ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Time-lapse deselection model for human day 3 in vitro fertilization embryos: the combination of qualitative and quantitative measures of embryo growth

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Objective: To present a time-lapse deselection model involving both qualitative and quantitative parameters for assessing embryos on day 3.
Design: Retrospective cohort study and prospective validation.
Setting: Private IVF center.
Patient(s): A total of 270 embryos with known implantation data (KID) after day 3 transfer from 212 IVF/intracytoplasmic sperm injection (ICSI) cycles were retrospectively analyzed for building the proposed deselection model, followed by prospective validation using an additional 66 KID embryos.
Intervention(s): None.
Main Outcome Measure(s): Morphological score on day 3, embryo morphokinetic parameters, abnormal cleavage patterns, and known implantation results.
Result(s): All included embryos were categorized either retrospectively or prospectively into 7 grades (A+, A, B, C, D, E, F). Qualitative deselection parameters included poor conventional day 3 morphology, abnormal cleavage patterns identified via time-lapse monitoring, and <8 cells at 68 hours postinsemination. Quantitative parameters included time from pronuclear fading (PNF) to 5-cell stage and duration of 3-cell stage. KID implantation rates of embryos graded from A+ to F were 52.9%, 36.1%, 25.0%, 13.8%, 15.6%, 3.1%, and 0 respectively (area under the curve [AUC] = 0.762; 95% confidence interval [CI], 0.701–0.824), and a similar pattern was seen in either IVF (AUC = 0.721; 95% CI, 0.622–0.821) or ICSI embryos (AUC = 0.790; 95% CI, 0.711–0.868). Preliminary prospective validation using 66 KID embryos also showed statistically significant prediction in Medicult (AUC = 0.750; 95% CI, 0.588–0.912) and Vitrolife G-Series (AUC = 0.820; 95% CI, 0.671–0.969) suites of culture media.
Conclusion(s): The proposed model involving both qualitative and quantitative deselection effectively predicts day 3 embryo implantation potential and is applicable to all IVF embryos regardless of insemination method by using PNF as the reference starting time point. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Embryo, time-lapse, implantation, pronuclear fading, deselection

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After the initial attempts at applying time-lapse embryo selection to clinical IVF practice (1, 2), a number of publications have shown promising results using morphokinetic data to predict embryo implantation (1,3–6). One of the most influential morphokinetic grading algorithms was published by Meseguer et al. (1) in 2011. Regarded as one of the foundation clinical studies of human embryo morphokinetics, this algorithm has, however, been questioned more recently regarding its transferability between different laboratories (7–10). Embryo morphokinetic results are thought to be the subject of a number of factors, such as culture media (11), oxygen concentration in culture (12), patient population (13, 14),

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ovarian stimulating protocols (15), hormone levels in the female partner (16), or even sperm DNA fragmentation (17). As the transferability of previously published morphokinetic algorithms is likely to be influenced by these factors, one should be cautious when implementing an embryo morphokinetic algorithm that was constructed in a different laboratory. In addition, some qualitative deselection parameters identified via time-lapse imaging of embryos have been reported, showing an encouraging potential to identify embryos with a low probability to implant (18–21). The advantage of using such parameters for embryo deselection is their qualitative nature, with the measurement being either positive or negative and also independent of absolute cell cleavage timings. As a result, interlaboratory transferability may possibly be improved using algorithms encompassing such parameters.

The majority of published time-lapse studies to date are based on embryos fertilized via intracytoplasmic sperm injection (ICSI) (1, 4) rather than conventional IVF, largely due to the difficulty in defining sperm entry time in the latter. However, even in ICSI cases, the sperm entry time point for each individual embryo may also be imprecise as seen, for example, in particular time-lapse equipment such as the Embryoscope (Vitrolife), where all embryos cultured on the same slide share one single starting time point (22). Furthermore, nuclear mature oocytes were shown to extrude the second polar body at various timings (ranging from 0.70 to 10.15 hours post-ICSI) (23), indicating that metaphase II oocytes may have different degrees of cytoplasmic maturity at the point of sperm injection. Recently, pronuclear fading (PNF), which is a biological time point, has been proposed as an alternative reference starting time point, rather than insemination, which is a procedural time point (24). Using PNF enables IVF and ICSI embryos to be integrated into the same algorithm and minimizes the variations in early stage timings owing to the procedural issues (24). Based on the above findings, the current study aims to present a time-lapse deselection model for predicting the implantation potential of embryos regardless of insemination method, including both qualitative and quantitative measures of the growth of early cleavage human embryos.

