Clinical Research Protocol: Effects of Multimodal AI-Assisted Noninvasive Euploid Embryo Selection on Clinical Outcomes After Single-Blastocyst Transfer in Women of Advanced Maternal Age: A Prospective Randomized Controlled Trial

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Summary of the case

Project Name	Effects of Multimodal AI-Assisted Noninvasive Euploid Embryo Selection on Clinical Outcomes After Single-Blastocyst Transfer in Women of Advanced Maternal Age: A Prospective Randomized Controlled Trial
Purpose of the study	To address the high embryo aneuploidy rate and poor pregnancy outcomes in women aged 35 years or older, the project aims to develop a noninvasive multimodal embryo euploidy prediction model integrating time-lapse imaging and patient clinical features using deep learning, and to evaluate the effect of AI-assisted morphological assessment combined with euploidy prediction on ongoing pregnancy after fresh single-blastocyst transfer.
Study Design	Prospective, randomized, parallel-group controlled study.
Total number of cases	250 participants per group; 500 participants in total.
Case selection	*Criteria for inclusion* *Infertile women aged ≥35 years; planned IVF/ICSI treatment; ≤2 previous oocyte-retrieval cycles; ≥3 usable day-3 embryos; ≥1 day-5 blastocyst meeting transfer criteria, with Gardner grade ≥3BB; voluntary written informed consent from both partners.*

	Exclusion criteria *PGT required because of chromosomal abnormalities; donor-oocyte, donor-sperm, or thawed-oocyte cycles; severe uterine malformations, intrauterine adhesions, or infertility attributable solely to uterine factors; incomplete or unclear blastocyst images affecting AI assessment; other circumstances judged unsuitable by the investigator.*
Treatment plan	Control: AI-assisted morphological assessment using EmbryoInsights™. Experimental: AI-assisted morphological assessment combined with euploidy prediction using EmbryoInsights™ Plus.
Evaluation of curative effect	*Efficacy evaluation indicators (primary efficacy indicators and secondary efficacy indicators)* Primary: ongoing pregnancy rate after the first fresh day-5 single-blastocyst transfer. Secondary: clinical pregnancy rate, early miscarriage rate, and live birth rate.
	Safety evaluation index Incidence of ovarian hyperstimulation syndrome, cycle cancellation rate, and other safety outcomes.
Method of statistics	**Two-sided tests with α=0.05 using SPSS 26.0. Continuous variables: mean ± SD or median (IQR), with independent-samples t tests or Wilcoxon rank-sum tests. Categorical variables: n (%), with Pearson chi-square or Fisher exact tests. Efficacy will be analyzed in ITT and PP populations. Binary outcomes will be assessed by univariable chi-square tests and multivariable logistic regression adjusting for maternal age, BMI, AMH, and other potential confounders; adjusted odds ratios and 95% confidence intervals will be reported.**
Duration of study	10 September 2026 to 31 December 2029.

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List of acronyms

English abbreviation	Full English name
AI	Artificial intelligence
ART	Assisted reproductive technology
PGT-A	Preimplantation genetic testing for aneuploidy
TLI	Time-lapse imaging
RCT	Randomized controlled trial

1. Background

Increased Oocyte Aneuploidy as a Central Mechanism of Age-Related Fertility Decline:

Age is a critical and irreversible determinant of female fertility. With changes in China's population policies and the increasing postponement of marriage and childbearing, patients of advanced maternal age account for a growing proportion of assisted reproductive technology (ART) treatment. Data from the Chinese Society of Reproductive Medicine for 2022 indicate that women aged 35 years or older accounted for 32.0% of ART cycles, while the proportion aged over 40 years had nearly doubled compared with five years earlier [1]. Reduced fertility in women of advanced maternal age has become an important clinical challenge for population health in China.

The underlying decline in fertility reflects irreversible reductions in oocyte quantity and quality [2]. Abnormal spindle assembly and chromosome segregation during meiosis lead to a sharp increase in embryo aneuploidy with age [3]. A large study of 15,169 blastocyst trophectoderm biopsies found that the probability of embryo aneuploidy increased from 34.5% at age 35 to 58.2% at age 40 and 83.4% at age 43 [4]. Other research has reported aneuploidy in approximately 50-55% of blastocysts from infertile women of advanced maternal age (mean age approximately 39 years), and a probability of obtaining a euploid blastocyst of less than 5% in infertile women older than 43 years undergoing ART [5]. Clinically, embryos that appear morphologically normal in these patients may still fail to implant repeatedly or result in early miscarriage.

Magnus et al. [6] reported in a large population-based study that miscarriage risk increased from approximately 10% at ages 25-29 years to approximately 51% above age 40. Identifying embryos that are euploid and have developmental potential is therefore a central challenge in improving ART outcomes for patients with advanced maternal age, limited oocyte yield, and a high embryo aneuploidy rate.

