</th>
<th>Number of blastomeres day 2 ⁴</th>
<th>Number of blastomeres day 3 ⁴</th>
<th>Morphological score day 3</th>
<th>Morula on day 3</th>
<th>Total score</th>
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<tr>
<td>Embryo 1</td>
<td>no</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>no</td>
<td>67</td>
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<tr>
<td>Embryo 2</td>
<td>no</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>no</td>
<td>76</td>
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<tr>
<td>Embryo 3</td>
<td>no</td>
<td>4</td>
<td>12</td>
<td>2</td>
<td>no</td>
<td>81</td>
</tr>
<tr>
<td>Embryo 4</td>
<td>no</td>
<td>5</td>
<td>7</td>
<td>2</td>
<td>no</td>
<td>87</td>
</tr>
<tr>
<td>Embryo 5</td>
<td>no</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>yes</td>
<td>89</td>
</tr>
<tr>
<td>Embryo 6</td>
<td>yes</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td>no</td>
<td>92</td>
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<tr>
<td>Embryo 7</td>
<td>no</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>no</td>
<td>93</td>
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<tr>
<td>Embryo 8</td>
<td>yes</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>yes</td>
<td>94</td>
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<tr>
<td>Embryo 9</td>
<td>no</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>no</td>
<td>98</td>
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<tr>
<td>Embryo 10</td>
<td>yes</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>no</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ The number of blastomeres are transformed to: abs (number of blastomers - 4)
² The number of blastomeres are transformed to: abs (number of blastomeres - 8), morula= 0
NA: Not applicable
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DISCUSSION

In this study we developed a prediction model to rank embryos within a single IVF/ICSI cycle of a couple according to their ongoing implantation potential. The model had moderate discriminative capacity and calibrated well, both in the development and in a separate validation set, with data that had not been used for the development of the model.

One of the strengths of our study is that we evaluated seven embryo predictors in consecutive transfers, using only embryos with exact traceability. We developed our model in a large data set (>6,000 embryos) and we validated our model thoroughly using more recent data, collected at the same clinic after the development of the model.

The moderate discriminative capacity implies that the model is not able to distinguish perfectly between embryos with small differences in ongoing implantation potential. Yet perfect prediction is not the goal of this embryo selection model: the primary goal is not to predict with absolute certainty whether an embryo will implant, but to rank embryos based on their ongoing implantation potential within a single treatment cycle of a couple. Although the ideal outcome of the model would be the number of live births, our study used ongoing implantation rate as the outcome of interest. We have not yet evaluated whether implementation of this model ultimately improves ongoing implantation rates and time to pregnancy, a topic for future research. Since < 2% of all ongoing pregnancies result in late miscarriage or still birth, we do not expect that our model will fundamentally change when using the number of live births as outcome measure (30).

When scoring the embryos, there was a range in timing of scoring of maximally 5 hours. This range could potentially lead to different classification of embryos. Yet despite the range in timing of scoring the embryos, the model had an acceptable discriminative capacity and calibrated perfectly even after external validation. We used data of a single center only, so the generalizability of the model to other clinics has to be evaluated more extensively in future studies (geographical validation). As the aim of the model was to rank the embryos acquired after an IVF/ICSI cycle of a couple and not to calculate the exact implantation rate of an individual embryo, higher or lower implantation rates should not influence the performance of the model. Over the years the data in this study were collected, there were two significant changes in our center: the embryo transfer policy shifted more towards single embryo transfer (as seen by the lower number of embryos transferred in the validation set), and a switch in culture media was made (HTF to Vitrolife). Despite these changes our model still had near-perfect calibration and acceptable discriminative capacity both after internal and external validation, indicating that these changes did not affect the performance of the model.
Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential

As indicated in the introduction, several other embryo implantation models have been developed in the past. Previous studies used much smaller datasets and not all used data of embryos with exact traceability of the individual embryos (11-17). Several studies did not validate their model (11-14;17). As prediction models may not perform as well in a new dataset as in the development set, external validation of models is essential to support general applicability of the model (26). It also enables further fine tuning of the model by updating the weight of each variable.

An additional problem with some other embryo implantation models is that they included patient characteristics into the model (13;18). As patient characteristics for each of these embryos are identical - all embryos are from the same couple - it is misleading to include these characteristics. They will seem to improve model fit, without actually contributing to the ranking potential. Some of these patient characteristics, such as age, are much stronger predictors than embryo parameters, so including these in a model would result in an overestimation of the discriminative capacity of the model in distinguishing between embryos of the same women, in which female age is identical.

The association between the five identified embryo factors and embryo implantation is biologically plausible: embryos that demonstrate early cleavage are known to be more likely to implant because they are likely to cleave more evenly, which is strongly correlated with a lower incidence of mitotic chromosomal errors, and therefore a higher chance of implantation (31). Our analyses showed that faster and slower cleaving embryos have a lower chance of implantation. The biological explanation could be that embryos that cleave directly into more than two cells and embryos that cleave too slowly or too fast also have significantly more chromosomal abnormalities (32;33). In addition, embryos that cleave faster have recently been shown to have greater perturbations in genomic imprinting and metabolic marker expression (34). The degree of fragmentation of an embryo is strongly correlated with chromosomal mosaicism and embryos that display fragmentation are less likely to implant (35).

