Clinical Research Protocol: Application of deep learning automation based on Time-lapse imaging to jointly assess embryo development to improve pregnancy outcome of single blastocyst transfer

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Summary of the case

Project Name	A clinical study based on Time-lapse imaging application of deep learning automated joint evaluation of embryo development to improve the outcome of single blastocyst transplantation pregnancy
Purpose of the study	Based on Time-lapse imaging application of deep learning, this project explores the automatic evaluation model of different developmental stages of embryos, and establishes an automated joint evaluation system for the whole process of embryonic development. In addition, the patients with single blastocyst transplantation are prospectively collected, and the pregnancy outcome of the patients is followed up by TLI+AI evaluation and morphological evaluation, and the pregnancy outcome of the two groups is compared.The project aims to explore key features that play a role in the evaluation of AI embryos to provide a theoretical basis for further screening of high-quality embryos, thereby increasing the implantation rate of single embryo transfer patients, reducing multiple pregnancies and ensuring the safety of mothers and children.
Study Design	This study is an observational prospective study after a retrospective analysis. It is a single-center study without randomization or blindness.In the early stage, 1000 patients are collected from three periods of embryo culture through Time-lapse to establish an automated joint evaluation system for the whole process of embryo development. At the later stage, the patients are divided into two groups: TLI+AI assessment group and morphological assessment group. 100 patients with 5D single blastocyst transplantation are carried out to follow up the pregnancy outcome.

Total number of cases	1000 (retrospective study)+100 (prospective study)
Case selection	*Criteria for inclusion* 1) < 40 years of age; 2) Routine IVF cycle; 3) Period number ≤ 2; 4) The number of eggs harvested was 5-15. 5) follicle stimulating hormone（FSH) ≤ 12 IU/L on the third day of menstruation; 6) Patients with more than 3 high-quality embryos per Day 3 and feasible single blastocyst transplantation per Day 5; 7) Patient factors affecting embryo implantation such as endometrial factors.

	Exclusion criteria 1) Preimplantation Genetic Testing (PGT) is needed for male infertility, ovulation cycle and chromosome abnormality. 2) Pictures of unformed or non-available blastocysts; 3) Incomplete or unclear images were collected during prokaryotic, mitotic and blastocyst phases, which affected the AI evaluation.
Treatment plan	Through conventional ovulation induction and time-lapse culture, the embryo development was observed dynamically, pictures were collected to establish an automated joint evaluation system for the whole process of embryo development, and the embryos were selected for daily 5 single blastocyst transplantation.
Evaluation of curative effect	*Efficacy evaluation indicators (primary efficacy indicators and secondary efficacy indicators)* Precision rate, embryo planting rate
	Safety evaluation index The harmonic mean of accuracy rate and recall rate, abortion rate
Method of statistics	(1) Statistical analysis of the evaluation system with accuracy and recall rate; (2) $\chi 2$ test was used to compare the rates among control groups, and $p < 0.05$ was considered statistically significant.
Duration of study	December 30, 2022-June 15, 2024

Directory

List of acronyms

English abbreviation	Full English name	Full Chinese name
Assisted Reproductive Technology	ART	Assisted reproductive technology
Single embryo transfer	SET	Single embryo transfer
Time-Lapse imaging	TLI	Time difference imaging technique
Artificial Intelligence	AI	Artificial Intelligence
Deep learning	-	Deep learning

1. Background

Embryo selection is the core of single embryo transfer and an important factor for the success of assisted reproductive therapy

Multiple pregnancies are one of the most common and serious complications of Assisted Reproductive Technology (ART).Foreign evidence-based medical evidence shows that the risk of a twin pregnancy is 20 times that of a natural pregnancy, and the risk of a pregnancy with three or more children rises to 400 times. Multiple pregnancies may be related to the woman's age, previous reproductive history, ovulation induction drugs, fertilization methods and transplant days, but the most direct influencing factors are the rate of embryo implantation and the number of transplanted embryos. With the extensive application of ART technology, usually two or more embryos are transferred to ensure the pregnancy outcome, resulting in the multiple pregnancy rate in recent 30 years as high as 30-40%. With the increase of the number of transplanted embryos, the maternal cardiac output, oxygen consumption, basal metabolic rate, incidence of "physiological anemia" and postpartum hemorrhage significantly increased. For fetuses, the excessive uterine tension of twins increases the risk of premature, low birth weight infants and perinatal death, and the family economic burden also increases correspondingly.

