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ARTICLE

Cytoplasmic movements of the early human embryo: imaging and artificial intelligence to predict blastocyst development

BIOGRAPHY

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KEY MESSAGE

This study suggests that movement of cytoplasmic particles of the human early embryo is a novel and valuable source of data for predicting further development, after advanced imaged analysis and artificial intelligence. This is not immediately translatable into clinical embryology practice, but opens up new perspectives for non-invasive embryo assessment.

ABSTRACT

Research question: Can artificial intelligence and advanced image analysis extract and harness novel information derived from cytoplasmic movements of the early human embryo to predict development to blastocyst?

Design: In a proof-of-principle study, 230 human preimplantation embryos were retrospectively assessed using an artificial neural network. After intracytoplasmic sperm injection, embryos underwent time-lapse monitoring for 44 h. For comparison, standard embryo assessment of each embryo by a single embryologist was carried out to predict development to blastocyst stage based on a single picture frame taken at 42 h of development. In the experimental approach, in embryos that developed to blastocyst or destined to arrest, cytoplasm movement velocity was recorded by time-lapse monitoring during the first 44 h of culture and analysed with a Particle Image Velocimetry algorithm to extract quantitative information. Three main artificial intelligence approaches, the k-Nearest Neighbour, the Long-Short Term Memory Neural Network and the hybrid ensemble classifier were used to classify the embryos.

Results: Blind operator assessment classified each embryo in terms of ability to develop to blastocyst, with 75.4% accuracy, 76.5% sensitivity, 74.3% specificity, 74.3% precision and 75.4% F1 score. Integration of results from artificial intelligence models with the blind operator classification, resulted in 82.6% accuracy, 79.4% sensitivity, 85.7% specificity, 84.4% precision and 81.8% F1 score.

Conclusions: The present study suggests the possibility of predicting human blastocyst development at early cleavage stages by detection of cytoplasm movement velocity and artificial intelligence analysis. This indicates the importance of the dynamics of the cytoplasm as a novel and valuable source of data to assess embryo viability.

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Declaration: The authors report no financial or commercial conflicts of interest.

KEYWORDS

Artificial intelligence
Artificial neural network
Blastocyst
Cytoplasm
Embryo
IVF

INTRODUCTION

One of the long-term, and among the most important, goals of medically assisted reproduction (MAR) is the achievement, in the shortest possible period of time, of a single healthy term pregnancy after the transfer of a single embryo. To this end, embryos need to be assessed by non- or minimally invasive methodologies and prioritized for transfer according to their developmental potential. In most MAR programmes until the early 2000s, embryos were being cultured until day 2 or 3 after insemination and selected for transfer according to static morphological criteria. More recently, significant paradigm shifts have occurred, based on the extension of embryo culture to day 5 or 6 to discriminate, by self-selection, embryos able to develop to the blastocyst stage. These blastocysts have, on average, higher developmental potential compared with cleavage-stage embryos, and can be used for embryo transfer with increased chances of achieving a viable pregnancy per transfer attempt (*Glujovsky et al., 2016*). To further increase efficiency, i.e. to identify more precisely and prioritize the most viable embryo in a cohort for transfer, blastocysts can be assessed according to static (The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011) or dynamic (*Gallego et al., 2019*) morphological criteria, or even by using a strategy of preimplantation genetic screening for aneuploidies (*Griffin and Ogur, 2018*). Collectively, these approaches based on blastocyst culture assure a higher treatment efficiency compared with day 2 or 3 embryo transfer. They cannot be considered ideal, however, for several reasons. A major drawback of such methodologies lies in the culture to blastocyst stage *per se*. In fact, blastocyst culture is more expensive and more demanding for the management of the IVF laboratory workflow. Even more importantly, if not mastered properly, blastocyst culture may affect intrinsic embryo developmental potential, affecting overall treatment efficacy (*Swain, 2019*). This scenario clearly points towards the need for a more advanced, non-invasive embryo assessment methodology able to predict developmental potential at earlier stages, without necessarily resorting to extended culture.

Progress in the realm of artificial intelligence and in image analysis offers unique opportunities to develop novel embryo assessment approaches. Recently, promising attempts have been made to harness the potential of deep learning to predict embryo implantation potential (*Khosravi et al., 2019*; *Miyagi et al., 2019*; *Tran et al., 2019*). All such cases, however, involved the use of static images or videos of blastocyst stage embryos, leaving the question of prediction at earlier stages unanswered.

