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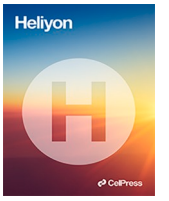

Research article

A novel non-invasive embryo evaluation method (NICS-Timelapse) with enhanced predictive precision and clinical impact

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A B S T R A C T

The selection of the finest possible embryo in in-vitro fertilization (IVF) was crucial and revolutionary, particularly when just one embryo is transplanted to lessen the possibility of multiple pregnancies. However, practical usefulness of currently used methodologies may be constrained. Here, we established a novel non-invasive embryo evaluation method that combines non-invasive chromosomal screening (NICS) and Timelapse system along with artificial intelligence algorithms. With an area under the curve (AUC) of 0.94 and an accuracy of 0.88, the NICS-Timelapse model was able to predict blastocyst euploidy. The performance of the model was further evaluated using 75 patients in various clinical settings. The clinical pregnancy and live birth rates of embryos predicted by the NICS-Timelapse model, showing that embryos with higher euploid probabilities were associated with higher clinical pregnancy and live birth rates. These results demonstrated the NICS-Timelapse model's significantly wider application in clinical IVF due to its excellent accuracy and noninvasiveness.

1. Introduction

A healthy embryo, a receptive endometrium, and molecular synchronization between the embryo and endometrium are necessary for a successful continuous pregnancy with assisted reproduction technology (ART) [1,2]. In order to boost the likelihood of pregnancy and speed up the process of giving birth to a living child, double or triple embryo transfers have typically been used in clinical in-vitro fertilization (IVF) for the past few decades. But that led to a significant increase in multiple pregnancies, which have been identified as the biggest threat to the health of both mother and fetus with a wide range of detrimental complications [3–5]. With a substantially reduced rate of multiple pregnancies in earlier studies, single embryo transfer (SET) was a practical treatment for multiple pregnancies [6]. Given the worries about the longer times for pregnancies, higher costs, and the potential for a decrease in overall pregnancy chances, it is noteworthy that the choice of the best available embryo to transfer is crucial and most difficult in SET. Therefore, advancements in techniques for evaluating embryos' ability for implantation were essential to the success of SET.

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Embryo morphological evaluation has traditionally been used to choose the possible embryos based on a small number of microscopic single-point observations [7]. However, microscopic morphological observations are prone to significant operator subjectivity, and observations made at particular time points were unable to capture the embryos' continual dynamic growth. Even morphologically healthy embryos do not always implant successfully during IVF without taking into account the embryo's chromosomal variations [8–10].

A non-invasive technology called Timelapse imaging (TLI) system using embryo morphokinetics is suggested to evaluate embryo potential in order to develop a better system for grading embryo quality, compared with the conventional morphologic assessment [11–14]. Timelapse parameters were found to be predictive of embryo ploidy status, blastocyst formation, and embryonic development potential. Models for selecting the "best embryo" were created as a result of these studies [10,15–22]. However, its true influence on clinical outcomes is still debatable [20,22]. In order to find the useful information buried in the vast amounts of data that TLI collected for the purpose of evaluating the potential of embryos, an appropriate technique is therefore required.

Preimplantation genetic testing for aneuploidy (PGT-A) is frequently used to choose embryos free of chromosomal abnormalities, the most significant cause of IVF implantation failure and miscarriage [2,23–25]. Recently, a technique known as noninvasive chromosome screening (NICS) was developed and applied to determine the aneuploidy of embryos by sequencing the cell-free DNA secreted into the spent culture media (SCM) from human blastocysts and does not call for invasive embryo manipulation, pricey biopsy tools, or difficult biopsy techniques [26]. Because genetic material from both inner cell mass (ICM) and trophectoderm (TE) cell lineages is released into culture conditions, researchers even claimed that cell-free DNA from SCM is more accurate than that acquired from TE biopsies. The maternal cell contamination, different embryo culture protocols, embryo manipulation, whole-genome amplification techniques, and sequencing data analysis, however, limit the capacity of NICS to predict embryo aneuploidy in the clinical setting, suggested that additional efforts should be made to encourage the clinical implementation of NICS.

The application of artificial intelligence (AI) technologies to ART has revolutionized embryos assessment and chosen. When given enough information, it can do back-propagation indefinitely to determine the best way to represent the supplied dataset. In the present study, with the application of AI technologies, we created a new non-invasive embryo evaluation and selection approach integrating Timelapse morphokinetics and NICS, which performed better than Timelapse morphokinetics or NICS alone. A cohort of 121 intracytoplasmic sperm injection (ICSI) patients was used to further assess the model's efficacy.

2. Materials and methods

2.1. Experimental model and study participant details

Between September 2020 and September 2021, the Reproductive Medicine Center at Tongji Hospital enlisted 213 donated frozen blastocysts from female patients (aged 23 to 50). In order to test the effectiveness of the novel model, 121 ICSI patients were prospectively recruited and divided into three groups. 36 Group A individuals received frozen blastocyst transfers. Early in the morning, the blastocysts were thawed. After 6 h of culture, the blastocyst was transferred to another G2 plus media to await transfer, and the SCM was obtained. 44 Group B patients with fresh blastocyst cultured, the SCM gathered, and the blastocyst was then frozen in preparation for a prospective frozen blastocyst transfer. 41 Group C patients who had both fresh blastocysts transplanted and fresh blastocyst SCM harvested in the same manner as Group A. This study was approved by the Ethics Committee of Tongji Medical College (Reference number:TJ-2020). All embryos used for this research were donated with the patients' written informed consent.