MATERIALS AND METHODS

Patient Management and Embryo Culture

The retrospective part of this study included a total of 212 treatment cycles (84 IVF and 128 ICSI cycles; females age 34.63 $\pm$ 4.41 vs. 34.45 $\pm$ 4.51 years, not significant) performed at Fertility North between February 2013 and December 2014, with all transferred embryos having known implantation data (KID) (21). In total, 270 (105 IVF and 165 ICSI embryos) fully annotated KID embryos that had reached at least the 5-cell stage were analyzed after culture in the Medicult media suite (Origio). The prospective part of this study included [1] 36 KID embryos cultured in the Medicult media suite from 30 IVF/ICSI cycles (females ages 35.11 $\pm$ 4.03 years) performed between May and July 2015 and [2] 30 KID embryos cultured in the G-Series media suite (Vitrolife) from 23 IVF/ICSI cycles (females ages 35.97 $\pm$ 5.31 years, not significant) performed between July and September 2015. The use of the Embryoscope was registered as an innovative procedure with the Reproductive Technology Council (Department of Health, East Perth, Australia), and accordingly all participating couples gave consent to use the Embryoscope as an incubator for embryo culture. Retrospective data analysis was approved by the Human Research Ethics Committees at both Joondalup Health Campus and Edith Cowan University.

Ovarian stimulation, gamete collection, and insemination using either conventional IVF or ICSI were performed as described elsewhere (19), with oocyte collection being day 0. Gametes were prepared in the Universal IVF (Origio) or G-IVF Plus medium (Vitrolife). Fertilized oocytes were placed in the Embryoscope for 3 days of culture in either ISM1 (Origio) or G-1 Plus medium (Vitrolife) before uterine transfer. Culture conditions were set at 37°C with 6% CO_2, 5% O_2, and balanced N_2, with images taken every 10 minutes across seven focal planes of the embryos.

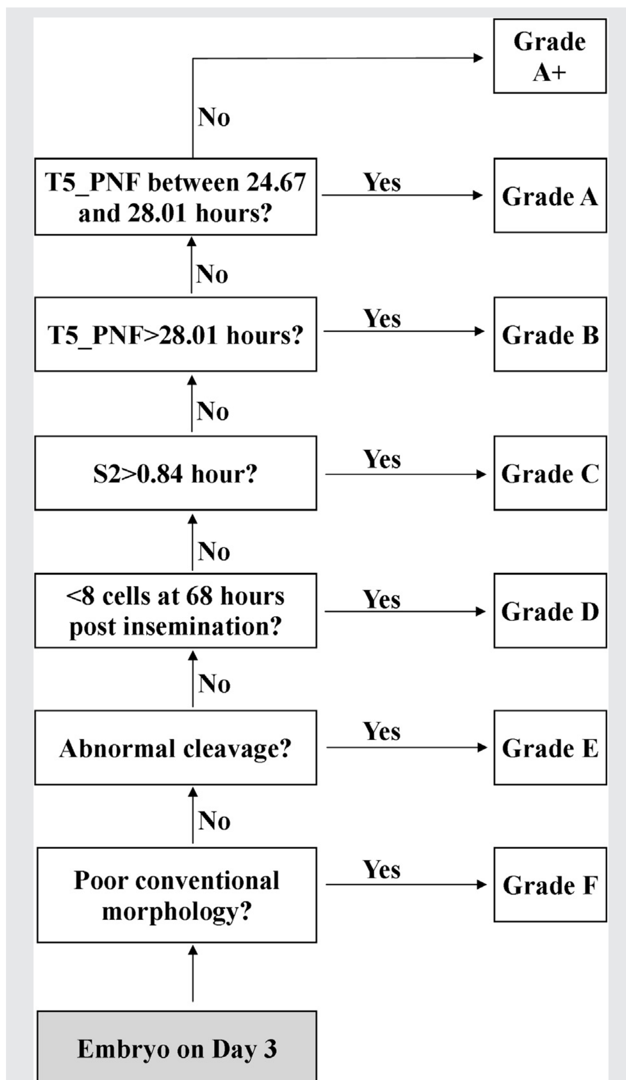
Morphokinetic and Conventional Assessment of Embryo Development