In this proof-of-principle study, the option of advanced image analysis of early development to predict the ability of blastocyst formation was approached. Sequential images collected by bright-field time lapse microscopy (TLM) over fewer than 2 days of culture were subjected to particle image velocimetry (PIV) analysis to detect the dynamics of cytoplasmic movements. The resulting data were elaborated by neural network-based artificial intelligence. The strategy to investigate cytoplasm dynamics as a novel source of data to assess oocyte function and embryo viability finds justification in studies carried out in the mouse model (*Ajduk et al., 2011*; *Yi et al., 2013*; *Bui et al., 2017*). Nevertheless, to date, movement of cytoplasmic particles has been totally neglected in attempts to predict human embryo viability.

MATERIALS AND METHODS

This retrospective proof-of-principle study included 230 embryos generated in intracytoplasmic sperm injection (ICSI) cycles carried out between October 2015 and May 2018. Approval for the study was obtained from the local Institutional Review Board (V.d.A. Prog. A.I. Ref. PR01/2013 Rev. 0 – Data appl. 11 March 2013) Laboratory non-clinical data were used for research purposes only. Cases suitable for analysis were selected according to the rule of having two sibling embryos from the same cohort (cycle), of which one developed to blastocyst stage and one arrested to an earlier stage. Therefore, the aim was to limit possible biases caused by patient variability.

Diagnosis of infertility included various causes, including male factor, tubal factor and polycystic ovary (but not polycystic ovary syndrome), with or without chronic anovulation. Such inclusion criteria were selected as a requirement of the study design consistent with the expectation of having a relatively high number of embryos in a cohort and the ensuing possibility of extending embryo culture to the blastocyst stage. Ovarian stimulation was carried out as previously described (*Zacà et al., 2018*).

Semen preparation and ICSI procedures

Discontinuous PureSperm (Nidacon, Göteborg, Sweden) gradient was used to select semen for ICSI (*Borini et al., 2006*). After preparation, spermatozoa were evaluated for concentration, total and progressive motility and morphology, according to *World Health Organization procedures (2010)*.

Embryo culture and time lapse image acquisition

EmbryoScope equipment (Vitrolife, Göteborg, Sweden) was used to culture oocytes and embryos. This is an integrated time-lapse technology incubator system for embryo culture, carried out in a $N_2/CO_2/O_2$ (89:6:5, volume per volume) atmosphere at 37°C without control of humidity. Microinjected oocytes were placed inside pre-equilibrated slides (EmbryoSlide) (Vitrolife, Göteborg, Sweden), each containing 12 droplets of 25 µl of cleavage medium (Cook IVF, Sydney, Australia) covered by 1.2 ml of mineral oil (SAGE, Biocare Europe, Rome, Italy). On day 3, cleavage medium was replaced with blastocyst medium (Cook IVF, Sydney, Australia) to extend embryo culture until day 5, where appropriate (*Lagalla et al., 2017*). Images were acquired over a period of 125 h starting from the time of ICSI, with a 15-min interval between consecutive picture frames.

Operator-based prediction of development to blastocyst stage

The outcome of artificial intelligence analyis was first comparatively assessed and then combined with standard operator-based prediction of development to blastocyst. Embryo assessment by standard morphology was preferred to alternative assessement approaches, i.e. morphokinetic algorithms, because static evaluation is routinely carried out in accordance with the recommendations of an international consensus (The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, *2011*) and remains the 'standard of care'

in clinical embryology. On the other hand, although it has great potential, morphokinetic evaluation has not yet reached a similar degree of consensus, perhaps also as an effect of biases derived from different technological platforms and applicative protocols. On day 2, at 42 h after ICSI, a single embryologist with over 20 years of experience in clinical embryology assessed each embryo according to the folllowing criteria: blastomere number, size and mutual position; degree (percentage of total volume) of fragmentation; multinucleated blastomeres; and cellular dysmorphisms, e.g. large vacuoles (*The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011*). On the basis of such morphological assessment conducted statistically, i.e. at a single time point, the operator formulated a binary prediction (yes/no) of development to blastocyst stage according to the recommendations of the Alpha and ESHRE Istanbul Consensus (*The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011*).

Definition of blastocyst

At 116 h after ICSI, embryos were categorized as developed at the blastocyst stage if they showed at least the following minimal requirements: at least a grade 1 blastocoel expansion; a discernible cluster of cells forming the inner cell mass; and a wall of epithelial-like cells delimiting the blastocoel and enclosing the inner cell mass (*The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011*).

