Reproductive Biology 18 (2018) 355–360

Contents lists available at ScienceDirect

Reproductive Biology

journal homepage: www.elsevier.com/locate/repbio

Predicting live birth by combining cleavage and blastocyst-stage time-lapse variables using a hierarchical and a data mining-based statistical model

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ARTICLE INFO

Keywords:
Time-lapse monitoring
Blastocyst culture
Predictive models
Data mining
In-vitro fertilization

ABSTRACT

Prolonged embryo culture is increasingly used as a way of improving pregnancy rates, especially in the context of single embryo transfer. So far, only a handful of studies examined the relation between implantation potential and time-lapse parameters extracted from later stages (morula and blastocyst) of embryo development. For this retrospective study all 285 single vitrified-thawed blastocyst transfers (SVBT) from all consecutive unselected patients whose fertilized oocytes were submitted to time-lapse monitoring (TLM) from a two-year cohort were analysed. Two different statistical models were created; a hierarchical one including the two strongest live birth (LB) predictors (t2 and texpB_2) and a more complex model based on principal component analysis (PCA) and logistic regression methods. The first, four-category, hierarchical model effectively distinguished between blastocysts of increasing LB rates (8, 30, 40, 53%). For the second data-mining model quartiles of the created Sc parameter had increasing LB rates (12, 19, 40, 49%). AUC values were comparable for both models (0.723, 95CI %:0.66–0.79 versus 0.717, 95CI%:0.65–0.78). The combination of cleavage- and blastocyst-stage variables through hierarchical or data mining-based algorithms was used successfully to predict live birth. However, due to the lack of internal / external validation the predictive capacities of this model could differ largely in different datasets.

1. Introduction

In recent years time-lapse monitoring (TLM) technology has emerged as a potentially promising way to improve the classical morphological selection of embryos. Since the publication of the first hierarchical model predicting embryo implantation [1] several groups tried to establish sophisticated algorithms that could be used to predict blastocyst formation, clinical pregnancy or aneuploidy status [2]. Most of these focused on cleavage-stage morphokinetic variables only, despite the fact the prolonged embryo culture is increasingly used as a way of improving pregnancy rates especially in the context of single embryo transfer [1,3,4]. Although some newly developed algorithms (such as KidScoreD5) do incorporate blastocyst-stage parameters, time-lapse variables extracted from later stages of embryo development are still relatively under-investigated [5]. So far, only a handful of published models examined the relation between implantation potential and blastocyst-stage morphokinetic parameters. Whereas a UK model has only used two closely-related blastocyst variables (tSB and tB), a Spanish algorithm incorporated a combination of two variables from different developmental stages (s3 and tEB) [6–8].

Another important issue is related to the fact the almost all published predictive algorithms were developed in a single centre ("in-house") and only few of them (KidScore, Eeva) involved datasets from multiple centres [4,8–10]. By now, several studies have shown that published models could not be directly applied to different clinical settings although they perform well on the dataset where they were developed [11–14]. We think that this is especially relevant for the clinical setting of our centre which is fundamentally different from the routine clinical practice of other European or US centres. In fact, our patient population is highly biased towards advanced-aged, low ovarian responders with a history of previous unsuccessful fertility treatments who are routinely submitted to an innovative protocol of mild ovarian stimulation, elective vitrification and subsequent vitrified-warmed blastocyst transfer [15]. Consequently, we have felt a strong need for building a centre-specific time-lapse prediction model based on our own data, because it is unlikely that published models would perform adequately in our unique Japanese setting.

Regarding the choice of potential statistical methods for developing

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https://doi.org/10.1016/j.repbio.2018.10.006
Received 25 April 2018; Received in revised form 17 October 2018; Accepted 19 October 2018
Available online 31 October 2018
1642-431X/

a successful centre-specific predictive algorithm one approach is to establish hierarchical scores where: 2 or 3 of the strongest (categorical) morphokinetic variables alongside with potential other deselection parameters are selected and split the analysed embryo cohort into 4–10 subgroups. The drawback is that the order of predictors is somewhat arbitrary affecting the performance of the model. More recently even more robust statistical methods involving data-mining approaches were also developed that could potentially improve the between-centre applicability of resulting algorithms [9,16,17].