All the embryos cultured in the Embryoscope were retrospectively annotated on day 3 by one embryologist (Y.L.), using the Embryoviewer (Vitrolife) software. The timing parameters considered in the present study included time from pronuclear fading to 5-cell stage (T5_PNF, hour) and duration of 3-cell stage (S2, hour), which had been regarded as two major implantation predictors (t5 relative to sperm injection instead of PNF and s2) in previous publications (1, 5, 8). Qualitative parameters used for deselecting embryos were also recorded, including [1] direct cleavage (DC) where either the 2- or 4-cell stage was less than 5 hours (20, 21), [2] reverse cleavage (RC) where either daughter cells fused after cleavage division or the blastomere failed to divide after karyokinesis (19), and [3] <6 intercellular contact points (ICCP) at the end of the 4-cell stage (21). Conventional morphological assessment was also performed based on the embryo image captured at 68 hours postinsemination (hpi) according to criteria previously published (25), analyzing cell count, symmetry, and degree of fragmentation.

Grading of Day 3 Embryos using the Proposed Model

Day 3 embryos were graded either retrospectively or prospectively using a series of questions as illustrated in Figure 1. Briefly, [1] if one was determined to be a poor-quality embryo (PQE) according to conventional morphology assessment at 68 hpi, it is categorized a grade F, otherwise subject to further criteria; [2] if one had displayed abnormal cleavage pattern(s) such as DC, RC, or <6 ICCP at the end of the 4-cell stage, it is categorized a grade E, otherwise subject to further criteria; [3] if one had <8 cells at 68 hpi, it is categorized a grade D, otherwise subject to further criteria; [4] if one had S2 > 0.84 hour, it is categorized a grade C, otherwise subject to further criteria; [5] if one had T5_PNF > 28.01 hours, it is categorized a grade B, otherwise subject to further criteria; [6] if one had T5_PNF

FIGURE 1



Flow chart for embryo classification using both qualitative and quantitative parameters to assess implantation potential.

Liu. Time-lapse embryo deselection. Fertil Steril 2015.

between 24.67 and 28.01 hours, it is categorized a grade A, otherwise A+. Steps 1–3 are the qualitative component, while steps 4–6 are the quantitative component. Rather than using insemination as the reference starting time point, PNF was used to minimize early-stage timing variations between IVF and ICSI embryos (as reported elsewhere) (24).

ET and Confirmation of Implantation

One or two embryos from the cohort per treatment cycle were selected for uterine transfer at the end of 3 days in culture, judged using the conventional grade in the retrospective analysis period or using the proposed model in the prospective period. All ET procedures were performed using a stipulated standardized technique under ultrasound guidance. Viable implantation was confirmed at 7 weeks of pregnancy by the detection of fetal heartbeat under ultrasound. Aiming at comparing embryos displaying different features instead of treatment cycles, the present study included only KID embryos as previously defined (21).

Statistical Analysis

Proportion data were analyzed using Fisher's exact test. Continuous parameters were compared via Student's t test. The prediction power of the proposed model on the implantation outcome of embryos was tested via receiver operating characteristic (ROC) with area under the ROC curve (AUC) test. Logistic regression was used to assess the contributing strength of parameters that are potentially associated with implantation outcome. All statistical analysis was performed using Statistical Package for the Social Sciences 20.0 (SPSS), and $P<.05$ was considered statistically significant.