Numerous studies have shown that the Single embryo transfer (SET) strategy has become one of the most effective ways to reduce multiple pregnancies in the field of assisted reproduction. Although both doctors and patients are aware of the superiority of SET in eugenics and procreation, they are concerned about reducing the number of embryos transferred and reducing the pregnancy rate, which is the main reason that makes it difficult to popularize SET in China.

In order to reduce multiple pregnancies, it is imperative to implement SET, and embryo optimization is the guarantee of single embryo transfer pregnancy.

It is challenging to optimize embryo selection based on morphological parameters to improve pregnancy outcome of single embryo transfer

Static morphological evaluation is the most commonly used method for embryo evaluation. It follows the ESHRE consensus to observe the embryo morphology at several fixed time points, which has the advantages of simple operation, low cost and no trauma to the embryo. Since the embryo itself is a complex three-dimensional structure, the focal plane needs to be adjusted repeatedly to obtain more comprehensive information during observation.For embryologists, it takes years of training to accurately and quickly judge these quantitative indicators under the microscope.On the other hand, errors brought by subjective factors or errors caused by fatigue cannot be avoided.In addition, a great deal of useful information about the development of the embryo is lost because of the timing of the assessment.The above defects of artificial observation not only restrict the scale development of embryo laboratory in terms of human resources, but also affect the further improvement of implantation rate, which is recognized as a technical difficulty to be improved in the field of assisted reproduction.

Embryonic development is a dynamic process.Time-Lapse imaging (TLI) is an advanced method for dynamic evaluation of embryo morphology, overcomes the disadvantages of static morphological evaluation, which not only ensures the stability of embryo culture microenvironment, but also continuously obtains a series of data of biological morphological changes during embryo development, providing more comprehensive and effective dynamic information for embryo evaluation.However, TLI technique is still limited to morphological evaluation, which can not accurately reflect the quality and developmental potential of embryos.Recent studies have shown that the TLI system currently in use and the accompanying embryo quality assessment software do not significantly improve the success rate. The reason may be that the current morphodynamic algorithm does not consider the influence of many external variables on the algorithm.

Artificial intelligence (AI) combined visualization technology opens up new ideas for selecting embryos

In order to optimize the embryo assessment process and make clinical work efficient and fast, embryologists have long been collaborating with computer scientists to use computer technology to assist embryo quality assessment.Artificial Intelligence (AI)· The branch of computer science that attempts to understand the nature of intelligence and produce new kinds of intelligent machines that react in a similar way to human intelligence.With the development of AI technology and computer processing power, more and more researchers are combining artificial intelligence with deep learning (deep learning) technology applied to the medical field.Deep learning is a new field in the research of machine learning. Its motivation is to build and simulate the neural network of human brain for analysis and learning. It mimics the mechanism of human brain and forms more abstract high-level representation attribute categories or features by combining low-level features to discover distributed feature representation of data, mainly for image, sound, text and other types of information.Convolutional neural network is a kind of depth feedforward artificial neural network. In recent years, it has been successfully applied to image recognition.Convolutional neural networks include one dimensional, two dimensional and three dimensional convolutional neural networks.Two-dimensional convolutional neural networks are mainly used in medical image data recognition, such as detection of diabetic retinopathy 18 kinds of skin diseases· And diagnosing diseases. In the field of reproduction, the measurement, evaluation and analysis of embryos have also been preliminarily explored.

Deep learning-based computer vision technology has the following significant advantages: 1) automatic end-to-end processing.For example, for the scoring of embryos, you can directly input an original picture to get the scoring result, the intermediate process does not need manual assistance;2) The model has strong generalization ability.When the number of learning samples reaches a certain level and the diversity is enough to reflect the actual scene, the trained artificial intelligence model can effectively adapt to different image formats (including photos taken by different brands of microscope equipment, or photos with different focal segments) and different image quality.3) It does not depend on artificially specified characteristics.Deep learning technology carries out autonomous learning and feature extraction on the basis of labeled samples, without the need to specify features of medical or visual significance in advance.So.Some unknown or hidden features can be found to improve accuracy.Many scientists at home and abroad have applied deep learning to each stage of embryonic development.

In the prokaryotic phase, Beuchat A uses traditional image algorithms to semi-automatically analyze prokaryotic images and provide accurate morphological measurement parameters.Zhao et al. use the segmentation system based on convolutional neural network to segment the images in the prokaryotic stage and achieve consistency (98 ± 1.02%). .Vikrant Reddy used CNN network (VGG16,AlexNet,ResNet-18) to classify the biprokaryotes (2PN), 3PN and germinal vesicle (GV) with an accuracy of 88% .In 2015, Aisha Khan used CNN +CRF t1, t4, t4+ (265 embryos) to predict the number of cells with an accuracy of 94% .In 2019, Tingfung Lau cut out the original cell region on 1309 videos, and used ResNet 50 + LSTM tsatrt tPnf t2 t3 t4 for division stage classification, with an average accuracy of 88.28% .**However, the accuracy of automatic labeling after the 4-cell stage was limited.**In China, Zhong Junjie et al. used the score of prokaryotic stage combined with cleavage stage to predict the developmental potential of embryos, and found that the comprehensive score could improve IVF implantation rate and pregnancy rate .