Cell_PIV analysis

Cell_PIV software (kindly provided by Dr Shane Windsor), based on the PIV algorithm, was used to detect embryo movement velocity (EMV) during the first two-cell division (44 h) of embryos at blastocyst stage and embryos not at blastocyst stage. The movement velocity vectors, observed in the embryo, were calculated by cross correlation between the patterns of the pixels of adjacent video frames, extracting five features: direction, the mean of the inverse of the local standard deviation of the direction of the vectors; vorticity, the mean vorticity of the vectors in each frame; hybrid, a hybrid combination of the mean magnitude and inverse standard deviation data; meanmag, the mean magnitude of the vectors in each frame; and summed, the mean magnitude of the vectors summed over a set number of frames forward in time from the current point.

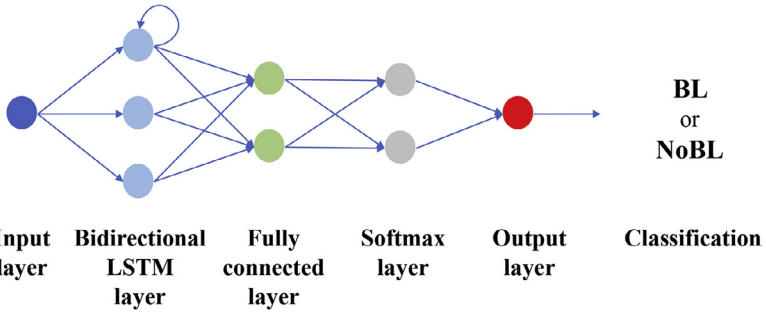

FIGURE 1 Long-Short Term Memory Neural Network (LSTM-NN) architecture. The structure of the LSTM-NN is made of five main layers. An input layer, in which the number of nodes is equal to the number of features used; a bidirectional LSTM layer, made with three nodes, of which its function is to bring out hidden patterns along the time series; a fully connected layer and a softmax layer, convert the temporal information stored in the LSTM layer in a classification probability; and an output layer provides the class with the highest probability.

Statistics

Differences in cytoplasmic movements in embryos at blastocyst stage and embryos not at blastocyst stage were evaluated using the two-sample Kolmogorov–Smirnov test with the MATLAB software (R2018B). Data were considered significantly different when $P < 0.05$.

Artificial intelligence

Two artificial intelligence approaches were used: the k-Nearest Neighbor (k-NN) and the Long-Short Term Memory Neural Network (LSTM-NN) Both algorithms have been exploited earlier in different biomedical classification contexts to reveal hidden patterns from complex biological data and to support the clinical decision process (*He et al., 2019*). The two approaches were implemented with MATLAB software (R2018B). In particular, the LSTM-NN was developed using the MATLAB Deep Learning Toolbox. The k-NN algorithm, largely used to solve time series classification problems (*Yakovitz, 1987*), classifies a new sample based on its distance similarity with the training set. As the computation of the distances between two time series is frequently subject to slight time shifts, the k-NN on Cell_PIV temporal series was preceded by the application of dynamic time warping, a pre-processing technique that produces the optimal alignment between sequences (*Geler et al., 2016*; *2020*; *Tran et al., 2019*). The LSTM-NN is a non-linear computational approach able to encode and store temporal information in its layers and, through subsequent nodes, convert it into a classification output (FIGURE 1). For this reason, LSTMs have been widely used to classify time series data (*Karim et al., 2019*).

A set of k-NN and LSTM-NN models, with empirically established parameters and different combination of features, was defined. Among them, four models (k-NN-1, k-NN-2, LSTM-NN-1, LSTM-NN-2) were used for final comparison with the operator on the test set, and were selected based on the best results of the cross validation on the training set. The selected models were then trained on the entire training set. To evaluate the models' generalization capabilities, a 10-fold cross validation on the entire dataset (230 embryos, 118 in the embryos not at blastocyst stage class and 112 in embryos at blastocyst stage class) was also carried out for the k-NN-1, k-NN2, LSTM-NN-1 and LSTM-NN-2 algorithms. Six embryos were excluded from the embryos at blastocyst stage group because of poor image quality. For the k-NN models, the number of neighbours equal to four were set, and the similarity between embryos was computed coupling the dynamic time warping with the Kullback distance. The LSTM-NN, whose architecture is shown in FIGURE 1, was trained with the Adam optimization function for 100 epochs, with a learning rate of 0.1 and a batch size of 20. Results of the artificial intelligence models on the test set were evaluated in terms of accuracy (fraction of correct predictions), sensitivity (fraction of embryos at blastocyst stage correctly predicted), specificity (fraction of embryos not at blastocyst stage correctly predicted), precision (fraction of true embryos at blastocyst stage identified among all the predicted