RESULTS

Embryo Deselection using Qualitative Parameters

A total of 270 transferred embryos with KID results were retrospectively annotated for different types of abnormal biological events identified via either conventional morphology assessment (PQE and <8 cells at 68 hpi) or time-lapse monitoring (DC, RC, and <6 ICCP at the end of the 4-cell stage). The implantation rate per embryo for those showing at least one of the above qualitative abnormalities was 7/104 (6.7%), and this was significantly lower than for those with no such abnormalities (57/166, 34.3%; $P<.01$). Logistic regression analysis showed that multinucleation (MN) at either the 2- (odds ratio [OR] = 1.222; 95% confidence interval [CI], 0.582–2.564; $P=.596$) or 4-cell stage (OR = 0.748; 95% CI, 0.137–4.069; $P=.737$) was not significantly associated with KID implantation outcome after considering abnormal biological events (OR = 0.062; 95% CI, 0.015–0.260; $P=.000$) including PQE, DC, RC, and <6 ICCP. Therefore, MN was not included into the deselection criteria in the present study.

Using Quartiles to Determine Optimal Ranges for Timing Parameters

After the removal of embryos (n = 104) showing abnormal biological events as described above, a total of 166 embryos were included for further morphokinetic analysis. Logistic regression analysis considering major morphokinetic parameters indicated no significant associations between implantation outcomes and T3_PNF (OR = 1.122; 95% CI, 0.512–2.460; $P=.773$) and CC2 (OR = 0.937; 95% CI, 0.418–2.102; $P=.875$) but significant with S2 (OR = 0.445; 95% CI, 0.221–0.898; $P=.024$) and nearly significant with T5_PNF (OR = 0.800; 95% CI, 0.628–1.019; $P=.070$). Therefore, S2 and T5_PNF were subsequently chosen as candidate parameters to be included into the deselection model.

Table 1 demonstrates the implantation rates of these embryos according to quartile ranges of S2, T5_PNF, T3_PNF, and CC2, where values dividing quartile ranges were determined by the SPSS software. Embryos with S2

TABLE 1

Quartile ranges of quantitative parameters and corresponding KID implantation rates after qualitative deselection (n = 166).

	S2		T5_PNF		T3_PNF		CC2	
Quartile	Range, h	Implantation rate (%)	Range, h	Implantation rate (%)	Range, h	Implantation rate (%)	Range, h	Implantation rate (%)
First	<0.17	14/43 (32.6)	<24.67	19/42 (45.2)[c]	<12.85	18/39 (46.2)	<10.34	15/38 (39.5)
Second	0.17–0.34	21/53 (39.6)[b]	24.67–25.99	14/45 (31.1)	12.85–13.67	16/51 (31.4)	10.34–10.84	14/47 (29.8)
Third	0.35–0.84	18/41 (43.9)[a]	26.00–28.01	19/53 (35.8)	13.68–14.51	15/45 (33.3)	10.85–11.67	22/49 (44.9)[d]
Fourth	>0.84	4/29 (13.8)[a,b]	>28.01	5/26 (19.2)[c]	>14.51	8/31 (25.8)	>11.67	6/32 (18.8)[d]
Total	–	57/166 (34.3)	–	57/166 (34.3)	–	57/166 (34.3)	–	57/166 (34.3)

Note: S2 = duration of 3-cell stage; T5_PNF = time from pronuclear fading to 5-cell stage; T3_PNF = time from pronuclear fading to 3-cell stage; CC2 = duration of 2-cell stage.
[a,b,c,d] Same superscript indicates statistical significance ($P< .05$)

Liu. Time-lapse embryo deselection. Fertil Steril 2015.