2.2. Methods details

2.2.1. Embryo thawing and spent culture medium collection

A total of 213 donated frozen blastocysts were recruited for embryo evaluation and selection model development. All of the frozen embryos came from ICSI and were developed to blastocysts utilizing a time-lapse microscopy system called Embryoscope Plus (Vitrolife, Denmark). The blastocysts were then defrosted and cultured. If there were any granular cells left, they would be gently eliminated by repeated blowing and sucking. Following three G2 plus medium washes, each blastocyst was grown in a 10 L G2 plus droplet. Following a 6-h culture, the SCM (20–25L) from each embryo was transferred into RNase–DNase–free PCR tubes, each of which contained 5 L of the cell lysis buffer (Yikon Genomics, Suzhou, People's Republic of China), and kept at −80 °C until use. Matched whole blastocyst was also transferred into 5 μL of the cell lysis buffer and stored at −80 °C until use.

2.2.2. Prospective patient recruitment and spent culture medium collection

In order to test the effectiveness of the novel model, 121 ICSI patients were prospectively recruited and divided into three groups. 36 Group A individuals received frozen blastocyst transfers. Early in the morning, the blastocysts were thawed. After 6 h of culture, the blastocyst was transferred to another G2 plus media to await transfer, and the SCM was obtained. 44 Group B patients with fresh blastocyst cultured, the SCM gathered, and the blastocyst was then frozen in preparation for a prospective frozen blastocyst transfer. 41 Group C patients who had both fresh blastocysts transplanted and fresh blastocyst SCM harvested in the same manner as Group A. A matched SCM sample was taken for NICS from the blastocyst that passed the criteria for embryo freezing (3BC) and had the best morphology. Due to missing Timelapse data, unquantified droplets, failed WGA for NICS analysis, or embryos not transferred, clinical outcomes of 75 patients (24 from Group A, 25 from Group B, and 26 from Group C) were finally recorded and gathered. Patients with ICSI-fertilized embryos, embryos with continuous cleavage-stage culture, and embryos with blastocyst culture during fresh cycles were the only ones included; those with blastocyst culture after thawing stored cleavage-stage embryos were excluded. Patients with

systemic immune illnesses such as thyroiditis, systemic lupus erythematosus, aberrant morphological oocytes, scarred uteri, uterine deformity, uterine adhesions, and other organic uterine problems were also disqualified.

2.2.3. Time lapse incubation and recording

The oocytes were then injected, put in a TL culture medium containing G1 plus, and continuously cultured for more than 3 days at 6 % CO2, 5 % O2, and 37 °C in a TL incubator (Embryoscope Plus, Vitrolife, Sweden). Following oocyte retrieval, all of the embryos were examined in the morning of day 3 according to the Istanbul Consensus, and the culture medium was changed to G2 plus for the subsequent blastocyst culture. The embryos were rated using the Gardner and Schoolcraft grading system on day 5 or 6 in the morning [40]. The morphologically best blastocyst was chosen, SCM samples were taken for NICS, and the embryo was either frozen or transplanted. The time of insemination is the embryonic development start time (t0). Each cleavage event of the embryo was timed at the moment of event completion. Morphokinetic parameters analyzed in this study included tPNa (Time of pronuclei appearance), t2 (Time to 2-cell stage), t3 (Time to 3-cell stage), t4 (Time to 4-cell stage), t5 (Time to 5-cell stage), t6 (Time to 6-cell stage), t7 (Time to 7-cell stage), t8 (Time to 8-cell stage), t9 (Time to 9-cell stage), tSC (Time from insemination to when the first cells of the embryo began to join together and compact), tM (Time from insemination to formation of a morula), tSB (Time from insemination to start of blastulation), tB (Time from insemination to formation of a full blastocyst), tEB (Time from insemination to expanded blastocyst), tpnf (Time when both pronuclei had faded), t2-t9 (Time of division to two-nine cells), cc2 (Time of second cell cycle (t3–t2), from 2 to 3 cells), cc3 (Time of third cell cycle (t5–t3), from 3 to 5 cells), s2 (Time of synchrony of the second cell cycle (t4–t3), from 2 to 4 cells), s3 (Time of synchrony of the third cell cycle (t8–t5), from 4 to 8 cells) were also recorded.

2.2.4. Whole-genome amplification and next-generation sequencing

Following library preparation with ChromInst (Yikon Genomics; EK100100724 NICS Inst Library Preparation Kit), whole-genome amplification was carried out utilizing culture media and entire blastocysts. On the MiSeq platform (Illumina, San Diego, CA, USA), next-generation sequencing was performed, producing approximately 2 million sequence reads for each sample.

2.2.5. Embryo grading by machine learning methods

As previously reported, the copy number variation (CNV) of each complete blastocyst and associated SCM were determined.[26] The genuine euploidy status of the embryo is determined by the complete blastocyst CNV results. Euploidy was described as an embryo with all 24 chromosomes without alterations in their numbers or structures, and aneuploidy was defined as an embryo having these variations.Euploidy prediction models were first constructed using a variety of machine learning techniques, including Logistic Regression, LightGBM, XGBoost, CatBoost, and Random Forest. The development of a euploidy prediction model was then carried out