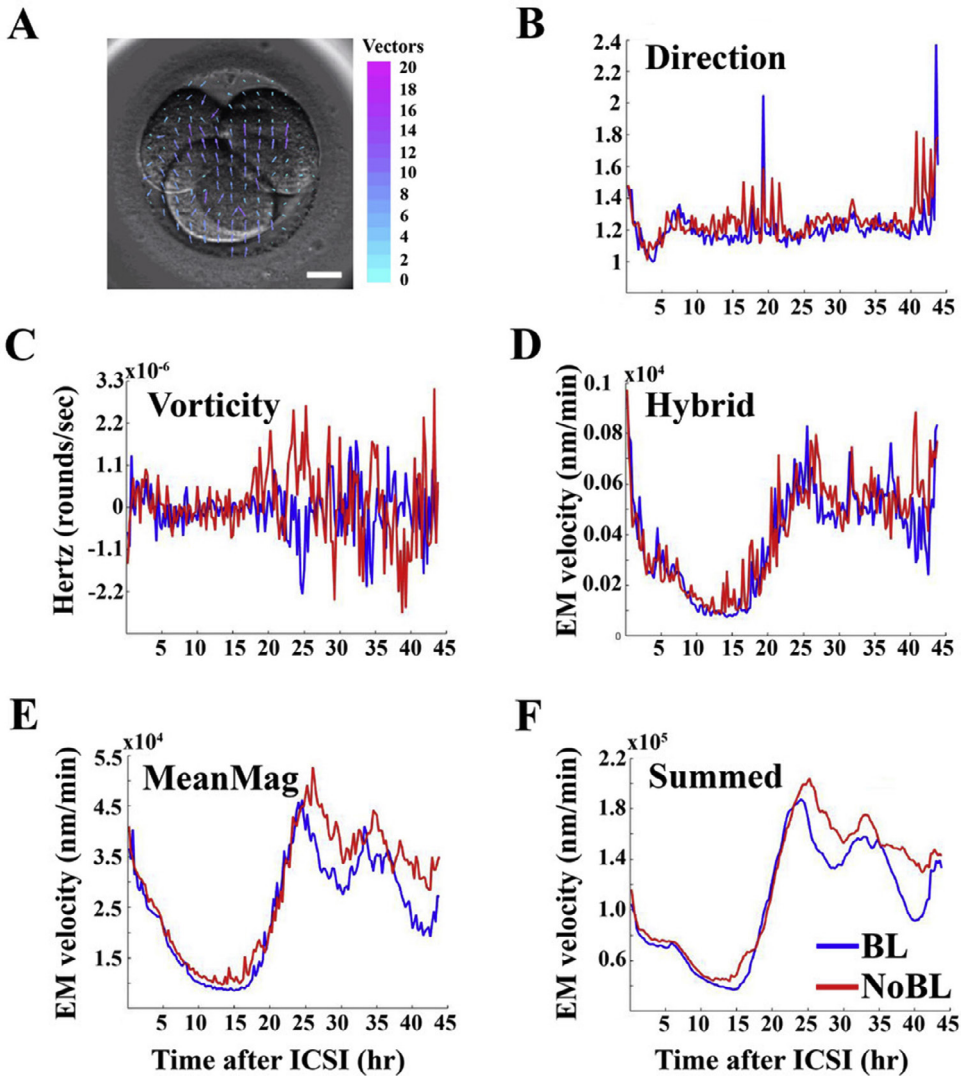

FIGURE 2 Embryo movement velocity profiles of embryos at blastocyst stage and embryos not at blastocyst stage. (A) Representative image frame of an embryo at 32 h after intracytoplasmic sperm injection (ICSI) showing the Cell_PIV velocity vectors. The colour and length of the arrows (vectors) indicate the velocity module of the movements when comparing the previous frame with that pictured. Coloured vector scale bar: blue, lower velocity; purple, higher velocity. Bar, 20 μm. (B–F) Embryo movement velicity profiles analysed using direction, vorticity, hybrid, meanmag or summed Cell_PIV feature, respectively. Y values (direction) in *Figure 2*B are absolute numbers.

embryos at blastocyst stage) and F1 score (harmonic mean of precision and sensitivity).