Single blastocyst transplantation has become the mainstream of single embryo transplantation strategy due to the advantages of high implantation rate. Blastocyst quality is mainly judged based on quantitative indicators such as the degree of sac expansion, trophoblast and inner cell mass quality .Pegah Khosravi et al. from the University of Conor took the lead in introducing deep learning into the field of embryo assessment. Based on the GoogLeNet Inception-V1 model, they trained 12,000 pictures of 1600 blastocysts. The classification accuracy was up to 96.94%, much higher than previous results. And far more accurate than embryologists.In the comparative analysis experiment, when five embryologists independently rated 394 blastocyst images (based on the same Gardner scoring method), only 89 embryos were identical .Jose Celso Rocha et al. used computer vision (CV) algorithm to extract relevant parameters from blastocyst pictures, and then modeled and classified them based on ANN neural network, achieving 76.4% accuracy .In China, some scholars have applied deep learning technology in the field of assisted reproduction.B. Huang et al. used multi-task deep learning with dynamic programming to classify the developmental stages of embryos in time-lapse photography videos, providing valuable information for embryologists .Taiwan embryologist Lan Huang et al. classified blastocyst development grade, inner cell mass and trophoblast quality, and combined the three classification results, the final embryo classification accuracy was 75.36% , the accuracy needs to be improved.In the past two years, it has been demonstrated that AI assessment can ensure the consistency of embryo quality assessment, and is better than embryologists .

In existing clinical studies, deep learning technology is mainly applied to automatically evaluate a single period of embryonic development, while comprehensive and continuous AI evaluation based on the dynamic process of embryonic development is rarely mentioned.This study is the first to propose the application of deep learning to the whole process of embryonic development, and to evaluate the embryo comprehensively at various developmental stages.In addition, although deep learning technology has made some progress in the evaluation of embryonic images, the black box mechanism is still a major obstacle to clinical application due to the lack of research on its classification mechanism.In 2017, Selvaraju recently proposed the Grad-CAM(Class Activation Map) method, that is, the weighted coefficients of the features were obtained by back propagation, the weighted weights of each feature map were obtained by the gradient return method, and then superimposed for visualization.Its application scope is wider, and it can be directly used for all kinds of convolutional neural network structures without retraining the model, and the visualization results are more accurate .This research team has made preliminary progress in the multi-focal length blastocyst quality evaluation model and visualization, in which the evaluation index AUC reached 0.936 And conducted in-depth communication and discussion with global experts in the field of reproduction at the ESHRE meeting in Europe 2020.**Recently, based on multi-focal length blastomere tracking technology, our research team has made a breakthrough in the identification of 8 cells and above in the division stage, and the identification accuracy of blastomere has reached more than 85%.The evaluation index of blastocyst region segmentation based on semantic segmentation method was also close to 90%, which reached the highest level of similar recognition results in the world.**

In conclusion, we believe that the establishment of AI-time lapse automatic system for embryo development evaluation not only overcomes the defects of traditional computer graphics algorithms, but also ensures the consistency of embryo evaluation.The quality assessment of the whole process based on the dynamic development of embryos can overcome the defects of single period quality assessment and improve the accuracy of assessment.The wide implementation and popularization of this technique can improve the evaluation accuracy, and at the same time, it can effectively promote the single blastocyst transplantation technology, reduce the rate of multiple pregnancies, protect the safety of mothers and children, reduce the personal and social economic burden, and create a good atmosphere for the establishment of a harmonious society.

2. Research objectives

2.1 Main objective: To continuously and automatically evaluate and track the development process of embryos using the latest deep learning technology.In this way, the human input of the laboratory can be reduced, and the objectivity, stability and accuracy of embryo evaluation can be improved, so as to improve the implantation rate of single embryo transfer patients, reduce multiple pregnancies, and ensure the safety of mother and child.

2.2 Secondary purpose: Based on the images taken by TLI system, explore new indexes and methods of embryo quality assessment by using the latest deep learning algorithm and combining embryo morphology method and embryo dynamics method.