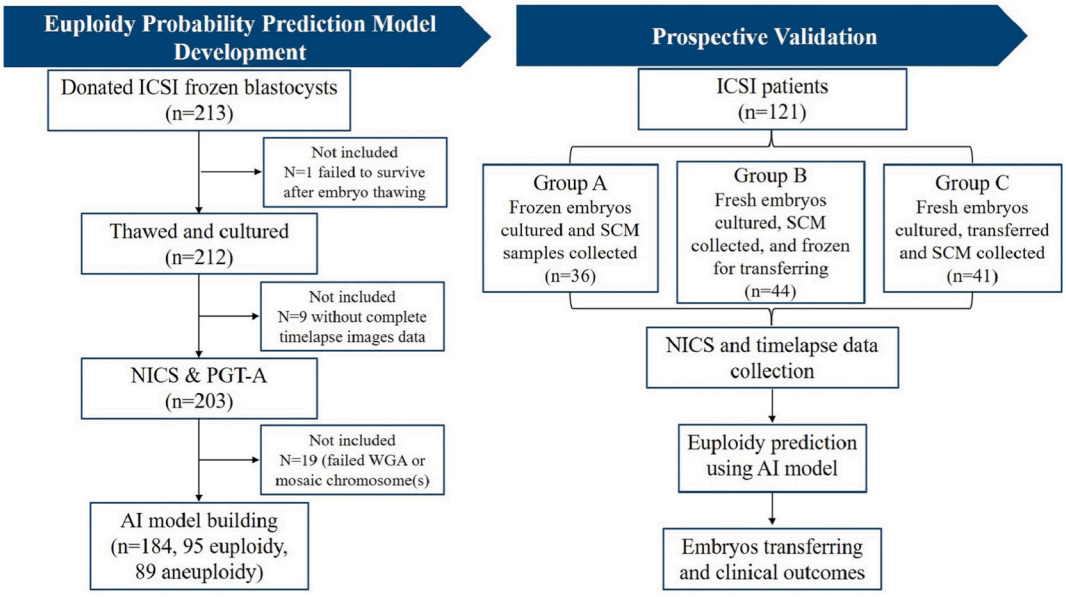

Fig. 1. Overeview of the study design. Left panel: 213 donated frozen blastocysts were ogrinially enlisted for model development. After ten blastocysts disqualified, NICS and PGT-A were performed on 203 pairs of complete blastocyst samples and the accompanying spent culture medium (SCM) samples. 19 embryos were further disqualified and a total of 184 blastocysts and the corresponding SCM and time-lapse morphokinetic characteristics were trained for embryo evaluation model using artificial intelligence algorithms. Right panel: 121 ICSI patients were prospectively recruited and were divided into three groups: those who received frozen blastocyst transfers with frozen harvested SCMs (Group A), those who received frozen blastocyst transfers with fresh harvested SCMs (Group B), and those who received fresh blastocyst transfers with fresh harvested SCMs (Group C). All the embryos were evaluated by NICS-Timelapse model. Clinical pregnancy rates and live birth rates served as the clinical endpoints.

using ensemble learning. Each machine learning technique's hyperparameters were tuned using Bayesian optimization. The performance of each machine learning model was assessed using the 10-fold cross-validation method, and the top performing model was chosen for further analysis.Euploidy prediction models were created using a combination of machine learning techniques, NICS data from corresponding SCM samples of embryos, Time-lapse morphokinetic parameters of cultured embryos, and combined NICS results and Time-lapse morphokinetic parameters, respectively. The euploidy probability determined by machine learning methods was used to calculate the embryo rating. The clinical pregnancy outcome of each selected embryo was followed to validate the clinical utility of the model.

2.3. Quantification and statistical analysis

Testing categorical variables is done using the chi-square test or the fisher's exact test, whereas testing continuous variables is done using the Welch *t*-test. To address the issue of multiple comparisons, all P values were two-sided and modified using the Holm-Bonferroni technique. A P value of less than 0.05 is regarded as statistically significant. The experiments above were performed using R software (4.0.0).

2. Results

3.1. Study design and participants

The study design was described in Fig. 1. First, a model for selecting and evaluating embryos was developed. We enlisted 213 donated frozen blastocysts from patients aged from 23 to 50. After embryo thawing, 212 of their blastocysts persisted. Nine blastocysts that had insufficient Timelapse imaging data were also disqualified. This led to the collection of 203 pairs of complete blastocyst