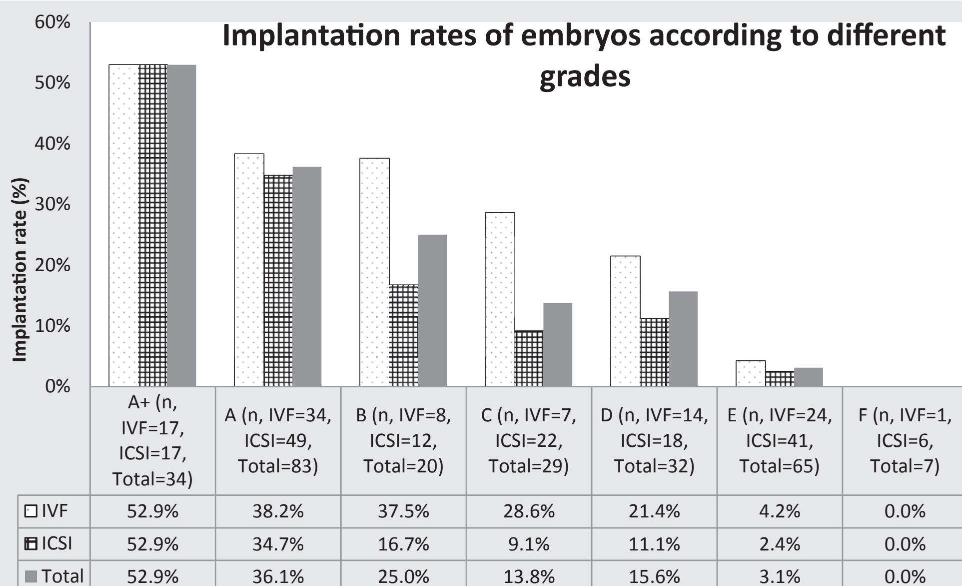
within the fourth quartile range had a significantly reduced implantation rate compared with those within the second (13.8% vs. 39.6%, $P<.05$), third (13.8% vs 43.9%, $P<.05$), or first three quartile ranges (13.8% vs. 38.7% [53/137], $P<.05$); whereas embryos with T5_PNF within the first quartile range had a significantly higher implantation rate (45.2% vs. 19.2%, $P<.05$) than those with T5_PNF within the fourth quartile range. As a result, the third quartile (0.84 hour) for S2 and the first and third quartiles (24.67 and 28.01 hours, respectively) for T5_PNF, were used to categorize ranges for these two timing parameters.

Implantation Prediction of the Proposed Model

A total of 270 KID embryos were retrospectively categorized into 7 grades (A+ to F) as described in Figure 1, and the implantation rates of embryos in each grade are shown in Figure 2. Both IVF and ICSI embryos showed similar descent in implantation rates from the top grade (A+) to the lowest grade (F). After converting the grades (from F to A+) to numeric ranking (from 1 to 7), ROC analysis showed significant prediction of the proposed model on implantation outcome in the IVF model (AUC = 0.721; 95% CI, 0.622–0.821; P=.000), ICSI model (AUC = 0.790; 95% CI, 0.711–0.868; P=.000), or a combination of two (AUC = 0.762; 95% CI, 0.701–0.824; P=.000).

The proposed model was also prospectively validated using two different culture media suites. KID implantation rates for embryos with different grades and ROC analysis are presented in Table 2. The prediction value of the proposed model showed a similar pattern in either the Medicult (AUC = 0.750; 95% CI, 0.588–0.912; P=.033) or G-Series medium (AUC = 0.820; 95% CI, 0.671–0.969; P=.006).

FIGURE 2



Implantation rates of KID embryos (n = 270) fertilized via IVF (n = 105) or ICSI (n = 165) according to different grades assessed using the proposed model.

Liu. Time-lapse embryo deselection. Fertil Steril 2015.

TABLE 2

Prospective validation of proposed day 3 embryo deselection model in two different culture media suites (n = 66).