RESULTS

Immediately after ICSI, each of the obtained 230 zygotes was transferred to a single Embryoscope well and cultured for a total of 125 h (5.2 days). Of these, 112 reached the blastocyst stage (48.7%), whereas 118 arrested sometime earlier, but never before the second cleavage cycle.

On the basis of a single picture frame taken at 42 h culture after ICSI, an expert operator blindly classified each of the 230 embryos, according to previously established morphological criteria (Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, *2011*) as developmentally competent (embryos at blastocyst stage) or incompetent (embryos not at blastocyst stage), reaching a 75.4% accuracy, 76.5% sensitivity and 74.3% specificity.

The time-lapse sequence recorded during 44 h (175 frames), corresponding to completion of the second embryonic cell cycle division, was analysed using PIV software Cell_PIV (FIGURE 2A), with five different image processing features: direction, vorticity, hybrid, meanmag or summed.

Analysis of the first three parameters highlighted noisy and overlapping EMV profiles of embryos at blastocyst stage and embryos not at blastocyst stage (FIGURE 2B–2D); instead, the meanmag (FIGURE 2E) and, more notably, the summed (FIGURE 2F) features two identified main and consistent time intervals with statistically different EMVs: the former between 12 and 21 h (*P*-value between 0.0001 and 0.042) and the latter between 39 and 43 h (*P*-value between 0.006 and 0.049).

When the summed EMV temporal profiles of embryos at blastocyst stage and embryos not at blastocyst stage were assessed according to known key

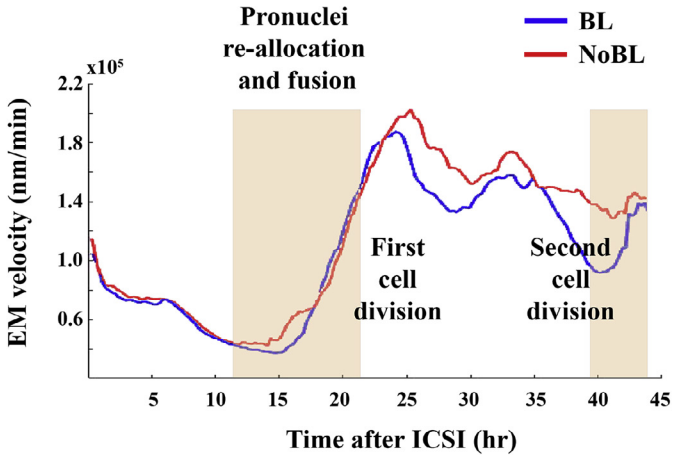

FIGURE 3 Cytological events occurring during the first two segmentation divisions. The summed embryo movement velocity profile of embryos at blastocyst stage embryos not at blastocyst stage resulted significantly different during the time intervals corresponding to pronuclei re-allocation (*P*-value between 0.0001 and 0.042) and the second embryonic division (*P*-value between 0.006 and 0.049). ICSI, intracytoplasmic sperm injection;

events occurring during the first 44 h of development (FIGURE 3), the first time interval overlapped with pronuclear re-positioning and ended shortly before the time of the first embryonic cell division. The second time interval coincided with the second cleavage cycle, until the end of the time-lapse frames analysed.

Although the morphodynamic patterns measured by Cell_PIV suggest, at least at these two time-intervals, the potential to discriminate between the two embryo classes, their high cytoplasm movement velocity variability restricts their possible use for a single embryo classification in the clinical practice. Nevertheless, this limitation was resolved by the subsequent artificial intelligence analysis.