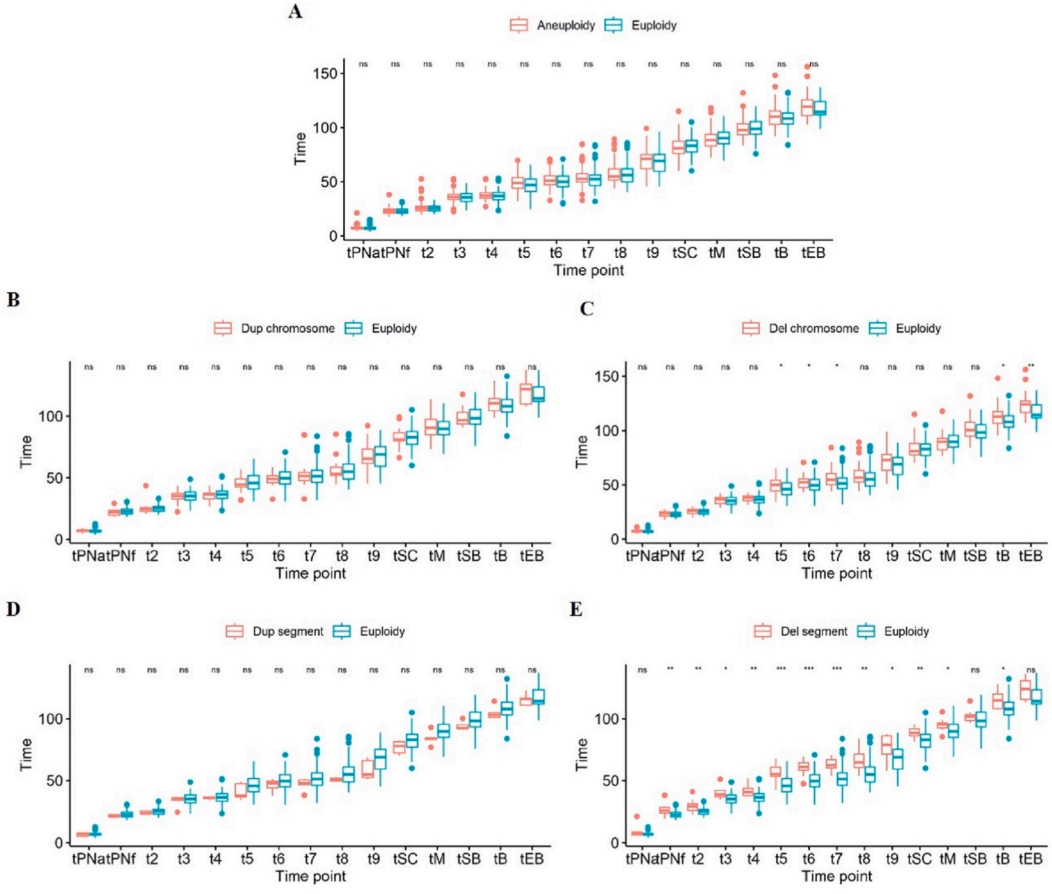

Fig. 2. Embryo aneuploidy delays embryonic development. (A) Aneuploidy embryos typically grew more slowly than euploidy embryos, notably at the t6, t7, and tEB time points. (B) Aneuploidy embryos with chromosomal duplication(s) displayed similar growth to euploidy embryos. (C) Aneuploidy embryos with chromosomal deletion(s) significantly postponed embryonic development. (D) Aneuploidy embryos with segmental duplication(s) displayed similar growth to euploidy embryos. (E) Aneuploidy embryos with segmental deletion(s) significantly postponed embryonic development.

samples with intact Timelapse data and the accompanying SCM samples. Following NICS and PGT-A due to failed whole-genome amplification (WGA) or mosaic chromosome(s), 19 embryos were disqualified. As a result, the model was created using artificial intelligence algorithms along with 184 blastocysts and the corresponding SCM and time-lapse morphokinetic characteristics. The effectiveness of the model for embryo evaluation and selection was then validated through the prospective recruitment of 121 ICSI patients. The patients were divided into three groups: those who received frozen blastocyst transfers with frozen harvested SCM samples (Group A), those who received frozen blastocyst transfers with fresh harvested SCM samples (Group B), and those who received fresh blastocyst transfers with fresh harvested SCM samples (Group C), in order to assess the performance of the model in various scenarios. Clinical pregnancy rates and live birth rates served as the clinical endpoints.

3.2. Embryo aneuploidy delays embryonic development

To assess the impact of embryo aneuploidy on embryonic development, we systematically evaluated the embryonic development of euploidy and aneuploidy embryos at various critical time periods. Aneuploidy embryos typically grew more slowly than euploidy embryos, notably at the t6, t7, and tEB time points, as illustrated in Fig. 2A. By type of aneuploidy, embryos with genomic instability were further divided into four categories. The development status of aneuploidy embryos with chromosomal or segmental duplications was similar to that of euploidy embryos (Fig. 2B and D). Aneuploidy embryos with segmental deletion(s), chromosomal deletion(s) significantly postponed embryonic development (Fig. 2C and E). These findings revealed that aneuploid embryos took longer than euploid embryos to reach the same developmental stage.

3.3. Euploidy probability prediction using Timelapse morphokinetic parameters

We developed an embryo evaluation model according to the euploidy probability of embryos using time-lapse morphokinetic parameters from 184 cultured blastocysts combined with machine learning random forest (RF) algorithm. We employed the chromosomal ploidy status of the entire blastocyst determined by PGT-A as the gold standard and discovered that 95 of the 184 were classified as euploidies while the other 89 were aneuploidies. Every embryo's euploidy probability score was determined by the model using 10-fold cross validation. Timelapse model got the performance with an area under the curve (AUC) of 0.76 and an accuracy of 0.72 in euploidy prediction (Fig. 3, Table 1). According to their individual euploidy probabilities, we further subdivided the embryos into different groups: 0.7–1.0, 0.3–0.7, and 0.0–0.3 (Fig. 4). We further discovered that 95 % group 0.7–1.0 embryos were euploidies, 100 % group 0.0–0.3 embryos were aneuploidies, while 48 % group 0.3–0.7 embryos, including 86.4 % blastocysts (159/184), were euploidies. These findings suggested that embryos with higher euploidy probability scores presented higher percentage of euploidies, and ambiguity in the classification of euploidy and aneuploidy in group 0.3–0.7.

3.4. Embryo euploidy probability prediction using NICS results

We also created an embryo evaluation model using NICS data from 184 blastocyst SCM samples solely. The chromosomal ploidy status of whole blastocyst identified by PGT-A was used as gold standard. Every embryo's euploidy probability score was determined

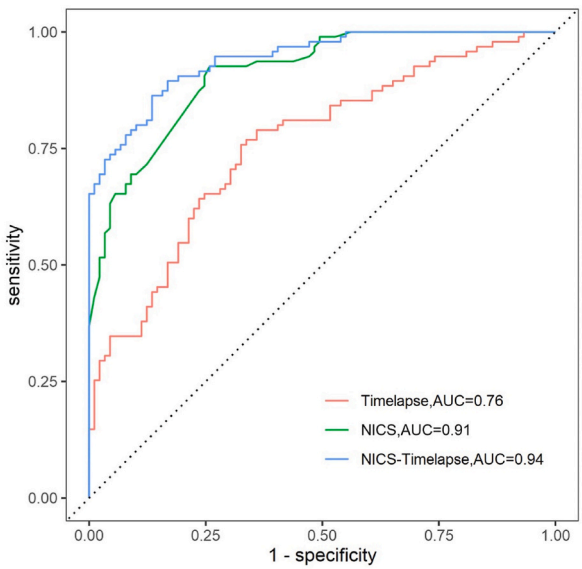

Fig. 3. Euploidy probability prediction performance by Timelapse model, NICS model and NICS-Timelapse model. The AUCs of Timelapse model, NICS model and NICS-Timelapse model were 0.76, 0.91, and 0.94, respectively.

Table 1
Performance of Timelapse, NICS, and NICS-Timelapse model.