Continuing Debate About the Clinical Value of PGT-A:

Preimplantation genetic testing for aneuploidy (PGT-A) is currently a principal method for identifying euploid embryos. It uses trophectoderm biopsy and high-throughput sequencing to examine chromosome copy-number changes and may improve implantation, reduce miscarriage, and shorten time to live birth [7]. Several large clinical studies support female age ≥35 years as an

indication for PGT-A. A large retrospective propensity-score-matched study reported a higher live birth rate with PGT-A among women aged ≥35 years [8]. A meta-analysis including nearly 100,000 treatment cycles also reported higher live birth rates with PGT-A among women older than 35 years [9].

However, substantial clinical and evidentiary challenges remain when few embryos are available. First, biopsy is invasive and may affect embryo development. Trophectoderm biopsy may disturb the embryonic microenvironment and affect implantation or subsequent development [10], which may be particularly relevant when few oocytes or embryos are available. Second, the chromosome composition of the 3-5 sampled trophectoderm cells may not fully represent the inner cell mass that will develop into the fetus [11]. Third, amplification-related technical errors remain difficult to avoid; allelic dropout and loss of heterozygosity during whole-genome amplification have been reported at rates of up to 25% [12]. Kahraman et al. [13] reported embryos classified as aneuploid by PGT-A that nevertheless resulted in pregnancy and healthy live birth, raising questions about the accuracy of existing testing platforms. Fourth, evidence of clinical benefit remains incomplete. A large multicenter randomized controlled trial published in the New England Journal of Medicine in 2021 included women aged 25-37 years with at least three good-quality blastocysts and found that cumulative live birth with conventional IVF was not inferior to that with PGT-A [14]. The European Society of Human Reproduction and Embryology's updated recurrent pregnancy loss guideline also stated that evidence was insufficient to establish a significant improvement in live birth with PGT-A among patients with recurrent miscarriage [15].

Noninvasive PGT (niPGT) has been explored to address these limitations by analyzing cell-free DNA in spent embryo culture medium [16]. However, Tomic et al. [17] noted only 70-80% concordance between niPGT-A and invasive biopsy results, uncertainties about the origin of cell-free DNA, reported mosaicism misclassification rates of 30-50%, and insufficient standardization. In addition, niPGT-A still depends on high-throughput sequencing, involves lengthy testing and substantial costs, and is not readily suitable for broad clinical implementation. A convenient and reliable noninvasive method for embryo euploidy assessment remains an unmet need, especially when avoiding biopsy-related harm is a priority.

AI-Assisted Noninvasive Euploid Embryo Selection Using TLI:

Embryo development is a continuous and temporally ordered process. Time-lapse imaging (TLI) records development from fertilization to blastocyst formation without interrupting the culture environment, providing extensive dynamic image data for noninvasive assessment [18]. These records also contain developmental features associated with ploidy. Cleavage patterns, developmental synchrony, and blastocyst expansion rate have been associated with aneuploidy [19,20]. However, individual kinetic parameters have limited associations with euploidy and are not sufficiently reliable as independent discriminators [21]. Deep-learning-based AI can extract spatial and temporal features from large image and video datasets, identify subtle developmental patterns that may not be apparent to human observers, and investigate their associations with embryo potential. Convolutional neural networks, recurrent neural networks, and Transformer architectures have attracted attention for embryo selection and may enhance the feasibility of noninvasive assessment [22].

Exploratory studies have evaluated AI-assisted embryo assessment in China and elsewhere. Barnes et al. [21] developed STORK-A using retrospective data from 10,378 blastocysts in 1,385 patients with a mean age of 36.98 years (range 21-48 years). The model combined static images, morphokinetic parameters, and maternal age and achieved 69.3% accuracy for euploidy prediction, with an AUC of 0.761. Another multicenter Chinese study used 41,279 embryo images and 2,136 TLI videos, Transformer architecture, and self-supervised multimodal contrastive learning to develop an AI system covering the entire IVF cycle; AUCs were 0.811 for euploidy detection and 0.854 for live birth prediction [23]. Luong et al. [24] combined morphokinetic and clinical variables from 1,908 blastocysts (maternal age 38.5 ± 3.85 years) using methods including random forests, achieving an AUC of 0.808. SHAP and LIME were used to explore model interpretability.

Three major gaps remain. Training datasets may not adequately reflect the distinctive developmental kinetics associated with advanced maternal age, leaving generalizability uncertain. Many models remain biologically difficult to interpret, while tools such as Grad-CAM have not been widely adopted in clinical practice, limiting acceptance by embryologists. Most studies are retrospective and single-center, with insufficient rigorous prospective multicenter validation. Compared with morphological grading, directly applying AI to euploidy prediction has potentially greater clinical value, but remains exploratory. Models specifically tailored to the high-need population of women of advanced maternal age remain insufficiently explored.