Thus, the aim of the present retrospective analysis, in a single-centre cohort of unselected Japanese infertile patients undergoing single blastocyst transfer following mild ovarian stimulation, was to establish and compare different statistical models that could predict live birth with a high predictive power.

2. Materials and methods

2.1. Study patients and follow-up

All consecutive infertile patients whose embryos were submitted to prolonged embryo culture in a time-lapse monitoring incubator between October 2012 and December 2014 at our centre were included in this retrospective analysis. The outcome of subsequent blastocyst transfers (n = 326) performed until December 2016 was followed-up until the last live birth occurred (August 2017). After excluding repeat cycles (n = 27) and fresh transfers (n = 14), 285 single vitrified-thawed blastocyst transfers (SVBT) were analysed. Institutional Review Board approval was not required for the present study, because in our centre all patients undergoing IVF treatment gave informed consent to the anonymous use of their data for retrospective reviews.

2.2. Natural cycle IVF and minimal ovarian stimulation protocols, oocyte retrieval and fertilization with conventional IVF or ICSI

Clomiphene citrate (CC) or letrozole-based minimal stimulation was used in most of cycles whereas unstimulated natural cycle IVF or other mild stimulation protocols represented only a smaller proportion of cases [18,19]. Transvaginal ultrasound guided oocyte retrieval was performed without anaesthesia using a very thin 21–22 G needle (Kitazato, Japan). Mature (MII) oocytes were inseminated by conventional IVF or ICSI.

2.3. Prolonged embryo culture in a TLM incubator, elective vitrification and embryo transfer

In our centre elective blastocyst culture, vitrification and subsequent vitrified-warmed single blastocysts transfer (SVBT) are routinely practiced [18]. Prolonged embryo culture was performed in a time-lapse incubator (EmbryoScope, Vitrolife, Gothenburg, Sweden) according to previously described methodology [1]. Briefly, normally fertilized 2PN zygotes were incubated at 37 °C in a 5% O_2 atmosphere and cultured individually until day 2–3 in a Quinn's Advantage Cleavage Medium (SAGE, United States) and subsequently until day 5–7 in a Quinn's Advantage Blastocyst Medium (SAGE, United States) [20]. During cleavage-stage selection was performed by classical morphology criteria according to published consensus criteria [21]. If reaching a uniform expansion of approximately 170 μm s blastocysts were electively vitrified using the Cryotop® vitrification method (Kitazato, Japan) [22,23] and were transferred in the following 1–3 months after oocyte retrieval. As general clinic policy, only single embryo transfers are performed at our centre.

2.4. Time-lapse annotations

Images in several focal planes were taken and recorded every 20 min shortly after insemination up until a period of approximately 160 h. Early (PNf, t2-t9) and late (full compaction at morula stage, start of blastulation and full blastocyst) morphokinetic time points as well as evenness at the 2-cell stage were scored in accordance with published consensus criteria [24]. To determine the degree of blastocyst expansion more objectively, two additional variables (texpB_1 and $_2$) were also introduced: the time point when the horizontal diameter of the expanded blastocyst reached 130 and 160 μm s, respectively. The exact degree of expansion was assessed by looking at a few consecutive frames and choosing the one where the horizontal diameter - as measured conveniently by the drawing tool of the EmbryoViewer - first reached the desired threshold. Annotations were performed retrospectively using EmbryoViewer by two experienced embryologists and re-checked by a third person. To control for different insemination techniques static morphokinetic variables were standardized to the common post-fertilization time point of pronuclear fading (tPNf) [20,25,26].

2.5. Outcome measures and the development of two statistical models

The main clinical outcome measure was live birth per single blastocyst transfer. Baseline cycle characteristics were compared between groups with and without live birth. $P < 0.01$ was considered statistically significant.

