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ASSISTED REPRODUCTION TECHNOLOGIES

Performance of Day 5 KIDScore™ morphokinetic prediction models of implantation and live birth after single blastocyst transfer

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Abstract

Purpose While several studies reported the association between morphokinetic parameters and implantation, few predictive models were developed to predict implantation after day 5 embryo transfer, generally without external validation. The objective of this study was to evaluate the respective performance of 2 commercially available morphokinetic-based models (KIDScore™ Day 5 versions 1 and 2) for the prediction of implantation and live birth after day 5 single blastocyst transfer.

Methods This monocentric retrospective study was conducted on 210 ICSI cycles with single day 5 embryo transfer performed with a time-lapse imaging (TLI) system between 2013 and 2016. The association between both KIDScore™ and the observed implantation and live birth rates was calculated, as well as the agreement between embryologist's choice for transfer and embryo ranking by the models.

Results Implantation and live birth rate were both 35.7%. A significant positive correlation was found between both models and implantation rate ($r = 0.96$ and $r = 0.90$, $p = 0.01$) respectively. Both models had statistically significant but limited predictive power for implantation (AUC 0.60). There was a fair agreement between the embryologists' choice and both models (78% and 61% respectively), with minor differences in case of discrepancies.

Conclusions KIDScore™ Day 5 predictive models are significantly associated with implantation rates after day 5 single blastocyst transfer. However, their predictive performance remains perfectible. The use of these predictive models holds promises as decision-making tools to help the embryologist select the best embryo, ultimately facilitating the implementation of SET policy. However, embryologists' expertise remains absolutely necessary to make the final decision.

Keywords IVF · Blastocyst · Time-lapse · Prediction model · Implantation

Introduction

Despite the large number of morphological evaluations of embryo quality described in the literature in order to select the embryos with the highest implantation potential for transfer or freezing [1, 2], the discriminating power of these evaluations for implantation remains moderate, both for the early stage and for the blastocyst stage [3]. In addition, embryo morphology assessment suffers from a significant inter-individual variability [4].

The implementation of time-lapse technology in clinical embryology in the late 2000s brought new insights into embryo development and embryo quality assessment, without compromising embryo culture conditions [5]. Although its clinical value is still debated by some in the literature [6], the shift from daily static observation to dynamic and continuous embryo monitoring in undisturbed culture conditions led

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to improved clinical outcome in many studies. These promising results were emphasized in a recently updated meta-analysis conducted in randomized controlled trials, which concluded that time-lapse leads to improved clinical outcome compared with classical morphological embryo evaluation [7, 8]. However, this was challenged in another recent meta-analysis leading to contradictory results [9, 10].

Given the large amount of data generated for each embryo, several predictive models for implantation based on morphokinetic parameters have been developed to facilitate, standardize, and improve embryo selection. Since the first model reported by Meseguer in 2011 [11], many others have been presented with various aims, i.e., to detect the risks of aneuploidy [12, 13], to predict blastocyst formation [14, 15], or implantation [16–18]. However, most of these algorithms (except in [17, 18]) are based on relatively little data (98 to 754 embryos) and few have been tested in a randomized prospective study [18–20], or validated in other ART centers [21–23]. The heterogeneous inclusion criteria (age, number of oocytes, oocyte origin) as well as the differences in patients' characteristics and laboratory settings and culture conditions including use of reduced oxygen between IVF centers limit the generalizability of these models [23–26], highlighting the need for huge multicentric dataset in almost unselected population in order to build a robust inter-laboratory model.

The KIDScore™ Day 5 (version 1 and version 2) is a predictive model for implantation after embryo transfer on day 5, developed for Embryoscope™ devices and based on very large multicentric datasets. It assigns a score that increases with the embryo's potential for implantation, and aims at ranking embryos from the same cohort in order to select the one with the highest implantation potential for transfer. In the first version (v1), it is based on 3 morphological parameters (normal ploidy status of the zygote (2PN) on day 1 and blastocyst morphology (ICM and TE) on day 5) and 8 morphokinetic parameters (tPNf, t2, t3, t4, t5, t8, tSB, tB), with hierarchical analysis assigning a categorical score between 0 and 6. Input was reduced to 5 morphokinetic and 2 morphology parameters in the second version (v2) based on regression analysis, assigning a linear score between 0 and 9.9. The mathematical details of these day 5 algorithms are protected by copyright and have not been published yet, and their respective performance in clinical setting has not been reported up to now.