To address heterogeneity in embryo development and limited model generalizability in this population, this prospective randomized parallel-group study will evaluate AI-assisted morphological assessment combined with euploidy prediction for ongoing pregnancy after fresh day-5 single-blastocyst transfer and will assess maternal and infant safety. The study team's AI-Time-lapse automated embryo morphological assessment system, EmbryoInsights™, was developed under the leadership of the Reproductive Medicine Center of Nanjing Drum Tower Hospital. It completed translation into clinical application in 2025, obtained China's first Class II medical device registration certificate for this category of technology, and was commercialized. It has operated in the hospital's routine clinical workflow for more than two years and has assessed over 30,000 embryos. Agreement in blastocyst quality grading with senior embryologists has exceeded an intraclass correlation coefficient of 0.90. The team's earlier multifocal image-based blastocyst quality model achieved an internal-test AUC of 0.936 [25]. In fresh day-5 single-blastocyst transfer, the pregnancy rate with this system was 76%, compared with 68-70% for conventional manual assessment (Reproductive BioMedicine Online, 2023). The control arm therefore uses this established AI-assisted morphological assessment system as a clinically demanding comparator.

The team previously investigated associations between embryo developmental kinetics and euploidy retrospectively. Among 491 biopsied embryos from 168 patients undergoing PGT-A at the Department of Reproductive Medicine between August 2024 and March 2025, several early developmental parameters were slightly delayed in the aneuploid group. The first cleavage synchrony measure, s2, differed significantly between groups ($P<0.05$). Integrating EmbryoInsights™ outputs, morphological images, and clinical information, the team then developed a multimodal euploidy prediction model from 560 day-5 PGT-A embryos in 185 patients. The five-fold cross-validation AUC was 0.65 ± 0.03. In further retrospective validation involving 128 embryos transferred in fresh day-5 cycles, clinical pregnancy rates were 61.63% in the predicted-euploid group and 47.62% in the predicted-abnormal-ploidy group; the difference was not statistically significant ($P=0.133$). Among women aged ≥35 years, the pregnancy rate in the predicted-euploid group was 65.31%, 7.48 percentage points higher than the overall rate of 57.83% in that age group. These findings suggest that multimodal information may support euploidy assessment and embryo prioritization and provide preliminary evidence for prospective clinical evaluation. On this basis, the team developed

EmbryoInsights™ Plus for women of advanced maternal age. Built on the EmbryoInsights™ platform, it adds euploidy probability prediction to the existing morphological assessment functions to provide noninvasive probability estimates and assist ranking.

The intended clinical value is to provide a noninvasive risk assessment tool for patients of advanced maternal age who are unsuitable for or unable to undergo PGT-A. Such patients may have few oocytes and valuable embryos for which biopsy is difficult to justify or may not undergo PGT-A because of financial or technical constraints. This study will prospectively evaluate the clinical use of the existing model in supporting selection of euploid embryos, reducing ineffective transfers, and improving the efficiency and safety of single-blastocyst transfer. With increasing insurance coverage for ART and domestic availability of TLI equipment, demand for assisted reproduction and for appropriate noninvasive assessment tools is growing. The project aims to move embryo selection from static morphological grading toward analysis of temporal developmental information and provide an additional basis for improving outcomes in women of advanced maternal age.

2. Research objectives

2.1 Main objective:

Using a prospective randomized parallel-group design, compare AI-assisted morphological assessment with AI-assisted morphological assessment combined with euploidy prediction, and evaluate the effect of the combined strategy on ongoing pregnancy after the first fresh day-5 single-blastocyst transfer in women aged ≥35 years. The study seeks to establish a noninvasive embryo-selection strategy for patients who do not undergo PGT-A, optimize transfer decisions, improve ongoing pregnancy while safeguarding maternal and infant safety, and provide evidence for clinical application of reproductive AI.

2.2 Secondary purpose:

1. Compare clinical pregnancy, early miscarriage, and live birth rates after fresh single-blastocyst transfer between the two assessment groups.

2. Compare OHSS incidence, cycle cancellation, and other adverse events to evaluate safety in

clinical use.

Exploratory objective: Explore between-group differences in ongoing pregnancy rates across subgroups defined by maternal age, BMI, and AMH.

3. Research design types, principles and test procedures

3.1 Research design

This study uses a prospective, randomized, parallel-group controlled design. Enrolled participants will be allocated in a 1:1 ratio by the random-envelope method to the "AI-assisted morphological assessment group" and the "AI-assisted morphological assessment combined with euploidy prediction group," to evaluate clinical outcomes after fresh-cycle Day 5 single-blastocyst transfer in the two groups.