	KID implantation rates (%)		
Grade	**Medicult**	**G-Series**	**Total**
A+	37.5 (3/8)	66.7 (4/6)	50.0 (7/14)
A	36.4 (4/11)	38.5 (5/13)	37.5 (9/24)
B	16.7 (1/6)	0/2	12.5 (1/8)
C	0/0	0/1	0/1
D	0/2	0/2	0/4
E	0/9	0/5	0/14
F	0/0	0/1	0/1
Total	22.2 (8/36)	30.0 (9/30)	25.8 (17/66)
AUC	0.750	0.820	0.783
(95% CI)	(0.588–0.912)	(0.671–0.969)	(0.674–0.893)
P value	.033	.006	.001

Note: ROC analysis was performed after converting embryo grades (F to A+) to numeric rankings 1–7. Statistical results are expressed as AUC and 95% CI.

Liu. Time-lapse embryo deselection. Fertil Steril 2015.

DISCUSSION

Embryo selection is one of the most critical tasks in IVF laboratories. As the avoidance of multiple pregnancy after IVF treatment is gaining greater global consensus, elective single ET supported by improved embryo selection is being heavily promoted to maintain pregnancy rates while reducing the risk of multiple pregnancy (26–28). More than a decade ago, efforts were made in pioneer studies to model top-quality cleavage-stage embryos, with an up to 47% implantation rate of double ETs in women younger than 38 years (29), which suggests that the clinician should consider a single ET when a top-quality embryo is available (28). Alternatively, ETs using a lower number of blastocysts after further selection via extended culture have been shown to yield pregnancy rates similar to those of transfers using higher numbers of cleavage-stage embryos (30). Although this technique is being widely used today in IVF laboratories, reports with conflicting conclusions regarding the obstetric, perinatal, and neonatal outcomes after transfer of blastocyst and cleavage embryos are still present in the literature (31–35). However, systematic review and meta-analysis seem to show better cumulative clinical pregnancy rates after the transfer of cleavage-stage embryos owing to higher use rates of embryos and less cancelled transfer owing to no blastocysts being available (36).

Although relatively expensive, time-lapse technology enables the collection of significantly increased volumes of data regarding embryo development without interrupting the culture conditions. Associations were reported to exist between embryo morphokinetic parameters and their subsequent implantation potential (1, 4) or ploidy status (37). However, more recent evidence has shown potential issues in the transferability of Meseguer et al.'s algorithm between different clinics (7–10), possibly due to different embryo growth rates in diverse settings (i.e., oxygen concentration [12], culture media [11], or patient population [13, 14, 17]) in different laboratories. Therefore, embryo deselection using qualitative parameters rather than quantitative measurements may improve interlaboratory reproducibility as the qualitative parameters are independent of absolute cleavage timings (10), although further investigation is required to support this theory. It would be of great practical value for new time-lapse equipment users to start with while collecting KID data for determination of their own optimal ranges in the quantitative parameters. In the proposed qualitative deselection model, the parameters included were extended by adding an additional conventional measure (<8 cells at 68 hpi), based on the qualitative model we have recently published (21), where both conventional (i.e., PQE in conventional grading) and time-lapse parameters (DC, RC, <6 ICCP at the end of the 4-cell stage) were included. All parameters were significantly ($P<.01$) associated with reduced implantation, except for PQE ($P>.05$), largely due to the small number (seven) of embryos affected. So presumably, with the consideration of qualitative deselection parameters at transfer, the implantation rate of embryos included in the current data set could potentially be improved up to 34.3% (57/166) from 23.7% (64/270). Earlier studies have explored the potential impact of the presence of MN in cleavage-stage embryos on their subsequent implantation via either static observations (38) or time-lapse monitoring (39). MN was not included in the present study as one of the deselection criteria, because logistic regression analysis showed no significant impact of MN on implantation when considering abnormal biological events. The formation of MN could partially be explained by the occurrence of certain abnormal cleavage patterns (e.g., MN formation after RC as illustrated in a previously published video clip [19]), and its impact could be diluted by the inclusion of such parameters.