Early cleavage (day 1) and the number of blastomeres on day 2 are important predictors in our model, implying that embryo selection should not be solely based on embryo parameters assessed on day 3. Culturing embryos individually and scoring them on each day therefore allows for better embryo selection. Newly developed real-time embryo monitoring systems enable the continuous monitoring of embryos and could assist in accurate determination of the timing of all cleavages (36). In the absence of sufficiently large randomized clinical studies it remains to be elucidated whether embryo selection using dynamic parameters improves clinical outcome, or whether it has additional predictive capacity for implantation. Before such randomized controlled studies are performed morphological selection based on daily evaluation of the embryos seems to remain at the core of current laboratory practice in IVF/ICSI.
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Combining a multivariable model that takes both the prognostic profile of the patient and the ranking of the embryos into account could be an important step towards a ‘patient tailored’ embryo transfer strategy. Such a combined model would enable calculation of ongoing implantation chances and multiple ongoing implantation chances. This is especially relevant in the situation where a decision has to be made to transfer one or two embryos. Currently, embryo quality is mostly dichotomized into top quality and non-top quality embryos. Our study shows that embryos can be ranked more precisely, based on their ongoing implantation potential, and that dichotomizing embryo quality is most likely an oversimplification of reality.

In the meantime, the model presented here can be used by embryologists as an objective tool to rank embryos by their ongoing implantation potential, and to select the embryo(s) with the highest ongoing implantation potential for transfer. Furthermore, it can help to create a more uniform embryo selection strategy for all laboratories transferring embryos on day 3 after oocyte retrieval.
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REFERENCES


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Supplemental figure 1 | Flowchart on embryo transfers

1a. Development dataset

3143 transfers in total
- SET 605 transfers
- DET 2198 transfers
- TET 340 transfers

No implantation in 2430 transfers
- SET 485 transfers
- DET 1699 transfers
- TET 279 transfers

Less embryos implanted than transferred in 466 transfers
- DET 405 transfers
- TET 61 transfers

All embryos implanted in 247 transfers
- SET 120 transfers
- DET 127 transfers
- TET 0 transfers

2677 transfers included
- SET 605 transfers
- DET 1793 transfers
- TET 279 transfers

1b. Validation dataset

1467 transfers included
- SET 550 transfers
- DET 683 transfers
- TET 234 transfers

1666 transfers in total
- SET 550 transfers
- DET 837 transfers
- TET 279 transfers

No implantation in 1315 transfers
- SET 444 transfers
- DET 637 transfers
- TET 234 transfers

Less embryos implanted than transferred in 199 transfers
- DET 154 transfers
- TET 45 transfers

All embryos implanted in 152 transfers
- SET 106 transfers
- DET 46 transfers
- TET 0 transfers
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Supplemental figure 2 | Spline functions that visualizes the association of the prediction of ongoing implantation and embryo variables

A. Number of blastomeres on day 2
B. Morphological score on day 2
C. Number of blastomeres on day 3
D. Morphological score on day 3
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**Supplemental table I | Baseline characteristics development and validation set**

<table>
<thead>
<tr>
<th></th>
<th>Development set</th>
<th>Development set traceable embryos</th>
<th>Validation set</th>
<th>Validation set traceable embryos</th>
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<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Number of patients</td>
<td>1790</td>
<td></td>
<td>1569</td>
<td></td>
</tr>
<tr>
<td>Number of IVF cycles</td>
<td>3143</td>
<td></td>
<td>2677</td>
<td></td>
</tr>
<tr>
<td>Age (SD)</td>
<td>35.5 (4.5)</td>
<td></td>
<td>35.6 (4.5)</td>
<td></td>
</tr>
<tr>
<td>Indication for IVF/ICSI</td>
<td></td>
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<td></td>
<td></td>
</tr>
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<td>Tubal pathology</td>
<td>616</td>
<td>20%</td>
<td>538</td>
<td>20%</td>
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<td>Unexplained subfertility</td>
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<td>24</td>
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<td>297</td>
<td>105</td>
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<td>Twins</td>
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<td>54</td>
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<td>Triplets</td>
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<td>Number of ongoing implantations</td>
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Selection of embryos for transfer in IVF: ranking embryos based on their implantation potential

**Supplemental table II | Univariable analysis**

<table>
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<tr>
<th></th>
<th>Univariable analysis</th>
<th>Univariable analysis</th>
<th>95% CI</th>
<th>P-value</th>
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<tr>
<td>Pronuclear score</td>
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<tr>
<td>2 PN (reference)</td>
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<td>0.28</td>
<td>(0.07 - 1.17)</td>
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<td>(0.55 - 1.28)</td>
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<td>(0.04 - 2.32)</td>
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<td>Progression from 4 blastomeres on day 2 to 8 blastomeres on day 3</td>
<td>1.28</td>
<td>3.59</td>
<td>(2.89 - 4.45)</td>
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</tr>
</tbody>
</table>

* for analysis morula embryos were excluded

OR= odds ratio
CI= confidence interval
β= beta coefficient