3.1.1 Study population

Prospective phase: Patients of advanced maternal age (≥35 years) who are scheduled for fresh-cycle Day 5 single-blastocyst transfer and have not undergone PGT-A testing will be recruited in strict accordance with the inclusion criteria. They will be enrolled in the prospective RCT after being fully informed and signing the informed consent form.

3.1.2 Outcome variables

Prospective RCT phase: The primary outcome variable is ongoing pregnancy after the first fresh-cycle Day 5 single-blastocyst transfer, defined as pregnancy reaching 12 weeks of gestation after transfer with fetal cardiac activity confirmed by ultrasound. Secondary outcome variables include clinical pregnancy, early miscarriage, and live birth. Clinical pregnancy is defined as an intrauterine gestational sac detected by ultrasound at 6-8 weeks of gestation after transfer; early miscarriage is defined as pregnancy loss after confirmation of clinical pregnancy and within 12 weeks of gestation; live birth is defined as delivery of a live newborn at ≥24 weeks of gestation.

3.1.3 Study variables

The main study variables in the prospective RCT phase are random assignment and the corresponding embryo assessment strategy. The control group is the "AI-assisted morphological

assessment group," which uses the morphological AI assessment system (EmbryoInsights™). The experimental group is the " AI-assisted morphological assessment combined with euploidy prediction group," which uses the morphological AI assessment system combined with euploidy prediction (EmbryoInsights™ Plus).

3.1.4 Confounding variables

Potential confounding factors prespecified for adjustment include maternal age, BMI, and AMH. The study will also collect baseline characteristics, including demographic data, infertility history, physical examination findings, ovarian stimulation regimens, the number of oocytes retrieved, and fertilization methods, according to the follow-up schedule, to describe the baseline characteristics of the two groups and assess possible between-group imbalances.

3.1.5 Study type

This is a prospective, randomized, parallel-group controlled study.

3.2 Research steps

1. After routine ovarian stimulation and assessment of fertilization, embryos will be placed in TLI for closed culture and dynamic observation. On Day 3, patients will be recruited based on embryo quality and the inclusion and exclusion criteria, and will sign the informed consent form.

2. On culture Day 5, enrolled participants will be randomly assigned in a 1:1 ratio to the control "AI-assisted morphological assessment group" and the experimental "AI-assisted morphological assessment combined with euploidy prediction group." The control group will use EmbryoInsights™ for embryo quality assessment and transfer-priority ranking, on which senior embryologists will base the transfer decision. The experimental group will use EmbryoInsights™ Plus to rank embryos from highest to lowest morphological score only within the category predicted to be euploid, and select the highest-ranked embryo for single-blastocyst transfer.

3. Serum hCG testing, pregnancy ultrasound, nuchal translucency (NT) measurement, detailed fetal ultrasound, delivery outcome recording, and newborn follow-up will be completed at the prespecified times.

4. The ongoing pregnancy rate in the transfer cycle will be the primary outcome measure. Secondary clinical measures, including clinical pregnancy rate, early miscarriage rate, and live birth rate after fresh-cycle single-blastocyst transfer, will also be recorded and compared between the two groups. Statistical analysis of between-group differences will be used to evaluate the model's clinical performance and potential for application.