The main objective of this work was to evaluate the clinical value of these 2 commercial morphokinetic predictive models for the prediction of embryo implantation after day 5 transfer.

Materials and methods

This monocentric retrospective study was conducted on all intracytoplasmic sperm injection (ICSI) cycles followed by day 5 single blastocyst transfer (SBT) performed with the Embryoscope® between January 2013 when single blastocyst transfer policy was implemented and December 2016 when we started using KIDScore™. This allowed us to have all data available on subsequent live birth. There were no clinical or biological inclusion criteria, except the use of ICSI. Egg donation cycles and PGD cycles, as well as double blastocyst transfer cycles, were excluded. As time-lapse cannot be offered to all patients in our setting yet, this technology is randomly assigned to all ICSI couples whenever space is available in the device, independently of any clinical or biological characteristics.

All patients were stimulated using a controlled ovarian stimulation protocol with antagonist protocol. When at least 3 follicles > 17 mm were observed on ultrasound, ovulation was triggered by a single administration of rHCG (Ovitrelle®, Merck Serono®). Oocyte retrieval was performed 36 h later. The cumulus oocyte complexes collected were denudated with hyaluronidase (SynVitro Hyadase®, Origio®). Only mature oocytes were retained for microinjection and subsequently inserted into the Embryoscope® and grown up to the blastocyst stage in sequential culture media (G1-G2 media®, Vitrolife®) under controlled atmosphere (6% CO_2, 5% O_2) with image acquisition every 10 min. Embryos' morphokinetic parameters were annotated individually according to published guidelines [2, 27], but not used for embryo choice at blastocyst stage. According to the strategy of our center, embryos with abnormal cleavages during first cell cycle, i.e., reverse cleavage or direct cleavage from zygote to ≥ 3 daughter cells, were systematically discarded on day 2. On day 5, blastocysts were selected for transfer according to Gardner's criteria. Blastocysts ≥ B1 were transferred, with the exception of those with a grade C trophectoderm. Blastocyst morphological quality was graded as follows. Blastocysts ≥ B3 expansion and grade A trophectoderm were classified as top quality, blastocysts < B3 and/or blastocysts with grade B trophectoderm were classified as medium-quality blastocysts, B1 and B2 blastocysts with apparent poor morphology but with trophectoderm that could not be evaluated precisely where classified as poor quality. Embryologist's choice was mainly based on these conventional day 5 morphological criteria, apart from abnormal cleavage cited above.

KIDScore™ Day 5 v1 and v2 were retrospectively applied on all blastocysts transferred on day 5. The correlation between KIDScore™ day 5 (v1 and v2) and observed implantation rate was then calculated (with KIDScore™ Day 5 v2 presented as quartiles instead of a continuous variable). ROC curve analysis with implantation as main endpoint was also performed.

Pregnancy test was carried out 11 or 12 days after embryo transfer and was repeated every 48 h until reaching the threshold of 1000 IU/L. Clinical pregnancies were confirmed by ultrasound 4 to 5 weeks later, demonstrating gestational sacs

and cardiac activity. Implantation rate was defined as the number of gestational sac with cardiac fetal activity over the number of embryos transferred. Live birth rate was defined as the number of deliveries that resulted in a live-born neonate, expressed per 100 embryo transfers.

Ethical approval

All study parameters were extracted from our local database, which is regulated by a French national agency (the CNIL). All patients gave their informed consent for the anonymous use of the database for research purpose. This protocol was approved by local ethics committee (GNEDS).

Statistical analysis

Statistical analysis was performed with GraphPad Prism and MedCalc software. Chi-square was used to compare proportions. ROC curve analysis was performed to evaluate the predictive performance of both models for implantation. Linear regression was used to measure the association between KIDScore™ and implantation. Multiple logistic regression was performed to assess the respective association between both KIDScore™, female age, conventional embryo morphology (i.e., blastocyst expansion rate according to Gardner's criteria and overall blastocyst morphological quality) and implantation. A p value < 0.05 was considered statistically significant.