A major advantage of time-lapse culture compared with the traditional protocol of static observations is the continuous monitoring of embryos, which enables the observation of most biological events during early embryonic development (4, 6). However the precision of the previously published timing systems (22) is limited owing to [1] technical issues in the routine IVF laboratory protocol (i.e., uncertain sperm entry time point t0 in IVF embryos) and [2] the design of particular equipment such as the Embryoscope (i.e., only a single t0 can be defined per slide, each of which carries a number of oocytes) (24). Considering these issues, the current study presents a deselection model using an alternative biological reference starting time point of PNF for both IVF and ICSI embryos as reported previously (24), which greatly improves the certainty and precision in timing parameters. Retrospective data analysis in the present study showed consistent results in implantation rates of embryos between IVF and ICSI cases by using the same optimal ranges of T5_PNF and S2 (Fig. 2). However, it must be noted that embryo selection using morphokinetic parameters should involve individual laboratory specific optimal ranges, owing to potential interlaboratory variation in embryo growth as described elsewhere (10–17). Therefore, it is highly recommended that each IVF laboratory develop its own cutoff values for quantitative parameters based on KID data, although methodology may be adopted from published studies. This is illustrated by the parameter showing the duration of the 3-cell stage, S2, a

morphokinetic selection/deselection tool used by both Meseguer et al. (1) and ourselves. Meseguer et al.'s original data had a cutoff of 0.76 hour, which compares well with the present study of 0.84 hour. However, this apparent agreement was the result of Meseguer et al. using the median value (1), but the present study uses the third quartile. Closer examination of the interquartile range shows that the embryos in the present study were growing faster (interquartile range of 0.17–0.84 hour) compared with Meseguer et al.'s (interquartile range of 0.3–1.5 hour), confirming the need for morphokinetic cutoffs to be laboratory specific. The different embryo morphokinetics in the two laboratories observed may be attributed to the different settings, including the oxygen concentration (12) used in the incubators, culture media (11), and patient factors (13, 14, 17). Furthermore, the reduced sample size in both studies should also be considered; therefore, differences or similarities in embryo morphokinetics ought to be further investigated in future large-scale studies. However, it is encouraging that the prospective validation of the proposed model in the present study was unaffected when simply switching between two different culture media suites within the same laboratory (Table 2).

Meseguer et al. (1) reported that the implantation rates of embryos followed a bell-shaped curve (16%, 37%, 40%, and 14%, respectively) according to four quartile ranges of t5 relative to sperm injection. The distribution of implantation rates of 270 KID embryos included in the present study followed a similar pattern according toT5_PNF (Supplemental Fig. 1A). Interestingly, however, after applying the deselection criteria, the implantation rates of the remaining embryos ($n = 166$) displayed a linear pattern (Table 1) along the timings of T5_PNF (Supplemental Fig. 1B). The explanation for this might be the removal of the DC embryos (the 2- or 4-cell stage is less than 5 hours) from the cohort, which has shortened T5_PNF values but led to limited implantation potential. Eliminating abnormal cleavage patterns such as DC may result in improved embryo selection for new time-lapse users when their own optimal ranges are not yet known. For example, embryo(s) with the shortest T5_PNF in the cohort could be selected after excluding embryos with abnormal cleavage patterns (including DC) without having to be restricted by the unknown optimal timing window.

Annotations of all embryos included in the present study were performed by one embryologist (Y.L.), to minimize the interobserver variations. Using intraclass correlation coefficients (ICCs) analysis, a previous study has shown good reproducibility in annotations of PNF and cleavage divisions up to the 8-cell stage of human embryos via time-lapse monitoring in both inter- (ICC ranging from 0.809 to 0.999) and intraobserver (ICC ranging from 0.87 to1) analysis (40). Apart from the above timing parameters, the proposed model also includes the conventional grading component and abnormal cleavage patterns. The conventional grading system was reported to have satisfactory inter- and intraobserver agreement, which could be further improved via training sessions (41, 42). However, the assessment of inter- and intraobserver reproducibility in identifying abnormal cleavage patterns has not yet been fully investigated and requires future large-scale studies. Similar to conventional grading systems, additional training and comparison of participating embryologists could improve the consistency of results. Furthermore, the small sample size of the present study requires large-scale prospective randomized controlled trials to validate the proposed model.