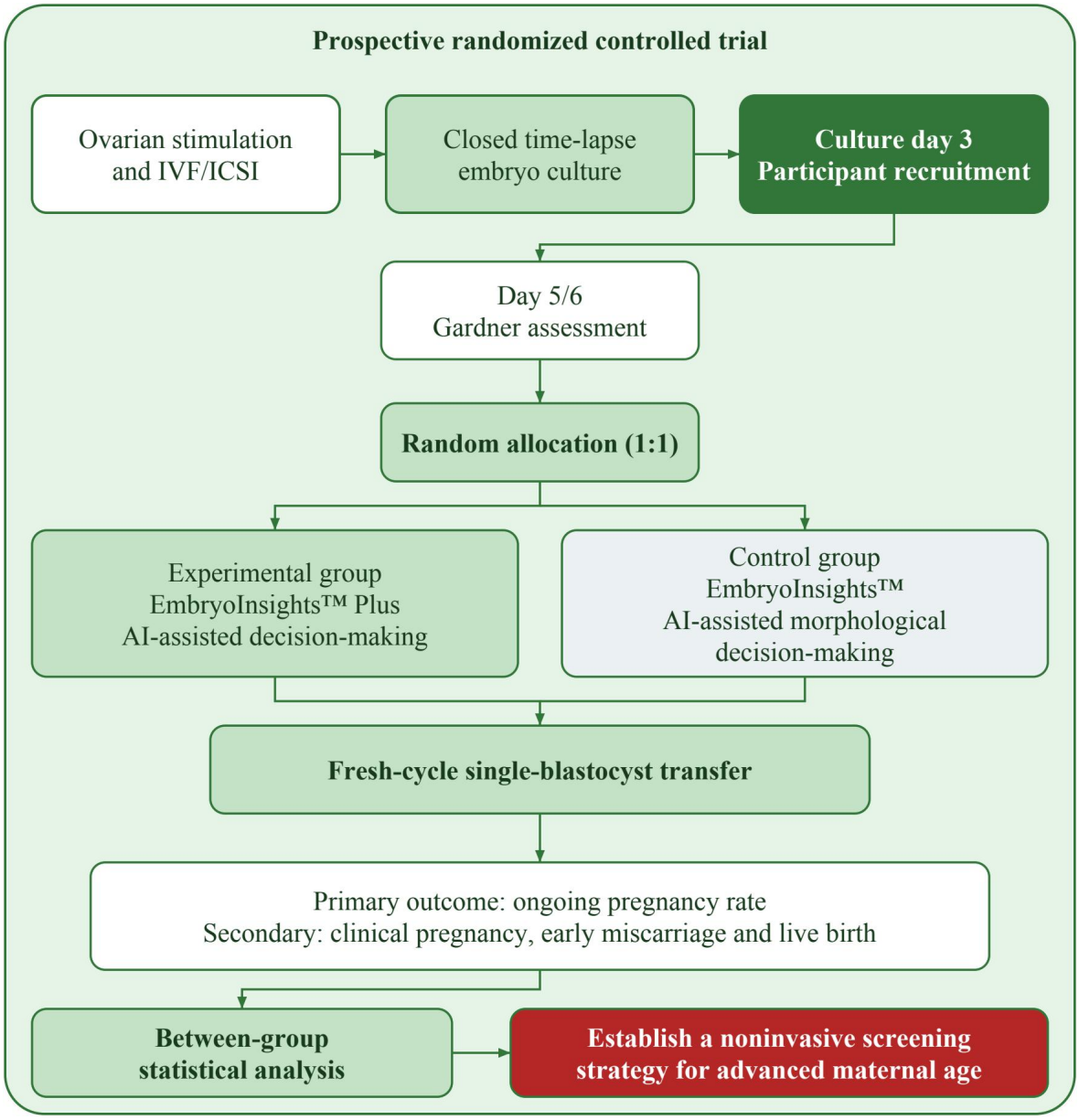


Figure 1. Study workflow

3.3 Indications

No investigational drug is involved, and no separate indication is specified.

3.4 Study endpoint

Primary: ongoing pregnancy rate after the first fresh day-5 single-blastocyst transfer. Secondary: clinical pregnancy rate, early miscarriage rate, and live birth rate.

4. Case selection

4.1 Number of cases

From September 2026 to December 2028, the prospective clinical validation phase will recruit infertile women aged ≥35 years who are scheduled for fresh day-5 single-blastocyst transfer without PGT-A at the Department of Reproductive Medicine of the Affiliated Drum Tower Hospital of Nanjing University Medical School. Each group requires 225 evaluable participants. Allowing for 10% attrition, 250 participants per group will be enrolled, for a total of 500, randomized in a 1:1 ratio.

4.2 Inclusion criteria

All criteria must be met:

1. Infertile women aged ≥35 years.

2. Planned IVF/ICSI treatment.

3. No more than two previous oocyte-retrieval cycles.

4. At least three usable day-3 embryos and eligibility for a day-5 single-blastocyst transfer.

5. At least one day-5 blastocyst meeting transfer criteria, with Gardner grade ≥3BB.

6. Voluntary written informed consent from both partners.

4.3 Exclusion criteria

Any of the following results in exclusion:

1. PGT required because of chromosomal abnormalities.

2. Donor-oocyte, donor-sperm, or thawed-oocyte cycles.

3. Severe uterine malformations, intrauterine adhesions, or infertility attributable solely to uterine factors.

4. Incomplete or unclear blastocyst images that interfere with AI assessment.

5. Other circumstances that, in the investigator's judgment, make participation inappropriate.

4.4 Eliminate criteria

After randomization in the prospective validation phase, an experimental-group participant will be excluded from the per-protocol (PP) analysis if all blastocysts in the cycle that meet morphological transfer criteria and have valid AI predictions are classified as aneuploid or mosaic, preventing protocol-specified ranking within the euploid category. The participant will remain in the intention-to-treat (ITT) analysis in her originally assigned group. Subsequent embryo selection and whether to transfer will be determined by senior embryology professionals and study clinicians according to routine clinical standards. Transfer will not automatically be cancelled on the basis of AI predictions. Reasons for failure to complete protocol-specified ranking and subsequent clinical management will be recorded accurately.