Results

We included 210 cycles leading to single blastocyst transfers in 210 couples. All couples were referred for ICSI because of male infertility factor, and received single blastocyst transfer on day 5. KIDScore™ Day 5 v1 and v2 were retrospectively applied on each blastocyst in order to evaluate their respective predictive value for implantation. Implantation occurred in 75 out of 210 cycles (implantation rate 35.7%). All clinical pregnancies ended up with live birth ($n = 75$, live birth rate 37.5%). Demographic characteristics, ovarian stimulation parameters, and embryo development are presented in Table 1. Mean KIDScore™ Day 5 v1 and blastocyst expansion rate were significantly different between implantation and non-implantation groups. The association between KIDScore™ Day 5 v1 and embryo implantation percentages is shown in Fig. 1a. A strong positive correlation between KIDScore™ Day 5 v1 and implantation rate was observed ($r = 0.96$, $p = 0.001$). A strong positive correlation between KIDScore™ Day 5 v2 and implantation rate was also observed ($r = 0.90$, $p = 0.01$) (Fig. 1b). ROC curve analysis was performed for both models and demonstrated that both models were significant predictors of implantation, with respective area under curves of 0.59 ([IC 95 0.52–0.66]; $p = 0.02$) and 0.60 (IC 95 0.51–0.67; $p = 0.005$) for KIDScore™ Day 5 v1 and v2 (Fig. 2). ROC curve analysis was also performed, blastocyst expansion rate (AUC 0.60, [IC 95 0.52–0.67]; $p = 0.018$) and blastocyst morphology score (AUC 0.61, [IC 95 0.54–0.69]; $p = 0.004$) (Supplementary Figure 1). Multiple logistic regression including female age, blastocyst morphology score, and KIDScore™ v1 and v2 showed that no variable was independently associated with clinical outcome.

Finally, we studied the concordance between the embryo chosen for transfer by the embryologist based on blastocyst morphology but not morphokinetic parameters (except direct cleavage), and the embryo retrospectively identified by KIDScore™ Day 5 v1 and v2 as having the highest implantation potential. This analysis was restricted to cycles where at least 2 blastocysts were eligible for transfer on day 5 ($n = 114$). Implantation occurred in 47 cycles in this subgroup (41.2%). The agreement between embryologist's choice and KIDScore™ day 5 v1 was 78% ($n = 89$, concordant group). Among the 25 cases of discordance for KIDScore™ day 5 v1, the majority ($n = 14$, 56%) had a score difference of 1, meaning that an embryo ranked 5 by the KIDScore™ Day 5 v1 was selected for transfer by the embryologist, whereas an embryo having a score of 6 was available. However, these 25 blastocysts not used for fresh transfer were systematically frozen anyway. The implantation rate was 41.6% in the concordant group ($n = 37/89$) and 40% in the discordant group ($n = 10/25$) ($p > 0.05$). The agreement between embryologist's choice and KIDScore™ Day 5 v2 was slightly lower ($n = 70$, 61.4%, concordant group) than with KIDScore™ Day 5 v1. However, KIDScore™ Day 5 v2 has a more detailed score, as it is continuous and not a categorical variable, and the mean score difference between the embryo selected for transfer and the one with the highest score was 0.37 on average (range 0.1–2.8). Among the 44 discordant classifications, the difference between the score of the embryo selected for transfer by the embryologist and the one retrospectively identified with the highest score by the model was less than 1 in 33 cases (75%). Once again, the 44 blastocysts with a higher score not initially chosen by the embryologist for fresh transfer were systematically frozen anyway. The implantation rate was 41.4% in the concordant group ($n = 29/70$) and 40.9% in this discordant group ($n = 18/44$) ($p > 0.05$).

Discussion

In this study, we demonstrated that both KIDScore™ Day 5 v1 and v2 were significantly associated with the chances of implantation and live birth after day 5 single blastocyst transfer, however, with perfectible performance.