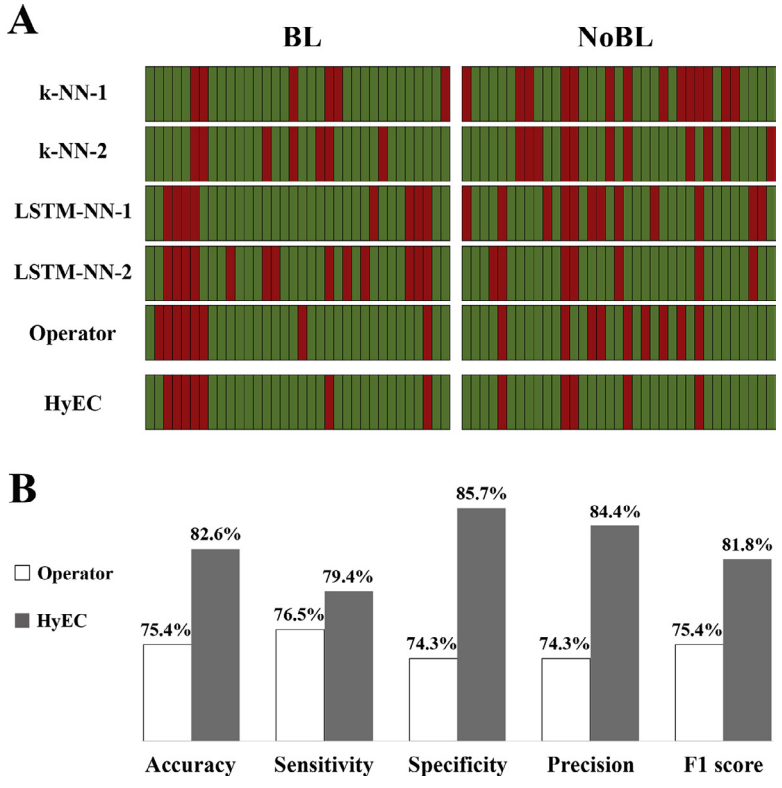

FIGURE 4 Improved embryo-quality classification with hybrid ensemble classifier. (A) Test embryos correctly (green) or incorrectly (red) classified by the artificial intelligence models (k-NN-1, k-NN-2, LSTM-NN-1 or LSTM-NN-2), by the operator or the hybrid ensemble classifier (HyEC); (B) performance metrics obtained by the operator alone or by the HyEC. K-NN, k-Nearest Neighbor; LSTM-NN, Long-Short Term Memory Neural Network.

Of the 230 embryos analysed, 161 (70%) were randomly selected as a training set of the artificial intelligence algorithms, whereas the remaining 69 (30%) were used as a test set. Both algorithms were fed with either the data of a single Cell_PIV feature, i.e. M or S, or with a combination, i.e. M + S or D + V + H + M + S.

k-NN

Of the four k-NN implementations carried out, the D + V + H + M + S (k-NN-2) showed the highest accuracy (72.5%), the best balance between sensitivity (76.5%) and specificity (68.6%), the highest precision (70.3%), and a F1 score equal to 73.2%; instead, the M + S (k-NN-1) approach gave a similar accuracy (71.0%), lower specificity (60.0%) and precision (66.7%), but a higher sensitivity (82.3%) and F1 score (73.7%). Implementation with a single feature, i.e. M or S, resulted in low accuracy (<65%) and, therefore, this approach was abandoned.

LSTM-NN

When trained on M + S features (LSTM-NN-1), LSTM-NN showed a 71.0% accuracy, 76.5% sensitivity, 65.7% specificity, 68.4% precision and 72.2% F1 score; instead, the D + V + H + M + S combination (LSTM-NN-2) displayed the same accuracy (71.0%), lower sensitivity (61.8%), higher specificity (80.0%), 75.0% precision and 67.7% F1 score. Implementation with a single feature, i.e. M or S, resulted in low accuracy (<70%) and, as before, this approach was abandoned.

Hybrid ensemble classifier

The results of the artificial intelligence models (k-NN-1, k-NN-2, LSTM-NN-1 and LSTM-NN-2), showed a similar classification accuracy to that reported by the operator (75.4%), although their comparison highlighted a high classification variability for each single embryo (FIGURE 4A).

Next, an ensemble classifier was used to integrate the results of the four artificial intelligence models together with the blind classification made by the expert

operator, assuming, as decision rule, the agreement of at least three out of five classifiers. For its characteristics, this classifier was named hybrid ensemble classifier (HyEC). The use of this strategy led to an improvement in all the performance metrics, with a final 82.6% accuracy, 79.4% sensitivity, 85.7% specificity, 84.4% precision and 81.8% F1 score (FIGURE 4B).

In addition, the generalization capability of each of the four models and of the HyEC was evaluated by 10-fold cross validation on the entire dataset (230 embryos, 118 in embryos not at blastocyst stage class and 112 in embryos at blastocyst stage class) (Supplementary Figure).