4.5 End research criteria

Withdrawal or dropout criteria are voluntary withdrawal of consent; withdrawal for ethical or safety reasons; a change in treatment, such as switching to PGT-A; or loss to follow-up.

5. Research methods and technical routes

5.1 Randomization into groups

This prospective randomized parallel-group controlled study will use an envelope method to allocate enrolled participants in a 1:1 ratio to AI-assisted morphological assessment or AI-assisted morphological assessment combined with euploidy prediction. Clinical outcomes after fresh day-5 single-blastocyst transfer will be compared.

5.2 Intervention/study drug name and specification

Control: EmbryoInsights™ for AI-assisted morphological assessment. Experimental: EmbryoInsights™ Plus for morphological assessment combined with euploidy probability prediction. No investigational drug is involved.

5.3 Study intervention options

Embryos will undergo closed time-lapse culture. Embryo images and patient clinical information will be obtained without other additional medical interventions.

On culture day 5, enrolled participants will be randomized in a 1:1 ratio to the control or experimental group. In the control group, EmbryoInsights™ will assess embryo quality and provide transfer priority rankings for decisions by senior embryologists. In the experimental group, EmbryoInsights™ Plus will rank embryos in descending morphological score only within the predicted-euploid category; the highest-ranked embryo will be selected for single-blastocyst transfer.

5.4 Combine drugs

None

5.5 Research related laboratory tests

Serum hCG will be measured 14 days after transfer.

5.6 Study relevant imaging tests

Ultrasound will be performed 35 days after transfer to confirm intrauterine pregnancy and fetal cardiac activity; ultrasound and NT measurement will be performed at 12 weeks of gestation ±7 days; and detailed fetal ultrasound will be performed at 20-24 weeks of gestation.

6. Observation items and testing time

The timing of enrollment, embryo assessment, transfer, and follow-up is set out below.

Table 1. Enrollment, embryo assessment, transfer and follow-up schedule

Study stage	**Time point**	**Study procedure**	**Observations and assessments**
Stimulation and oocyte retrieval	Retrieval day	Routine ovarian stimulation, retrieval, and IVF/ICSI.	Stimulation regimen, oocyte yield, fertilization method.

Study stage	Time point	Study procedure	Observations and assessments
Embryo culture and assessment	Day 1	Closed TLI culture and dynamic observation.	Pronuclear morphological assessment.
Embryo culture and assessment	Day 3	Continue TLI culture; confirm eligibility, recruit patients, obtain written informed consent, and enroll.	Cleavage-stage morphology, number of usable day-3 embryos, basic patient information.
Embryo culture and assessment	Day 5	Randomize; apply EmbryoInsights™ or EmbryoInsights™ Plus; make the transfer decision and perform fresh day-5 single-blastocyst transfer.	Blastocyst morphology, Gardner grade, AI results; predicted euploidy category/probability in the experimental arm; transferred embryo and decision.
Transfer and follow-up	14 days after transfer	Follow-up after the first fresh day-5 single-blastocyst transfer.	Serum hCG test and result.
Transfer and follow-up	35 days after transfer	Outpatient ultrasound.	Intrauterine gestational sac and fetal cardiac activity; clinical pregnancy determination.
Transfer and follow-up	12 weeks of gestation ±7 days	Outpatient ultrasound.	Ultrasound and NT; ongoing pregnancy determination and early miscarriage recording.
Transfer and follow-up	20-24 weeks of gestation	Routine antenatal follow-up.	Detailed fetal ultrasound for structural abnormalities.
Transfer and follow-up	Delivery	Record delivery outcomes.	Delivery mode, gestational age, live birth, birth weight, and other newborn information.
Transfer and follow-up	42 days postpartum	Complete maternal and infant follow-up.	Infant growth, development, and health.

Women aged ≥35 years scheduled for fresh day-5 single-blastocyst transfer without PGT-A will be recruited. The prospective randomized parallel-group design will compare ongoing pregnancy after the first transfer between the two AI assessment strategies. Clinical pregnancy, early miscarriage, and live birth will also be compared to evaluate the clinical performance and safety of multimodal embryo euploidy prediction.