Blastocyst is the ultimate developmental stage before implantation, occurring after embryo genome activation and

Table 1 Demographic characteristics, ovarian stimulation parameters, and embryo development in implantation and non-implantation groups. All cycles were performed by ovarian stimulation with antagonist protocol, followed by ICSI because of male factor and single blastocyst transfer on day 5. Results are presented as mean ± standard deviation or proportion when appropriate

		Implantation ($n = 73$)	No implantation ($n = 137$)
Demographic characteristics	Female age (years)	31.92 ± 4.38	32.67 ± 4.65
	Male age (years)	35.97 ± 7.55	36.04 ± 6.36
	Infertility duration (years)	3.45 ± 2.12	3.44 ± 2.10
	AMH (μg/L)	4.01 ± 0.56	3.51 ± 2.78
Ovarian stimulation parameters	Initial daily dose of gonadotropin (U)	233 ± 89	246 ± 115
	Total amount of gonadotropins (U)	2313 ± 929.20	2517 ± 1106
	Number of mature oocytes retrieved	9.7 ± 4	8.5 ± 3.5
Embryo development	Fertilization rate (%)	58.94%	57.24%
	Blastulation rate (blastocyst per 2PN)	68.3%	68.32%
	Blastocyst expansion rate at the time of transfer[§]	3.29 ± 1.06	2.87 ± 1.22*
	KIDScore Day 5 v1	4 ± 1.67	3.43 ± 1.74*
	KIDScore Day 5 v2	6.94 ± 1.76	6.38 ± 2.09

*$p < 0.05$. [§] Grading of blastocyst expansion according to Gardner's criteria

cellular lineage specification. The positive association between blastocyst morphological criteria [3] and/or late morphokinetic criteria [3, 28] and implantation chances has been extensively demonstrated. However, blastocyst implantation rate is far from 100%. Moreover, blastocyst morphological assessment remains slightly subjective and ranking several blastocysts can be difficult in routine practice. In this respect, algorithms combining early morphokinetic and late morphological criteria could be a promising approach to improve embryo quality assessment on day 5 before transfer. To our knowledge, this study is the first to evaluate the clinical value of KIDScore™ Day 5 (v1 and v2) for the prediction of implantation after day 5 embryo transfer. Although we found a good correlation between the KIDScore™ Day 5 and implantation rates, and a statistically significant predicting capability of both models for implantation and live birth, the area under curve obtained for both models was moderate and perfectible. These results are quite close to those recently reported for several day 3 algorithms, including KIDScore™ Day 3 [29]. Although ROC analysis is common and appropriate when evaluating a prognostic factor such as KIDScore™, its results should be interpreted with care. Indeed, KIDScore™ aims at ranking embryos within a cohort rather than finding their individual implantation potential. Moreover, the outcomes considered here are implantation and live birth, which are by definition multifactorial, depending not only on embryo quality, but also on several clinical factors.

We also retrospectively studied the concordance between KIDScore™ indication and embryologist's choice in order to evaluate to which extent the model would help the embryologist to objectively rank embryos and prioritize the one with the highest implantation potential. We found that the embryologist's choice was in agreement with the prediction model in most cases (78% and 61% for v1 and v2). It should be recalled here that only blastocyst morphology but not morphokinetic parameters (except direct and revers cleavages) were not used