To establish a first, hierarchical prediction model, each morphokinetic variable was checked for the existence of "optimal live birth ranges" with the highest proportion of LBs. The whole range of values for each TLM variable was displayed in histograms with bins of equal size (0.5 h for t2-t4, cc2a-b; 1.5 h for t6; 2.5 h for blastocyst-stage variables). For each bin, the percentage of LBs in each of the corresponding columns was calculated and those with an above average rate were grouped together Supplemental Fig. 1. In this way those (ten) TLM variables where such an optimal range could be observed were converted into categorical ones (inside versus outside of the optimal live birth range) for further multivariate logistic regression analysis (also adjusting for five patient-, and cycle-related confounders) Supplemental Table 1. Two of the strongest (texpB_2 and t2) were combined to develop a four-category, hierarchical model.

A second, more complex predictive model was also developed according to the methodology published in Milewski et al. [17]. Analysed morphokinetic parameters (except s2 and s3) did not have a monotonic character (very low as well as very high values were not good predictors of live birth) [2,16,17,27,28]. For all morphokinetic parameters (except s2 and s3) the distance between the parameter value and the median value in the live birth group (called, respectively, t2med, t3med, …) was calculated according to the formula:

$$tmed = | t - Median(t) |$$

Supplemental Table 2. shows correlations (Spearman coefficients) between morphokinetic parameters. The predictive model for live birth was constructed based on principal component analysis (PCA) and logistic regression analysis. The PCA technique is a data-mining method that relies on transformation of an initial correlated set of features into new uncorrelated variables called principal components. This method may be applicable to regression analysis, when it is impossible to include all variables in a model because of multicollinearity. The PCA method requires standardization of variables according to the mean and standard deviation values [17]. Therefore, for all tmed variables, as well as for s2 and s3, standardized parameters were created (t2st, t3st, …), according to the formula:

$$tst = (tm - Mean(tm)) / SD(tm)$$

After application of the PCA algorithm, the matrix of coefficients αnm was generated. Based on this matrix, seventeen principal components fn were calculated, as a linear combination of standardized tst parameters:

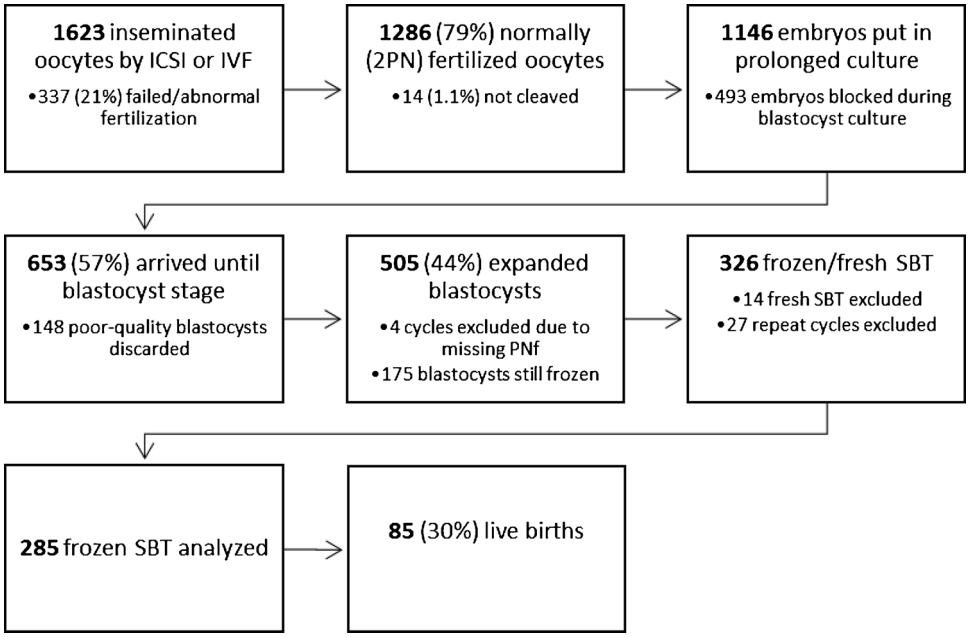

Fig. 1. Flow chart of study events.