DISCUSSION

The present study was designed to explore the use of a novel source of data to predict the ability of the human embryo to develop to the blastocyst stage. This aim was pursued by harmonizing different methodologies, i.e. TLM, advanced image analysis and artificial intelligence computational and elaboration tools. Collectively, the resulting data indicate that our approach can achieve a diagnostic accuracy classifiable as 'very good' (*Šimundić, 2009*). This is noteworthy, considering the proof-of-principle character of our study and the small size of the data set analysed. Such findings, however, should be interpreted only in a research context, lacking at present validation of their clinical applicability.

The current standard of embryo assessment in MAR relies on static morphological parameters (*The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011*). In most clinics worldwide, embryos are ranked according to their presumed developmental potential and prioritized for transfer at the cleavage (day 3) or blastocyst (day 5 or 6) stage. Although the two approaches generate comparable cumulative live birth rates after the transfer of fresh and cryopreserved embryos (*Glujovsky et al., 2016*), self-selection derived from blastocyst culture offers the advantage of a shorter time to pregnancy. Because blastocyst culture is not immune from technical and management drawbacks, however, early and robust prediction of development to blastocyst stage could significantly simplify and possibly improve the ranking of embryos according to their developmental potential. As shown also by our study, classical morphological criteria applied by an experienced embryologist have a measurable ability to predict development to blastocyst stage. Nevertheless, a higher predictive power is required to make short-term culture competitive with blastocyst culture. In a similar approach to that used in our study, *Conaghan et al. (2013)* reported that an algorithm applied to TLM-based automatic annotation of embryo morphokinetics improves the ability of experienced embryologists to predict development to the blastocyst stage. The potential of automatic morphokinetic annotation and embryo selection at cleavage stages was also reported in another study based on the same technology (*Kieslinger et al., 2016*). These studies introduced the concept of combined operator-automated assessment. Compared with the present study, however, in such cases, embryo assessment was extended to day 3.

The ability of morphokinetic algorithms generated by TLM technology to predict development to blastocyst and implantation has been extensively investigated as early as 2010 (*Wong et al, 2010*; *Meseguer et al., 2011*), albeit without reaching a consensus (*Armstrong et al., 2019*). This has prompted the need, recently tackled with the application of artificial intelligence, to harness the immense potential of TLM to generare morphokinetic data. An initial experience documenting the application of artificial intelligence to oocyte and embryo scoring in clinical embryology was published as early as 2013 (*Manna et al., 2013*). Although relevant as a proof-of-principle and based on static images, this study was not followed by more extensive, independent investigations. The first major studies combining TLM technology and artificial intelligence applied to human IVF were published in 2019. *Tran et al. (2019)* reported on a modality based on deep learning and raw data derived from TLM sequences of embryos developed to the blastocyst stage. In this research, the investigators showed that the probability of achieving a clinical pregnancy can be automatically predicted with an area under the curve of 0.93. This study, however, requires independent confirmation, especially because its methodology seems to be largely unreported. In another study, *Khoshravi et al. (2019)* demonstrated that an artificial intelligence platform trained on blastocyst images scored by experienced embryologists can predict blastocyst quality with an area under the curve greater than 0.98 and the chances of pregnancy in a range between 13.8% and 66.3%, depending on blastocyst and patient characteristics. In addition, *Rad et al. (2019)* reported that artificial intelligence can predict embryo implantation based on the analysis of a single blastocyst image. A similar approach was used more recently by *VerMilyea et al. (2020)*, who described an improvement produced by artificial intelligence of 42% over routine embryologist assessment. These studies, together with others (*Curchoe et al, 2019*; *Dirvanauskas et al., 2019*; *Kanakasabapathy et al., 2019*), have generated considerable interest in the field and will certainly be followed up by exciting developments.

The combination of different technical and analytical tools used in the present study to test the hypothesis that novel information derived from cytoplasmic movements occurring in the early embryo can be harnessed to develop predictive models of development.