3. Research design types, principles and test procedures

3.1 Research design

This study is an observational prospective study. It is a single-center study without randomization or blind grouping.In order to establish an automated joint evaluation system for the whole process of embryo development, it is necessary to collect images of 1000 patients in the prokaryotic, mitotic and blastocyst stages of single blastocyst transplantation in IVF cycles. According to the TLI+AI assessment and morphological assessment, at least 100 cases are required for fresh cycle 5 a Day single blastocyst transplantation.At present, the pregnancy rate of single blastocyst transplantation is basically maintained at 60%. Through this study, the pregnancy rate is expected to be increased to 80%. The test standard α is 0.05.

3.2 Research steps

(1) Establish an AI-time lapse automated combined embryo evaluation system to improve the accuracy of embryo evaluation

1) According to the entry and expulsion criteria, 1000 patients were enrolled and Time-lapse closed-loop culture embryo images of prokaryotic phase, mitotic phase and blastocyst phase were collected.

2) To establish the inclusion criteria for training samples, screen and sort out embryo picture samples;

3) Five embryologists annotated the image samples to generate training sample sets and test sample sets;

4) Convolutional neural network based on deep learning was used to build the prokaryotic detection network, the splintering period counting network, the splintering period segmentation network and the blastocyst region segmentation network, and train the detection models in each period respectively;

5) The convolutional neural network classification model is built to classify the embryo quality, and the AI-time lapse automatic joint embryo evaluation system is established by using the five-fold crossover method to train and verify the model.

(2) Debug the generalization ability of the model and improve the evaluation function of embryonic development

1) Test the generalization ability of the AI evaluation model by using embryo picture data, and visualize the features dependent on the model classification.

2) Comparative analysis of AI evaluation and manual evaluation, optimization of model with transfer learning method, and improvement of model accuracy.

(3) To verify the automated combined embryo assessment system to improve the pregnancy outcome of single blastocyst transplantation

1) The enrolled subjects undergoing monoblastocyst transplantation were screened, and informed consent was signed with the patients;

2) Routine ovulation, fertilization and embryo culture in time lapse system for dynamic observation;

3) The optimal blastocysts were selected by AI-time lapse system for combined evaluation and manual evaluation, and 100 cases with fresh cycle of 5 blastocysts per Day were prospective performed.

4) The pregnancy outcomes of the two groups, including the implantation rate, abortion rate and the rate of carrying babies home, were followed up for statistical analysis.

3.3 Indications

The patient meets the indications for IVF/embryo transfer therapy, as shown in the admission criteria

3.4 Study endpoint

Data collection and analysis were completed in 100 prospective 5 Day single blastocyst transplantation patients

4. Case selection

4.1 Number of cases

A total of 1000 patients were collected in Time-lapse closed-culture embryos for three periods to establish an AI-time lapse combined evaluation model, and 100 patients with monoblastocyst transplantation were prospectively carried out.All samples were approved by the Medical Ethics Committee and signed by the patient with informed consent.

4.2 Inclusion criteria

1) < 40 years old;

2) Routine IVF cycle;

3) Period number ≤ 2;

4) The number of eggs collected was 5-15;

5) FSH ≤ 12 IU/L on the third day of menstruation;

6) Patients with more than 3 high-quality embryos per Day and feasible 5 single blastocyst transplantation per Day;

7) Patient factors affecting embryo implantation such as endometrial factors.

4.3 Exclusion criteria

1) PGT should be performed due to male infertility, ovulation cycle and chromosome abnormalities;

2) Pictures of blastocysts not formed or not available;

3) Incomplete or unclear images were collected during prokaryotic, mitotic and blastocyst phases, which affected the AI evaluation.

4.4 Eliminate criteria

Patients who refused to undergo 5 Day single blastocyst transplantation

4.5 End research criteria

Patients may terminate the study due to the following reasons: 1) imaging system failure before the end of culture;2) No embryos could be transplanted after 5 days of culture;3) There is no valid embryo picture for AI analysis;4) The patient cancels the transplant;5) Subjects withdraw from the study at their own request;6) Subjects did not follow the study protocol.

5. Research methods and technical routes

5.1 Randomization into groups

This study was not a randomized controlled study

5.2 Intervention/study drug name and specification

None

5.3 Study intervention options

None

5.4 Combine drugs

None

5.5 Research related laboratory tests

None

5.6 Study relevant imaging tests

None

6. Observation items and testing time

1) The morphological parameters required by the embryos were observed from Day 1-5 during embryo culture;

2) Images of prokaryotic stage, cleavage stage and blastocyst stage were collected for the establishment of automated embryo evaluation models at different developmental stages;

3) Routine luteal support after embryo transfer, positive blood HCG detected 14 days after transplantation, and pulsation of gestational sac and original cardiac tube detected by B-ultrasound at 4-6 weeks were considered as clinical pregnancy until fetal delivery.