$$fn = \alpha n\ t2st * t2st + \alpha n\ t3st * t3st + \ldots + \alpha n\ s3st * s3st$$

Supplemental Table 3. shows coefficients of the new factors obtained using the PCA method. Then, a univariate logistic regression analysis for live birth as a dependent variable was conducted. Supplemental Table 4. shows results of the univariate logistic regression analysis. Next, considering the variables whose significance was confirmed in univariate logistic regression, a multivariate logistic regression model was created Supplemental Table 5. Based on the coefficients determined in the multivariate logistic regression model, parameter Sc was created as the sum of the products of the four parameters multiplied by the corresponding coefficients. It is described by the formula:

$$Sc = 0.184 * f1 + 0.161 * f2 - 1.047 * f12 - 0.103 * Female\_age$$

For both prediction models, the area-under the curve values (AUC) with 95% confidence intervals were calculated.

3. Results

3.1. Baseline cycle characteristics and clinical outcome

During the study period 501 time-lapse monitored expanded blastocysts were obtained from all consecutive infertile patients whose embryos were submitted to prolonged embryo culture in a time-lapse incubator. Subsequently, a total of 326 single blastocyst transfers were performed of which - after excluding repeat cycles or fresh transfers - 285 were analysed. The overall live birth rate per (single) embryo transfer was 30% (85/285). The flow chart of study events is summarized in Fig. 1. Baseline characteristics of the embryo transfer cycles according to live birth are summarized in Table 1.

3.2. Results of a hierarchical model

The examination of detailed histograms revealed that areas with a significantly higher proportion of LBs ("optimal LB ranges") existed for six cleavage-stage (t2, t3, t4, t6, cc2a, and cc2b) and four blastocyst-stage variables (tearlyB, tBfull, texpB_1 and texpB_2). Unadjusted and adjusted odds ratios showing the association between live birth and inside/outside categories are shown in Supplemental Table 1.

Table 1
Baseline cycle characteristics in groups with or without live birth.

Embryo transfer cycles	Live birth (n = 85)	No live birth (n = 200)	p
Female age, years	36.9 ± 3.7	38.7 ± 4.0	< 0.0001 [a]
BMI, (kg/m^2)	20.4 ± 2.2	20.7 ± 2.3	0.36 [a]
Basal FSH, (IU/mL)	8.9 ± 5.1	9.2 ± 4.9	0.57[a]
Primary infertility, n (%)	59 (69)	129 (65)	0.82 [b]
Nulliparous, n (%)	75 (88)	181 (91)	0.56 [b]
No IVF treatment (at other centers), n (%)	40 (47)	74 (37)	0.11 [b]
Current cycle rank			0.13 [b]
1–2 cycle, n (%)	27 (32)	57 (29)	
3–4 cycle, n (%)	36 (42)	67 (34)	
5 or higher, n (%)	22 (26)	76 (38)	
Stimulation type			0.35 [b]
Natural cycle, n (%)	8 (9)	13 (6.5)	
Clomiphene, n (%)	66 (78)	148 (74)	
Letrozole, n (%)	11 (13)	35 (18)	
Other, n (%)	0 (0)	4 (2)	
Retrieved eggs, n	6.5 ± 3.4	5.7 ± 3.4	0.05 [a]
Male partner age, years	38.4 ± 4.4	40.4 ± 5.2	0.003 [a]

[a] Mann-Whitney *U* test.
[b] Chi-squared test.

Combining two of the strongest variables (t2 and texpB_2) resulted in a simple, four-category ("A" to "D") hierarchical model Fig. 2. The model effectively distinguished between blastocysts with increasing live birth potential with an AUC value of 0.723, 95CI%: 0.66–0.79.