Model	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	Precision
Timelapse	0.76	0.76	0.67	0.71	0.72	0.72	0.71
NICS	0.91	0.93	0.74	0.79	0.90	0.84	0.79
NICS-Timelpase	0.94	0.86	0.87	0.87	0.86	0.86	0.87

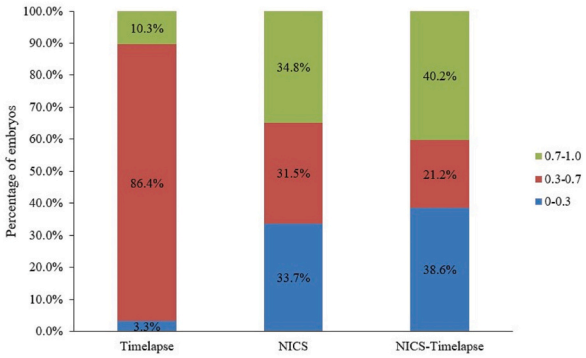

Fig. 4. Euploidy probability predictions of 184 embryos using Timelapse model, NICS model, and NICS-Timelapse model. According to predicted euploidy probability, all embryos were divided in to three groups: 0–0.3, 0.3–0.7.07-1.0. By Timelapse model, 3.3 % embryos were predicted with euploidy probability 0–0.3, 86.4 % were predicted with euploidy probability 0.3–0.7, and 10.3 % were predicted with euploidy probability 0.7–1.0. By NICS model, 33.7 % embryos were predicted with euploidy probability 0–0.3, 31.5 % were predicted with euploidy probability 0.3–0.7, and 34.8 % were predicted with euploidy probability 0.7–1.0. By NICS-Timelapse model, 38.6 % embryos were predicted with euploidy probability 0–0.3, 21.2 % were predicted with euploidy probability 0.3–0.7, and 40.2 % were predicted with euploidy probability 0.7–1.0.

by the model using 10-fold cross validation. The NICS model demonstrated blastocyst euploidy status prediction with an accuracy of 0.84 and an AUC of 0.91 (Fig. 3, Table 1). Following that, we classified embryos as different groups based on their relative euploidy probabilities of 0.7–1.0, 0.3–0.7, and 0.0–0.3 (Fig. 4). By entire blastocyst PGT-A results, euploidies were confirmed in 94 % of group 0.7–1.0 embryos, 50 % of group 0.3–0.7 embryos, and 10 % of group 0.0–0.3 embryos. In comparison to the Timelapse model, considerably more embryos were assigned to groups 0.7–1.0 (34.8 % vs. 10.3 %, $P = 2.58 \times 10^{-5}$) and 0.0–0.3 (33.7 % vs. 3.3 %, $P = 1.47 \times 10^{-9}$), and considerably fewer embryos (31.5 % vs. 86.4 %, $P = 1 \times 10^{-8}$) fell into group 0.3–0.7 by the NICS model (Fig. 4). These results suggested a better performance in euploidy probability prediction of NICS model than the Timelapse model.

3.5. Development of an embryo grading strategy based on NICS and Timelapse morphokinetic parameters

Finally, we created an embryo grading model based on NICS data from 184 blastocyst SCM samples and the associated Timelapse morphokinetic parameters together with the RF technique. With an AUC of 0.94 and an accuracy of 0.88, the combined NICS-Timelapse model demonstrated its ability to forecast blastocyst euploidy quality (Fig. 3, Table 1). According to their relative euploidy probabilities, 75 (40.8 %), 39 (21.2 %), and 70 (38.0 %) embryos were classified as group 0.7–1.0, group 0.3–0.7, and group 0.0–0.3 (Fig. 4). Compared to the Timelapse mode, significantly fewer embryos fell into group 0.3–0.7 (21.2 % vs. 86.4 %, $P = 1.13 \times 10^{-11}$), and significantly more embryos were classified in group 0.7–1.0 (40.2 % vs. 10.3 %, $P = 1.59 \times 10^{-6}$) and group 0.0–0.3 (38.6 % vs. 3.3 %, $P = 7.05 \times 10^{-11}$) (Fig. 4). When compared to NICS model, more embryos were assigned to group 0.7–1.0 (40.2 % vs. 34.8 %, $P = 0.531$) and group 0.0–0.3 (38.6 % vs. 33.7 %, $P = 0.57$) and less embryos were fell into group 0.3–0.7 (21.2 vs. 31.5 %, $P = 0.109$) similarly, although without statistical significance (Fig. 4). These results suggested a much higher consistent chromosome euploidy with whole blastocysts and a better performance in euploidy probability prediction of NICS-Timelapse model.

3.6. NICS-timelapse grading system as a predictor of clinical outcomes

To validate the prediction ability of euploidy probabilities of NICS-Timelapse model, 75 ICSI patients were recruited. The baseline of characteristics of patients were listed in Table S1. After embryo transfers, 75 patients' clinical pregnancies and live deliveries were recorded. Of those, 62.7 % (47/75) of the patients had clinical pregnancies. Specifically, 42.9 % (9/21) transferred embryos predicted with euploidy probabilities 0.0–0.3, 62.5 % (10/16) transferred embryos with predicted euploidy probabilities 0.3–0.7, and 73.7 % (28/38) transferred embryos predicted with euploidy probabilities 0.7–1.0 were found with clinical pregnancies, indicated a higher euploidy probabilities and a significantly higher clinical pregnancy rate (P trend = 0.01). 57.3 % (43/75) patients achieved live birth, which consisted 38.1 % (8/21) transferred embryos predicted with euploidy probabilities 0.0–0.3, 56.3 % (9/16) transferred embryos with predicted euploidy probabilities 0.3–0.7, and 68.4 % (26/38) transferred embryos predicted with euploidy probabilities 0.7–1.0, indicated a higher euploidy probabilities and a significantly higher live birth rate (P trend = 0.01).