7. Evaluation criteria for curative effect

In this study, deep learning method was used to evaluate the blastocyst quality based on neural network model and manual evaluation of blastocyst quality

In order to improve the accuracy and effectiveness of prediction, the optimal parameter range is adjusted timely according to the results.

The validity evaluation index in the study is the accuracy rate, which represents how many of the predicted positive samples are really positive samples.There are two possible predictions, one is to predict the positive class as positive class (TP), the other is to predict the negative class as positive class (FP), the accuracy rate is P=TP/(TP+FP), the value is not less than 0.9.The safety evaluation index is the harmonic average of accuracy rate and recall rate F=2*P*R/(R+P), that is, positive samples are not expected to be identified as negative samples, or negative samples are identified as positive samples, and the value is not less than 0.9.

In the pregnancy outcome, the clinical results of single embryo transfer were counted, and the data were statistically analyzed by the rate of embryo implantation, the rate of carrying babies home and the abortion rate of safety indicators.The pregnancy rate of single embryo transfer cycle was compared with that of conventional morphology.According to the results, adjust the optimal parameter range in time to improve the accuracy and effectiveness of the prediction.

8. Observation of adverse events

None

9. Quality control and quality assurance of the research (Determine whether this part is needed according to the specific situation of the project)

Embryo culture and transfer within the laboratory are performed in accordance with the standard SOP procedure, and the patient is given full informed consent to improve the subject's compliance.

10. Data security inspection

None

11.Statistical processing

11.1 Statistical methods include

Through deep learning method, a 16-layer convolutional neural network structure was built, which was used to extract the features of embryo images in different periods, and then the neural network model was used for statistical analysis.SPSS16.0 software was used for statistical analysis. Through the clinical results of single embryo transfer, the data were statistically analyzed with the embryo implantation rate, abortion rate and baby carrying home rate as indicators.The pregnancy rate of single embryo transfer cycle was compared with that of conventional morphology.According to the results, adjust the optimal parameter range in time to improve the accuracy and effectiveness of the prediction.To obey the normal distribution of measurement data using mean + / - standard deviation ($\bar{x}$ + s) was described, using samples t test, inspection rate comparing with x ^ 2.P < 0.05 was considered statistically significant.

11.2 Sample size calculation process

At present, the pregnancy rate of single blastocyst transplantation is basically maintained at 60%. Through this study, the pregnancy rate is expected to be increased to 80%. The test standard α is 0.05.

12. Ethics of clinical research

Clinical research will follow the relevant regulations such as the Declaration of Helsinki of the World Medical Congress.Clinical studies are conducted only after the protocol is approved by the Ethics Committee prior to the start of the study.Before each subject is enrolled in this study, the investigator is responsible to give a complete and comprehensive introduction of the purpose, procedure and possible risks of this study to the subject or his/her agent, and sign a written informed consent. Subjects should be informed that they have the right to withdraw from this study at any time, and informed consent should be kept as a clinical study document for future reference.Subjects' privacy and data confidentiality will be protected during the study.

13. Collection and storage of samples/specimens

In this study, images of embryos were collected every 15 minutes by time-lapse system, and all the images were stored in the computer hard drive.It is stored in the hard disk of the computer of the center. It is backed up regularly every year and kept completely indefinitely.

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Investigator Statement and Protocol Signature page

The researchers stated:

I agree to comply with the review opinions of the Ethics Committee and start the clinical trial after approval, report to the Ethics Committee in a timely manner any changes in the clinical trial activities, as well as any unexpected problems involving risks to subjects or other personnel, and carry out the trial after re-obtaining the approval of the ethical review.Follow up review and conclusion review as required by the Ethics Committee.

I agree to conduct clinical trials in strict accordance with the design and specific provisions of this protocol.

I understand that I can interrupt or terminate this clinical trial at any time if it is in the best interest of the subjects.

I agree that I will personally conduct or supervise the clinical trial and that all investigators in my organization who assist me in conducting the clinical trial understand their role in the clinical trial.

In carrying out this clinical trial, I will strictly abide by the current GCP and the Declaration of Helsinki.And promised that the whole process will be ethical, ethical and scientific requirements.

During the implementation of clinical trials, I will strictly abide by all laws and regulations related to clinical trials to protect the rights and interests of patients.

I agree to maintain adequate and accurate medical records and ensure that these medical records are readily available for inspection and inspection in accordance with relevant laws and regulations.

________________ ____________________ __________________

Name (block letter) Signature Date of signature