3.3. Results of a PCA-based, composite predictive model

After dividing the embryos into four groups according to quartiles and the median value of the Sc parameter (Q1-Q4), statistically significant differences ($p < 0.0001$) in live birth rates were found between the studied groups Table 2. The area under the ROC curve was AUC = 0.717 (95% confidence interval: 0.65–0.78). ROC curves of the hierarchical and data-mining based models are compared in Fig. 3.

4. Discussion

Our single-centre study, involving a retrospective cohort of single

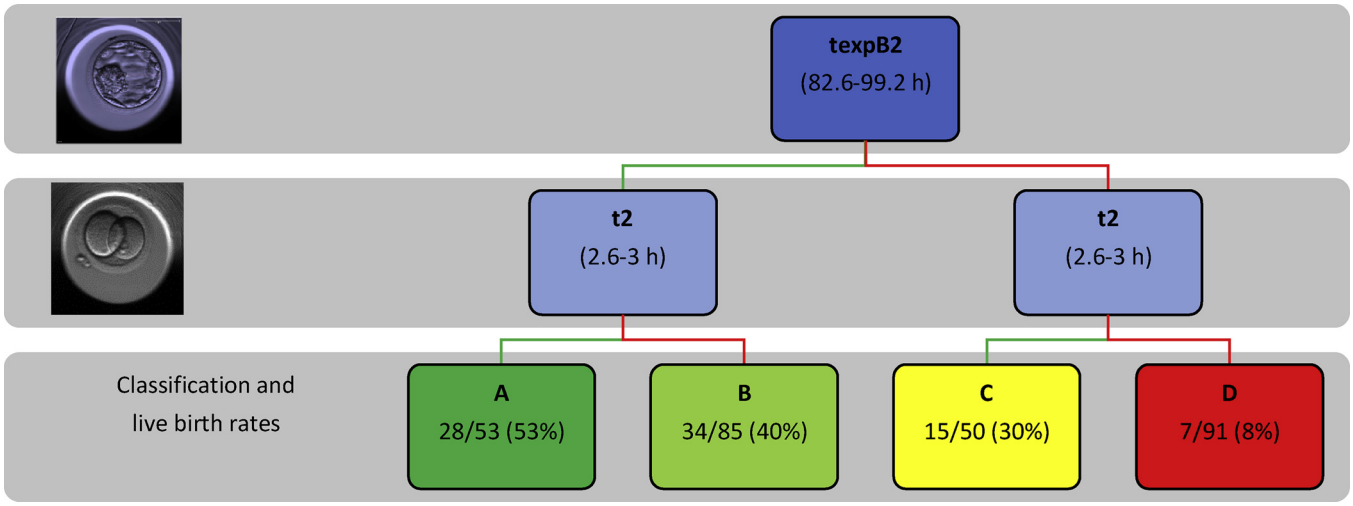

Fig. 2. Four-category, hierarchical model (the green and red lines indicate being inside or outside of the indicated range for each morphokinetic variable).

Table 2
Live birth rates for quartiles of the Sc parameter.

Quarter (N)		C1 (67)	C2 (67)	C3 (67)	C4 (67)
LB rate	N	8	13	27	33
	%	11.94%	19.40%	40.30%	49.25%

embryo transfers of time-lapse-monitored blastocysts, has found that by using two different methodologies cleavage- and blastocyst-stage morphokinetic variables could be successfully combined to create statistical algorithms for predicting live birth. Both the simpler, hierarchical and the more complex, data mining-based model had a similarly high predictive power and could be used effectively to distinguish between blastocysts of increasing implantation potential.