To conclude, both qualitative and quantitative deselections are powerful tools to aid embryo selection via continuous monitoring. The deselection model proposed in the present study has been shown to work in the one laboratory, thus the transferability of this model between laboratories with independent patient populations, particularly the qualitative component, requires further investigation.

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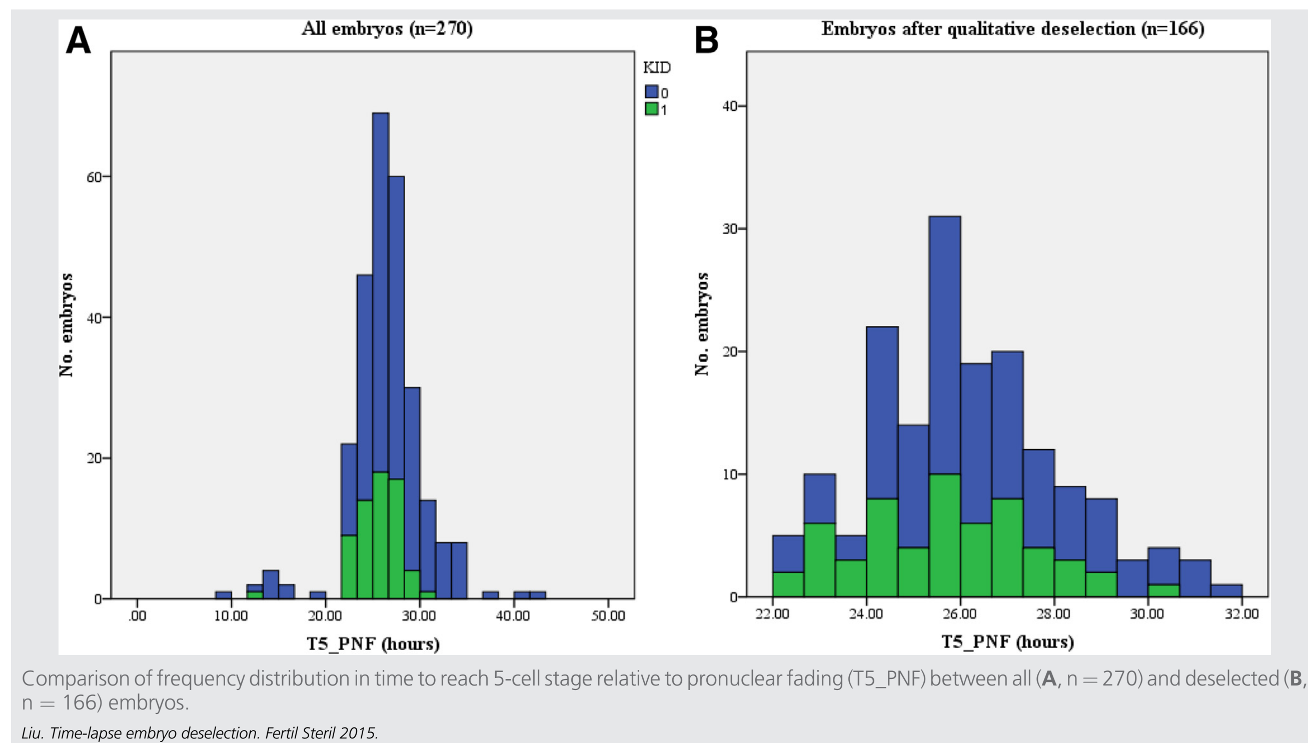
SUPPLEMENTAL FIGURE 1



Comparison of frequency distribution in time to reach 5-cell stage relative to pronuclear fading (T5_PNF) between all (**A**, n = 270) and deselected (**B**, n = 166) embryos.

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