Focus on cytoplasmic movements as a source of data to predict human embryo development is a novelty in MAR. In fact, all embryo evaluation models, both static and dynamic, rely on large-scale embryo morphological attributes, e.g. pronuclear position, cell number, degree of fragmentation, rate of blastocele expansion and times of specific events (cleavages, compaction, start of blastocel formation), leaving cytoplasmic dynamics totally neglected. Therefore, in PIV analysis of cytoplasmic movements interpreted with the power of artificial intelligence, we glimpsed a novel window of opportunity to improve early prediction of embryo developmental ability. Previous animal studies have highlighted the significance of cytoplasmic dynamics for oocyte and embryo function. In a mouse model, *Ajduk et al. (2011)* were the first to demonstrate that TLM associated with PIV can detect specific patterns of cytoplasmic flows occurring during fertilization, which can be then used to predict embryo viability. With the use of the same image detection technology, we previously showed that mouse oocyte developmental competence, as assessed

by chromatin rearrangement at the germinal vesicle stage, can be correctly predicted with a probability of 90% (*Bui et al., 2017*). Crucially, however, in this study, we made more far-reaching and effective use of the data generated by TLM-PIV, by training an artificial neural network. Therefore, we decided to exploit the excellent predictive power of this approach to assess embryo developmental competence *in vitro* in a MAR scenario. We recognize the limitations of our study, mainly represented by the small data set and the end point (blastocyst development), which is not the ultimate goal of infertility treatment. At the same time, however, we are encouraged by the significance and outcome of the present study, which should, however, be intended as a proof-of principle. First, compared with the artificial intelligence studies of *Tran et al. (2019)* and *Khoshravi et al. (2019)*, we used a completely different and new source of data, i.e. analysis of cytoplasmic movement detected by PIV, as discussed above. This demonstrates that non-invasive bright field microscopy can potentially offer more than simply static morphology or morphokinetic information. In particular, our data suggest that sophisticated tracking of subcellular characteristics (discrete cytoplasmic particles) can add a 'layer' of morphological information, qualitatively and quantitatively valuable, never used or even considered so far for the analysis of human or animal cleavage-stage embryos. Second, our data suggest that artificial intelligence has the potential to improve the predictive ability of an experienced embryologist, achieving 82.6% accuracy, 79.4% sensitivity and 85.7% specificity to predict development to blastocyst. Human intervention may be seen as an obstacle to full automation. On the contrary, we believe that valuable human skills should not be lost as an effect of technological development, but rather preserved and used. Third, and particularly significant for the practice of MAR, our study suggests that blastocyst development can be predicted by day 2, instead of day 3 as shown in other studies (*Conaghan et al., 2013*). Overall, however, two major limitations of our studies should be noted. The small sample size is compatible with the exploratory and preliminary nature of this investigation, but represents a weakness that prevents immediate application in a clinical scenario. Therefore, larger studies should be undertaken to confirm the validity of the present data and assure more stringent control of possible biases derived from clinical parameters. Indeed, we are in the process of setting up a larger study focused on clinical end-points. Not only will this require a larger study population and more participating clinics, but also an evolution of computational and information technology tools to automate crucial steps of data extraction and elaboration, of which length and manual operativity represent an impediment to routine use of our model.

From a biological perspective, the study confirms the importance of early development for the correct unfolding of later stages. Interestingly, our approach could be further focused on the fertilization window, to assess the specific predictive power of this developmental segment. Consistent with this proposition, PIV patterns of competent and non-competent embryos diverge significantly during the interval of fertilization comprised between pronuclear positioning and breakdown. Once again, this highlights the importance and predictive power of fertilization events, as reported in recently published studies (*Coticchio et al., 2018*). Alternatively, the present approach could be extended to the cytoplasmic activity of human unfertilized oocytes, mirroring our previous mouse study (*Bui et al., 2017*).

From a more practical perspective, should these preliminary data be confirmed, the possibility of limiting embryo culture to 2 days would entail a reduction in costs and complexity of the IVF process compared with day-5 culture, while preserving a clinical efficiency comparable with blastocyst transfer.

In conclusion, collectively, although not immediately translatable into clinical embryology practice, the present study suggests that movement of cytoplasmic particles of the human early embryo is a novel and valuable source of data to predict further development, after elaboration by advanced imaged analysis and artificial intelligence. This opens new perspectives for non-invasive embryo assessment. Indeed, we are in the process of gathering forces for a much larger study having live birth rate, as primary end-point, and other parameters of clinical outcome as secondary end-points.

ACKNOWLEDGMENTS

This work was made possible thanks to support of 9.baby Family and Fertility Center, the Italian Ministry of Education, University and Research (MIUR) Dipartimenti di Eccellenza Program (2018–2022) to the Department of Biology and Biotechnology 'L. Spallanzani', University of Pavia, and a grant from the University of Pavia (FRG 2018). The authors also thank Merck-MilliQ Laboratory Water Solutions for support.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.12.008.

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Received 7 May 2020; received in revised form 27 October 2020; accepted 18 December 2020.