In recent years an increasing number of TLM studies focused on finding morphokinetic variables that could be predictors of embryo implantation [1,3,4,29,30]. Most of these studies involved cleavage-stage embryos only and managed to identify several early cleavage morphokinetic variables (such as t3, t5, cc2, s2 and cc3) that were consistently associated with successful implantation. On the other hand, so far there is a lack of published studies involving time-lapse monitored blastocysts and morphokinetic parameters extracted from later stages are still under investigation (morula, early and expanded blastocyst stage) [5]. A UK group did manage to establish a relationship, although using an indirect approach. Campbell and co-workers at first developed an aneuploidy risk model, based on 98 time-lapse-monitored blastocysts that also underwent preimplantation genetic screening. This model came to include two closely related blastocyst-stage morphokinetic variables: tSB and tB [31]. Subsequently, the authors have applied their new model to 88 KID blastocysts without PGS and have observed that aneuploidy risk classes also correlated well with foetal heart beat implantation and even live birth [6]. In a more recent, large Spanish study involving 832 transferred blastocysts, a four-category implantation model was established using tEB and s3, that could distinguish between blastocysts of decreasing implantation potential

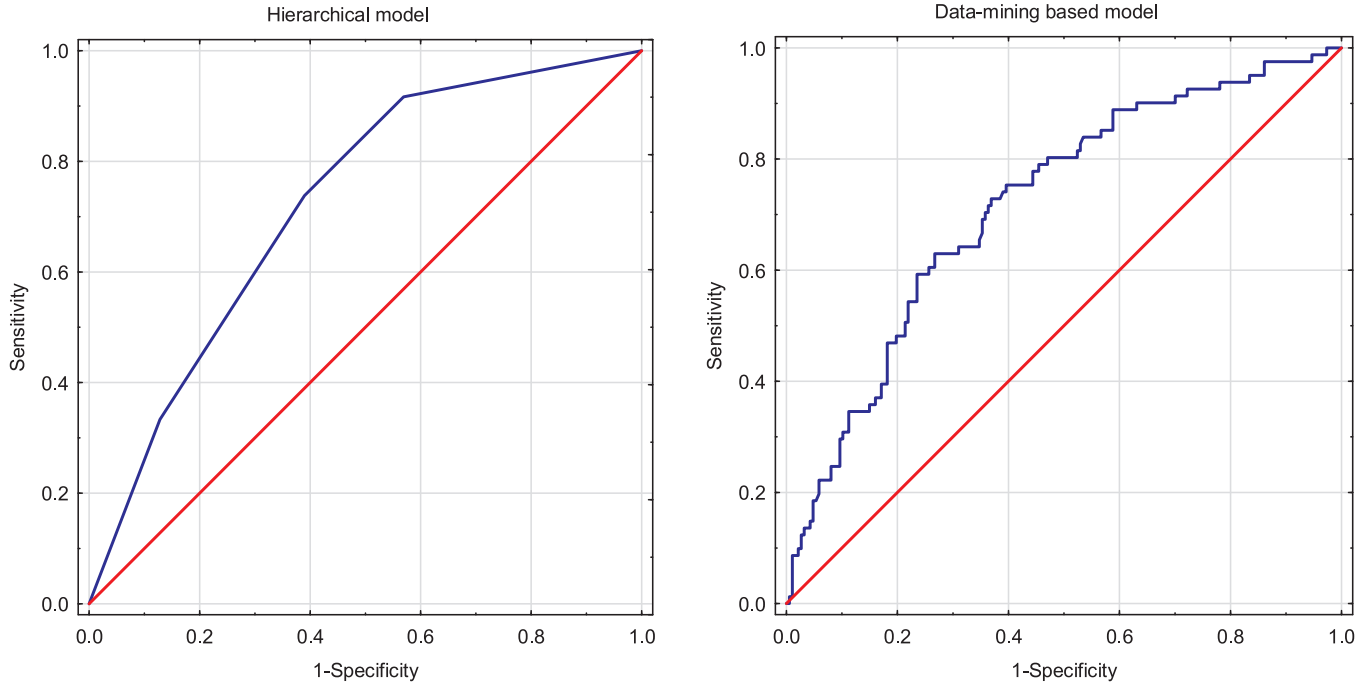

Fig. 3. ROC curves for the two statistical models.

(from 72.2 to 39.7%). However, the model's AUC was low (0.591) and remained similar (0.596) after internal validation on an independent dataset [8].