Three groups of 75 patients were created based on various clinical circumstances. 24 frozen embryos were transferred by Group A patients, and 50 % of these pregnancies were clinically successful (Fig. 5A). 25 patients in Group B transferred frozen embryos (with fresh circle SCMs), and 76.0 % of them had clinical pregnancies (Fig. 5B). 26 Group C patients transferred fresh embryos, and 61.5 % of them had clinical pregnancies (Fig. 5C). According to these findings, the NICS-Timelapse model can be used in a variety of clinical circumstances because there was no statistically significant difference in the clinical pregnancy rate across the three groups of patients (Group A vs. B vs. C, 50 % vs. 76.0 % vs. 61.5 %). There was a propensity for greater clinical pregnancy rates in embryos with higher euploidy probabilities within each group with Group B attained significance (Group A, P trend = 0.30; Group B, P trend = 0.03; Group C, P trend = 0.13).

41.7 % (10/24) of the transferred Group A embryos resulted in a live birth (Fig. 6A). 68.0 % (17/25) of the transferred Group B embryos resulted in a live delivery (Fig. 6B). 61.5 % (16/26) of the transferred Group C embryos resulted in live births (Fig. 6C). These results revealed no significant difference in the live birth rate between the three patient groups (Group A vs. B vs. C, 41.7 % vs. 68.0 % vs. 61.5 %), showing that the NICS-Timelapse model could be used in various clinical settings. There was a tendency within each group (with Group B reaching significance) that embryos with a higher likelihood of euploidy have higher live birth rates, which indicated better clinical outcomes (Group A, P trend = 0.30; Group B, P trend = 0.05; Group C, P trend = 0.13).

4. Discussion

The effectiveness of human assisted reproduction depends on a number of important and difficult procedures, including embryo cultivation, embryo selection and transfer, cryopreserve or discard [27]. Clinical embryo selection is based on the embryo's morphological scores and/or PGT-A's determination of its aneuploidy status. Embryologists may view embryos continually without modifying the culture conditions thanks to the non-invasive Timelapse image technology, which has recently been used in IVF. The non-invasive embryo aneuploidy analysis technology, NICS, avoids the embryo biopsy's intrusive harm, the sparse number of cells that must be biopsied, and the high technical requirements [28–32]. The use of the Timelapse image system or NICS in embryo selection, however, revealed conflicting data and requires additional research. As a result, in the current study, we carefully and creatively created and tested a non-invasive embryo selection model that integrated NICS and data on the morphokinetics of cultured embryos collected over time. This model performed better than either NICS or Timelapse data alone.

Morphokinetics information provided by Timelapse image system has been increasingly utilized in embryo selection since the first embryo selection algorithm (using first division cytokinesis duration, time between first and second mitosis, and time between second and third mitosis) described in 2010 (Fadon P et al. Semin Reprod Med. 2021) [20]. Studying the relationship between Timelapse derived morphokinetic parameters and IVF/ICSI outcomes, it is shown that time intervals between discrete fertilization events (such as intervals between disappearance of the cytoplasmic halo and PN breakdown) were strongly associated with embryo quality on Day 3, fertilization and cleavage morphokinetic parameters (such as tPNf, t2, t3, t4, t5, t8) were also related to embryo development, implantation and pregnancy rates, and blastulation kinetics parameters (rtSB, dB) were recently revealed to be associated with live-birth rates [33]. Despite contradictory evidence existed, it is promising that construction of powerful algorithms using Timelapse morphokinetic parameters to facilitate embryologists to select the best possible embryo for transfer by eliminating embryos with lower potential of embryo development, blastocyst formation, implantation, pregnancy or live birth.

Our NICS-Timelapse model was developed based on NICS results and 20 Timelapse morphokinetic parameters (including tPNa, tPNf, t1, t2, t3, t4, t5, t6, t7, t8, t9, tSC, tM, tSB, tB, tEB, cc2, cc3, s1, s2). With an AUC of 0.94, sensitivity of 0.94, specificity of 0.82, and accuracy of 0.88, the NICS-Timelapse model outperformed the NICS model, which had an AUC of 0.91, sensitivity of 0.74,