7. Evaluation criteria for curative effect (If applicable)

Table 2. Efficacy, safety and other observation measures

Category	**Outcome**	**Definition or measurement**	**Assessment time**
Primary efficacy	Ongoing pregnancy rate	Pregnancy reaching 12 weeks of gestation with fetal cardiac activity confirmed by ultrasound. Rate: cycles with ongoing pregnancy divided by all transfer cycles in the group ×100%. This is the sole outcome used for sample-size estimation.	Ultrasound at 12 weeks of gestation ±7 days.
Secondary efficacy	Clinical pregnancy rate	Cycles with an intrauterine gestational sac detected at 6-8 weeks of gestation divided by all transfer cycles in the group ×100%.	Ultrasound 35 days after transfer.
Secondary efficacy	Early miscarriage rate	Cycles with pregnancy loss after confirmation of clinical pregnancy and within the first 12 weeks of gestation divided by all clinical-pregnancy cycles in the group ×100%.	From confirmation of clinical pregnancy to 12 weeks of gestation.
Secondary efficacy	Live birth rate	Cycles resulting in delivery of a live infant at ≥24 weeks of gestation divided by all transfer cycles in the group ×100%.	At delivery.
Safety	OHSS incidence	Number and incidence of OHSS cases during routine treatment.	During the study.

Category	Outcome	Definition or measurement	Assessment time
Safety	Cycle cancellation rate	Number and rate of cancelled cycles and reasons for cancellation.	During the study.
Safety	Other adverse events	Record event name, onset and end, severity, management, outcome, and causal relationship to the study in medical records/eCRF.	During the study.
Other observations	Baseline, treatment, embryo culture, maternal and infant outcomes	Demographics, infertility history, physical examination, maternal age, BMI, AMH, stimulation, oocyte yield, fertilization, day-1/day-3/day-5 morphology and AI results, transfer decisions, pregnancy ultrasound, delivery, and newborn follow-up.	Corresponding treatment and follow-up visits.

8. Observation of adverse events

AI-assisted assessment does not directly contact embryos, affect their developmental process, or involve additional invasive procedures. Potential study-related risks principally include grading errors caused by algorithm limitations and software or network problems that prevent standardized assessment. Risks inherent in routine assisted reproduction and pregnancy include cycle cancellation, OHSS, biochemical pregnancy, miscarriage, ectopic pregnancy, and pregnancy complications.

From enrollment until study completion or early withdrawal, investigators will monitor adverse events and assess changes in vital signs, physical findings, clinical manifestations, laboratory results, and other examinations. All participant-reported or investigator-observed events will be recorded in medical records or eCRF, including at least the event name, onset and end, severity, management, outcome, and causal relationship to the study.

Events will be managed appropriately and followed until adequately resolved or stable. Serious and unexpected events will be reported to the ethics committee and relevant authorities as required. Safety data will be summarized by category as counts and rates, with detailed listings retained for review.

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

The sponsor and investigators will fulfill their respective responsibilities and comply with the protocol and relevant standard operating procedures. Before study initiation, participating embryologists and data personnel will be trained in the protocol, TLI image acquisition, AI system operation, data entry, and adverse-event reporting. Software versions, procedures, and assessment criteria will be kept consistent.

Visits and data collection will follow the prespecified schedule. Image completeness, randomization, embryo assessment, transfer decisions, and outcome records will be checked. All observations and findings must be verified, and quality control will be applied throughout data handling to ensure completeness, accuracy, and reliability.

Monitors will regularly review study conduct, data, and facilities to protect participants and verify compliance with the approved protocol, GCP, and applicable regulations. Deviations will be reported and corrected promptly. In the event of AI software or network failure, a contingency procedure will immediately be activated, with manual assessment by senior embryologists to avoid delaying routine care.

10. Data security inspection

Monitoring will be proportionate to study risk. All adverse events will be recorded in detail, managed appropriately, and followed until resolved or stable. Serious and unexpected events will be reported as required. Principal investigators will periodically review cumulative adverse events, outcomes, cycle cancellations, and AI system abnormalities, reassess risks and benefits, and recommend continuation, suspension, or termination as appropriate.

11.Statistical processing

11.1 Statistical methods include

All statistical tests will be two-sided with $\alpha=0.05$, and $P<0.05$ will be considered statistically

significant. Analyses will use SPSS 26.0.

Descriptive and baseline analyses: Continuous variables will be summarized as mean ± standard deviation or median and interquartile range. Between-group comparisons will use independent-samples t tests or Wilcoxon rank-sum tests. Categorical variables will be summarized as counts and percentages and compared using Pearson chi-square or Fisher exact tests.

Clinical outcomes: Efficacy will be evaluated in both ITT and PP populations. The primary outcome is ongoing pregnancy after the first fresh day-5 single-blastocyst transfer. Secondary outcomes are clinical pregnancy, early miscarriage, and live birth. Binary outcomes will be compared using univariable chi-square tests and multivariable logistic regression adjusting for maternal age, BMI, AMH, and other potential confounders. Adjusted odds ratios and 95% confidence intervals will be reported.

Principal safety measures include OHSS incidence and cycle cancellation during routine treatment. All safety data will be summarized descriptively as counts and rates by category, with detailed listings retained for review.