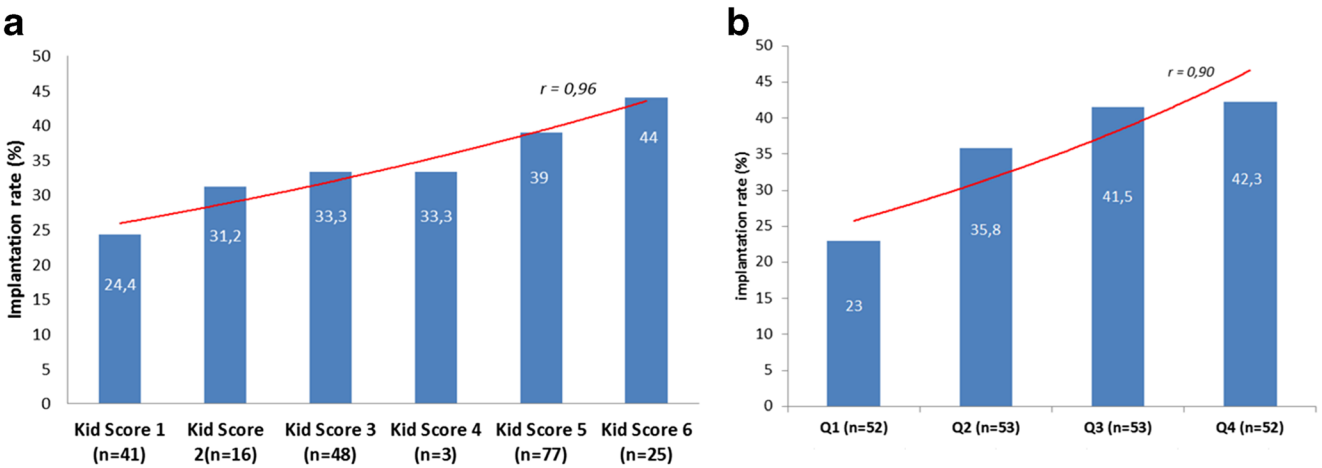

Fig. 1 Association between KIDScore™ Day 5 version 1 (**a**) or version 2 (**b**) and embryo implantation rates (linear regression, $n = 210$)

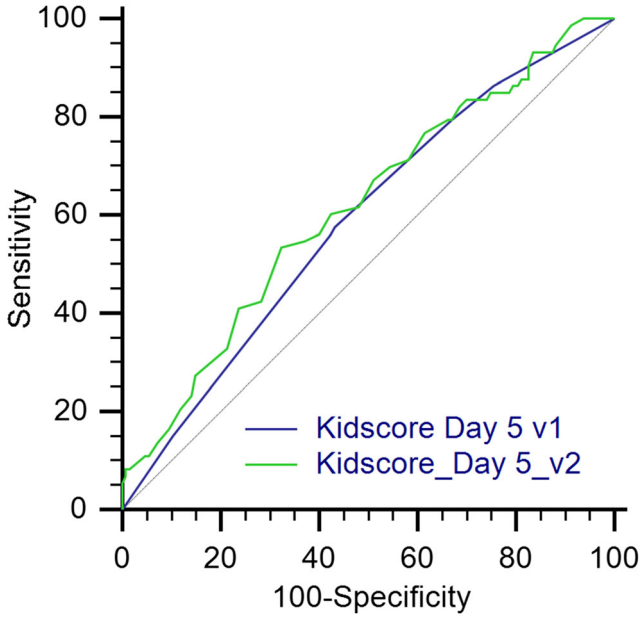

Fig. 2 Receiver operating characteristic (ROC) curve comparing KIDScore™ Day 5 version 1 and version 2 for the prediction of implantation ($n = 210$)

for selecting blastocysts for transfer. However, both trophectoderm morphology and direct cleavages are part of KIDScore™ and constitute thus some kind of overlap between both selection strategies. Although KIDScore™ Day 5 was not tested, such a relatively high agreement between algorithms and embryologists' choice was reported in an interesting recent comparative study [30]. Of interest, we observed that the gap between the model and embryologist's choice was always very limited, with the embryologist choosing the embryo with the second highest score for transfer and freezing the one with the highest score. Implantation/live birth rates were not statistically different between concordant and discordant groups, but the numbers in each group were limited. However, and owing to the positive association found between KIDScore™ Day 5 and implantation/live birth rate, it could be eventually postulated that the use of these predictive models would have led to a slightly higher implantation/live birth rate after fresh blastocyst transfer. This hypothesis obviously remains to be tested in properly designed prospective studies with appropriate power calculation. In any case, KIDScore™ might be considered as an "objective" second opinion on embryo ranking, helping embryologists to make their clinical decision more objectively. This could prove specifically useful for new or less experienced embryologists and in large staffs as a way to standardize or homogenize procedures. KIDScore™ Day 5 is directly available in the annotation software, does not need to be validated before clinical use, and gives add-on information helping the embryologist to evaluate embryo quality.