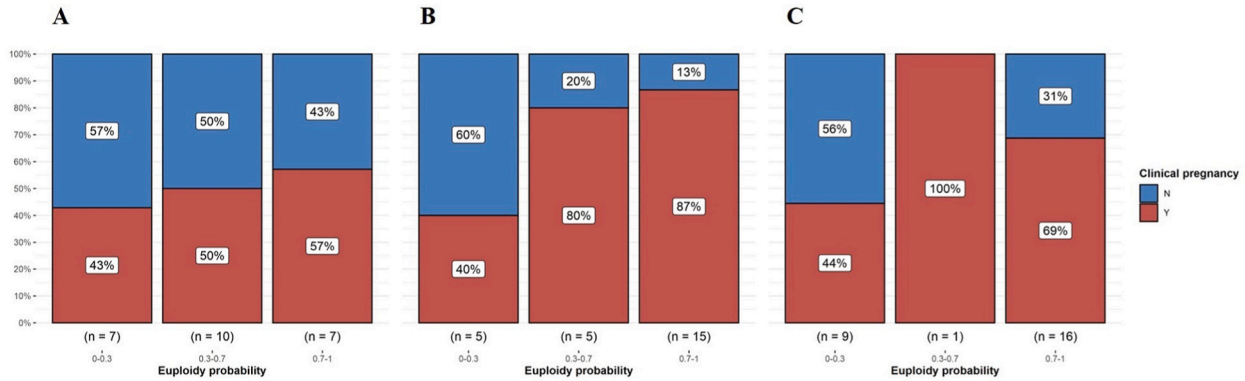

Fig. 5. NICS-Timelapse grading system as a predictor of clinical pregnancies. After the transfer of the embryos, 75 patients' clinical pregnancies status were recorded. (A) Among 24 Group A patients, 42.9 % (3/7) of the embryos predicted with euploidy probabilities 0.0–0.3, 50 % (5/10) with euploidy probabilities 0.3–0.7, and 57.1 % (4/7) with euploidy probabilities 0.7–1.0 were clinically successfully pregnant. (B) In 25 Group B patients, 40.0 % (2/5) embryos predicted with euploidy probabilities 0.0–0.3, 80 % (4/5) with euploidy probabilities 0.3–0.7, and 86.7 % (13/15) with euploidy probabilities 0.7–1.0 achieved clinical pregnancies. (C) Of 26 Group C patients, 44.4 % (4/9) of the embryos had euploidy probabilities of 0.0–0.3, 100 % (1/1) of 0.3–0.7, and 68.6 % (11/16) of 0.7–1.0 had clinical pregnancies.

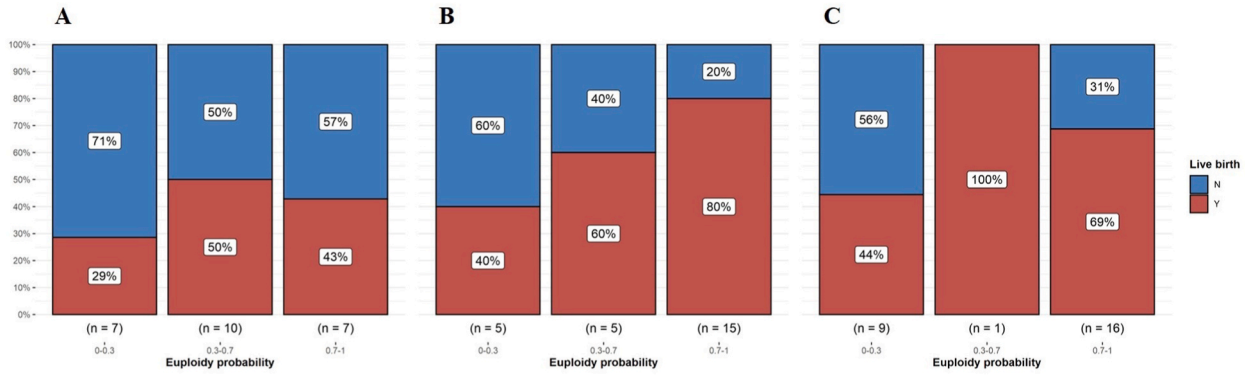

Fig. 6. NICS-Timelapse grading system as a predictor of live births. After the transfer of the embryos, 75 patients live births status were recorded. (A) Among 24 Group A patients, 28.5 % (2/7) of the embryos with predicted euploidy probabilities 0.0–0.3, 50 % (5/10) with euploidy probabilities 0.3–0.7, and 42.9 % (3/7) with euploidy probabilities 0.7–1.0 resulted in live births. (B) In 25 Group B patients, 40.0 % embryos of the euploidy probability 0.0–0.3, 60 % (3/5) of the euploidy probability 0.3–0.7, and 80.0 % (12/15) of the euploidy probability 0.7–1.0 achieved live births. (C) Of 26 Group C patients, 44.4 % (4/9) of embryos with euploidy probabilities 0.0–0.3, 100 % (1/1) with euploidy probabilities 0.3–0.7, and 68.6 % (11/16) with euploidy probabilities 0.7–1.0 resulted in live births.

specificity of 0.93, and accuracy of 0.84, as well as the Timelapse model, which had an AUC of 0.76, sensitivity of 0.67, specificity of 0.76, and accuracy of 0.72. In particular, by the NICS-Timelapse model, significantly more embryos were predicted with euploidy probabilities 0.7–1.0 and 0.0–0.3, and significantly fewer embryos were classified with unclear euploidy or aneuploidy 0.3–0.7 (Fig. 4). According to the results of PGT-A, 91.5 % (65/71), 90.3 % (56/62), and 100 % (6/6) embryos with predicted euploidy probabilities at 0.0–0.3 by the NICS-Timelapse model, NICS model, and Timelapse model were actual aneuploidies; 48.7 % (19/30), 50.0 % (29/58), and 48.4 % (77/159) embryos with predicted euploidy probabilities 0.3–0.7 by the above three models were true euploidies; and 94.6 % (70/74), 93.8 % (60/64), and 94.7 % (18/19) embryos with anticipated euploidy probabilities at 0.7–1.0 were true euploidies. Despite the fact that there was no discernible difference between the three models' aneuploidy ratios and euploidy ratios, the NICS-Timelapse model effectively weeded out a greater number of actual aneuploid embryos and a remarkably higher number of truly euploid embryos. These results demonstrated the NICS-Timelapse model's substantially higher consistency in chromosome euploidy with complete blastocysts and its superior performance in predicting euploidy likelihood.