Regarding the statistical methodologies used for establishing predictive algorithms so far most published models were hierarchical scores [1,4,6,32,33]. In these, relevant TLM variables are first converted to categorical ones (inside-outside optimal range) and organized hierarchically. The earliest model developed by the Spanish group pioneering TLM involved 247 KID embryos and lead to the creation of a selection score involving three cleavage-stage variables classifying embryos into 8 subsets (t5, s2, and cc2a combined with other exclusion criteria). With lower time-lapse categories implantation rates also decreased gradually from 52% to 8% and the predictive model has reached an AUC of 0.720 [1]. An update of the same model involving 754 KID embryos have slightly modified it (this time including t3, cc2a, and t5) but did not improve the predictive properties of the original model. When validated on a different set of 1137 KID embryos from the same centre a low AUC score of 0.61 was obtained [4]. Another Australian model developed on retrospective dataset of 270 transferred day-3 KID embryos reached an unusually high AUC value at 0.762. In this model apart from cleavage stage variables (standardized t5 and s2) qualitative deselection criteria were also included possibly increasing its predictive ability (poor day-3 morphology, abnormal cleavage patterns and < 8cells after 66 h) [33].

In some of the most recent TLM studies, more robust statistical methodologies were used to establish predictive algorithms for blastocyst formation or embryo implantation. In two consecutive studies Milewski et al used a PCA-component based statistical method, which effectively circumvents issues related to multicollinearity present in hierarchical models. In an initial study for the development of a blastocyst formation model an AUC of 0.806 (good distinction) was reached [28]. In a follow-up study involving cleavage-stage variables (as well as embryo fragmentation and female age) the predictive algorithm for embryo implantation reached a somewhat lower AUC value of 0.70 [17]. Unsurprisingly, the predictive power was higher for blastocyst formation due to the confounding effect of other variables affecting embryo implantation (e.g. endometrial receptivity).

Even more recently, the manufacturer of Embryoscope used a five-year, multi-centric dataset of 3.275 transferred day-3 embryos to develop predictive models for blastocyst formation and implantation potential [9]. Using sophisticated statistical methods (e.g. decision tree approach, recursive partition and five-fold cross validation) a five-step model (KIDScore D3) was developed involving a combination of cleavage-stage morphokinetic variables up until t8. When validated on a different dataset of 11.218 embryos cultured until day 5, blastocyst formation was predicted with an AUC: 0.745, and when compared it was also superior to most previously published algorithms. However, implantation potential in the original development dataset was only predicted with a smaller AUC of 0.650. The authors have claimed broad applicability of this model due to a large, diverse dataset used for calibration, however, it will still need external validation and testing in everyday clinical practice.

There are also substantial differences between the models in their applicability: the final hierarchical model included only two of the strongest morphokinetic variables (t2 at the beginning and texpB_2 at the end of the developmental process of an embryo), whereas the more complex PCA-based model required all 18 TLM variables as well as female age (although the number of included variables was based on our decision only and could be reduced).

The main limitation of our study is the relatively limited number of 285 single blastocyst transfers. This sample size however, which was achieved in a single centre during a 2-year study period, is comparable with those published in other relevant TLM articles and is certainly sufficient to develop an initial, centre-specific model which later could be improved on and validated internally or externally [12].

In conclusion, in a group of unselected infertile patients undergoing minimal ovarian stimulation, our findings suggest that the combination of cleavage- and blastocyst-stage morphokinetic variables either through hierarchical or data mining-based statistical models could be successfully used to establish predictive algorithms for live birth, often surpassing the predictive power of previously published models. However, due to the lack of internal / external validation the predictive capacities of this model could differ largely in different datasets.

Authors' contributions

DB contributed to study design, data acquisition, statistical analysis and interpretation, manuscript drafting, RM contributed to advanced statistical analysis, manuscript drafting, and critical review of the manuscript, YT and TS contributed to data acquisition, data interpretation and critical review of the manuscript, RK, TM and SK contributed to study design, data interpretation and critical review of the manuscript. All authors read and approved the final version of the manuscript.

Funding details

None.

Declaration of interest

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.repbio.2018.10.006.

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