One strength of our study lies within the use of a single embryo transfer dataset instead of the known implantation dataset (KID), avoiding the inherent confounding factors associated with double embryo transfers [29]. Furthermore, the availability of live birth data reinforces the clinical relevance of this work, even though KIDScore™ has been initially designed as a predictor of implantation and not live birth. Third, our study was conducted in an almost unselected ICSI population, making it theoretically be generalizable in other centers. However, we acknowledge that this statement should be nuanced. Indeed, not all patients are offered time-lapse and undergo single blastocyst transfer in our center. Moreover, our population is quite young, therefore questioning the robustness of our results in other settings, as female age could be considered as a confounding factor [29]. Another limitation lies within statistical robustness of our analysis. Indeed, no study reported the performance of these day 5 prediction models and concordance with embryologist's selection up to now, preventing from formulating relevant hypothesis and hence from performing an a priori power calculation. However, this retrospective cohort study might serve as a basis for further prospective trials. The issue of culture conditions should be addressed as well when considering the generalizability of a prediction model [31]. Although we acknowledge that our results should not be applied to IVF labs performing blastocyst culture in ambient oxygen atmosphere, the unquestionable evidence that blastocyst culture should be performed under reduced oxygen level [32] nuances this limitation. Finally, although some consensus guidelines have been proposed, one of the limitations of predictive models based on morphokinetic parameters lies within the lack of standardization of the starting point of image analysis and annotations [27, 33]. Most studies, including ours, use the middle time point of the whole ICSI procedure for the cohort as t0. We acknowledge that alternative starting point should be considered in the future, such as pronuclear fading, in order to minimize bias, although the impact of an eventual 10-min delay on the KIDScore™ result and subsequent clinical decision might be insignificant.

One obvious limitation of this study lies within its retrospective design. A prospective randomized study is obviously needed to confirm the clinical relevance of KIDScore™. Although there is no evidence in the literature that male factor is associated with specific morphokinetic pattern [34], we also acknowledge that our results only reflect cycles performed in male factor cases, and should therefore not be generalized to the whole infertile population. Furthermore, the very limited number of cycles performed with extremely severe oligospermia or surgically retrieved sperm did not allow relevant statistical subgroup analysis.

The issue of independent confounding factors, such as age, when building morphokinetic predictive models has been recently raised [29, 35]. Blastocyst morphology is significantly associated with implantation and should be considered as a confounder [29]. Trophectoderm grade is one of the variables

included in KIDScore™ Day 5 models. Predictive models based on large multicentric databases including various types of patients might partly overcome this risk of bias. In this respect, and as a perspective, these results might advocate for the development of fully automated annotation tool and deep learning approaches. Indeed, automated detection of cell cleavages could help lower inter-operator variability, improving lab workflow, and ultimately allow large multicentric database analysis. Thereby, a recent prediction model based on machine learning analysis of embryo development has been published and describes a random forest model able to predict implantation potential of a transferred embryo with a high AUC of 0.74 [36]. Moreover, a very promising and fully automated deep learning model, validated in 8 clinics, has just been described [37]. This model appears to be able to predict fetal heart pregnancy with an extremely high AUC of 0.93 [37].

Conclusion

KIDScore™ Day 5 morphokinetic-based predictive models are significantly associated with implantation and live birth rates after day 5 single blastocyst transfer. However, their predictive performance remains perfectible. Although this needs to be further confirmed in large prospective randomized studies in order to evaluate their real clinical added value, the use of these predictive models hold promises as decision-making tools to help the embryologist select the best strategy for embryo culture and the best embryo for transfer. It can be postulated that the association of time-lapse and morphokinetic decision support algorithms might lead to facilitated implementation of SET policy. Even if partial automation might be a desirable goal in IVF labs, embryologists' expertise is still absolutely necessary to have a global view of embryo development and make the final decision about embryo fate.

Compliance with ethical standards

All patients gave their informed consent for the anonymous use of the database for research purpose. This protocol was approved by local ethics committee (GNEDS).

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