We prospectively recruited 121 ICSI patients and used the NICS-Timelapse model to assess the euploidy probability of each embryo in order to confirm the model's effectiveness. In the end, 24 Group A patients underwent frozen blastocyst transfer and SCM collected after embryo thawing, 25 Group B patients underwent frozen blastocyst transfer and SCM collected after fresh blastocyst culture, and 26 Group C patients underwent fresh blastocyst transfer and SCM collected after fresh blastocyst culture. Clinical pregnancy rates and live birth rates in embryos predicted by the NICS-Timelapse model with a euploidy probability of 0.7–0.1 were 73.7 % and 68.4 % for the entire cohort, 57.1 % and 42.9 % for Group A, 86.7 % and 80.0 % for Group B, and 68.8 % and 68.8 % for Group C, respectively. These clinical pregnancy rates and live birth rates were equivalent to those from previous studies using PGT-A for embryos selection from other investigations [34,35]. Our study demonstrated a tendency that greater clinical pregnancy rates and live birth rates were connected across all groups with embryos diagnosed by the NICS-Timelapse model with higher euploidy probability. We also found that the model had better prediction for euploidy among Group B and C compared to Group A, as the percentage of embryos euploidy probability 0.3–0.7 was lower in group B (20 %, 5/25) and C (3.8 %, 1/26) compared to group A (41.7 %, 10/24). Besides, the differences in clinical pregnancy rate and live birth rate between different euploidy probability groups were most obvious in group B (0.0–0.3, 40.0 %, 40.0 %; 0.3–0.7, 80.0 %, 60.0 %; 0.7–1.0, 86.7 %, 80.0 %), followed by group C (0.0–0.3, 44.4 %, 44.4 %; 0.3–0.7, 100.0 %, 100.0 %; 0.7–1.0, 68.6 %, 68.6 %) and Group A (0.0–0.3, 42.9 %, 40.0 %; 0.3–0.7, 50.0 %, 50.0 %; 0.7–1.0, 57.1 %, 42.9 %).

The variations in the various groups were caused by a variety of circumstances. First, the percentage of patients undergoing their first ET cycles in Group A was significantly lower compared to Group B and C, and the times of previous ET in Group A were significantly higher than the other two groups. These differences could explain why Group A had a lower pregnancy rate and live birth rate than the other two groups. Even though we made a concerted effort to rule out potential confounding factors by establishing stringent inclusion and exclusion criteria, such as systemic immune disorders, organic uterine diseases, chromosomal disorders, and so forth, some bias may still exist, such as immunogenic factors linked to recurrent implantation failures. Second, our findings suggested that SCMs from fresh blastocysts were better able to accurately reflect the genetic status of the entire embryo than those from vitrified-thawed blastocysts. Before vitrification, the blastocysts are laser perforated to cause blastocyst collapse and the outflow of the blastocoelic fluid, enabling the exchange of the blastocoel fluid with the cryoprotectant solution and increasing cryopreservation survival. However, at the same moment, a little hole appeared in zone pellucida. Following the embryo's thawing, the fragment, apoptotic cells, and apoptotic cells outside the blastocoel were discharged into the culture media through the perforations, which could affect the results of the NICS. Finally, the key distinction between Group B and Group C was whether or not the embryos were transferred during fresh cycles. In contrast to group C, which received fresh embryo transfer, group B used frozen embryo transfer, shielding the patients from the negative effects of high levels of estrogen and progesterone and controlled ovarian hyperstimulation (COH) protocols and serving as a better exemplar of the model's usefulness.

Interestingly, we also discovered that aneuploidy embryos with chromosomal or segmental deletion(s) exhibited a considerable development delay compared to euploidy embryos, whereas those with chromosomal or segmental duplication(s) had a similar or slightly faster development status. According to earlier research, aneuploid embryos did not develop as normally as euploid ones [36–39]. According to Nogales MDC et al., trisomies exhibited similar kinetics to normal embryos, while multiple chromosomes affected exhibited noticeably faster division rates than normal embryos, and monosomies exhibited faster divisions than normal embryos and slower divisions than embryos with multiple chromosomes abnormalities [38]. We discovered that the development status of the single-chromosome duplication embryos in this study was comparable to the findings of our investigations, but the kinetics in the single-chromosome deletion embryos were in direct opposition to our findings. The disparity in results between our findings and those of the research by Nogales MDC et al. could have a number of factors [38]. First, the variations in the analyzed samples may be a factor in the inconsistent findings. In contrast to Nogales' research, which used cleavage-stage biopsies and only sampled one or two blastomeres, our study amplified and evaluated the entire blastocyst. The question of whether one or two blastomeres could be reflective of the genetic makeup of the entire blastocyst is still up for debate. Then, the two studies' disparate aneuploidy classification criteria may potentially provide results that are in conflict with one another.

In conclusion, we created and verified a non-invasive embryo selection algorithm, which integrated Timelapse and NICS data, performed better than either type of data alone. Patients who had previously experienced miscarriage but did not satisfy the criteria for PGT-A might find it helpful. If there were numerous viable blastocysts and a keen interest in genetic screening for embryos, this model might be used to rank the embryos. Furthermore, this model might be beneficial for embryos with fine ICM and TE that is too poor for biopsy.

5. Limitations of the study

The NICS-Timelapse approach still has some limitations. First off, the cohorts utilized for model training and evaluation were rather small, and because of this, the model's performance could have been negatively impacted and the error between expected and actual results could not have been reduced. Second, there were no clinical outcomes for the embryos in the training group, which may have potentially had an impact on the model's performance. Another major question it that it is unknown how these predicted calculations connected to other clinical parameters, such as female age, prior reproductive history, endometrial receptivity, environmental conditions, and others, which are known to impact embryo outcomes. These elements were not included in the computations. Therefore, it will be very useful to conduct additional research into the prognostic link between known clinical parameters and deep learning prediction.

Data availability statement

The datasets supporting the current study have not been deposited in a public repository but are available from the corresponding author on request.

CRediT authorship contribution statement

Hui He: Writing – original draft, Data curation. **Li Wu:** Methodology, Formal analysis, Data curation. **Yulin Chen:** Software, Methodology. **Tuan Li:** Validation, Supervision, Project administration, Methodology. **Xinling Ren:** Validation, Supervision. **Juan Hu:** Validation, Methodology, Data curation. **Jinming Liu:** Validation, Formal analysis, Data curation. **Wen Chen:** Supervision. **Bingxin Ma:** Validation, Formal analysis, Data curation. **Yangyun Zou:** Writing – review & editing, Software, Methodology. **Zhen Liu:** Project administration, Investigation, Formal analysis, Data curation. **Sijia Lu:** Supervision, Funding acquisition. **Bo Huang:** Supervision, Project administration. **Lei Jin:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30189.

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