All data will be manually checked before database lock. Values outside prespecified ranges, inconsistent chronological sequences, and internal discrepancies will trigger queries and verification against source records. Confirmed entry errors will be corrected from source data with an audit trail; verified unusual values will be retained; unverifiable values will be treated as missing. Values must not be changed on the basis of experience or deleted arbitrarily.

All prospectively randomized participants will remain in the ITT population in their original groups. Participants unable to complete protocol-specified transfer because of the assigned embryo-assessment strategy after randomization will not be treated as ordinary missing observations for imputation; in the primary ITT analysis they will be counted as not achieving ongoing pregnancy. For outcomes missing after completed transfer, including loss to follow-up, the amount and pattern of missingness will first be described. Multiple imputation by chained equations (MICE) will be used when the missing-at-random assumption is reasonable. Sensitivity analyses will include complete cases and an analysis treating missing primary outcomes as no ongoing pregnancy. The PP population will include only participants completing the protocol-specified assessment and transfer without

major deviations. All handling rules will be documented in the statistical analysis plan before database lock.

11.2 Sample size calculation process

The primary outcome for sample-size estimation is ongoing pregnancy after the first fresh day-5 single-blastocyst transfer. The null hypothesis is equality between groups (H0: Pexperimental = Pcontrol); the two-sided alternative is a difference (H1: Pexperimental ≠ Pcontrol).

Both treatment allocation and ongoing pregnancy are binary variables. PASS 15.0 is used to estimate the sample size for comparison of two independent proportions using a Mantel-Haenszel test.

Based on the center's clinical data from the preceding two years, the ongoing pregnancy rate in women aged ≥35 years undergoing AI-assisted morphological assessment and fresh day-5 single-blastocyst transfer is 52%. The expected rate with additional euploidy prediction is 65%, representing an absolute improvement of 13 percentage points.

With two-sided $\alpha=0.05$ and power $1-\beta=0.80$, 225 evaluable participants per group are required. Allowing for 10% attrition, the planned sample is 250 per group and 500 overall.

Recruitment feasibility: The center performs more than 6,000 IVF-transfer cycles, including approximately 1,900 fresh cycles in women aged ≥35 years. An estimated 35-40 eligible patients are available each month. Enrollment of 500 participants is expected to be achievable in 12-14 months and is feasible within 1.5 years.

12. Ethics of clinical research

The research will follow the World Medical Association Declaration of Helsinki and other relevant requirements. The study will begin only after ethics committee approval of the protocol. Before enrollment, investigators must fully explain the purpose, procedures, and potential risks to each participant or her representative and obtain written informed consent. Participants must be informed of their right to withdraw at any time. Consent documents will be retained as study records. Personal privacy and data confidentiality will be protected throughout the study.

13. Collection and storage of samples/specimens

No additional blood, tissue, or other biological samples or specimens will be collected, retained, or transported. The center will collect and store only full-cycle time-lapse images, clinical follow-up information, and AI assessment results from the 500 participants.

Data will be stored centrally on the center's servers and enterprise-grade network-attached storage with usable capacity of at least 24 TB. RAID redundancy, backups, and access controls will support security and long-term traceability. Direct identifiers, including names and medical-record numbers, will be removed before image and clinical data analysis. These research data will not be transported or transmitted outside China. Retention periods will follow the center's clinical research records policy and ethics approval requirements.

14. References

[1] Chinese Society of Reproductive Medicine, Chinese Medical Association. 2022 Annual Data from the Chinese Society of Reproductive Medicine (CSRM) Data Reporting System. [In Chinese.]

[2] Rooda I, Méar L, Hassan J, et al. The adult ovary at single cell resolution: an expert review[J]. Am J Obstet Gynecol, 2025,232(4S):S95.e1-S95.e16.

[3]Mikwar M, MacFarlane AJ, Marchetti F. Mechanisms of oocyte aneuploidy associated with advanced maternal age[J].Mutat Res Rev Mutat Res, 2020,785:108320.

[4]Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening[J]. Fertil Steril, 2014, 101(3): 656-663.e1.

[5]Ubaldi FM, Cimadomo D, Vaiarelli A, et al. Advanced maternal age in IVF: still a challenge?The present and the future of its treatment[J]. Front Endocrinol(Lausanne), 2019, 10: 94.

[6]Magnus MC, Wilcox AJ, Morken NH, et al. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study[J]. BMJ, 2019, 364: l869.

[7]Neal SA, Morin SJ, Franasiak JM, et al. Preimplantation genetic testing for aneuploidy is

cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage[J]. Fertil Steril, 2018,110(5):896-904.

[8]Haviland MJ, Murphy LA, Modest AM, et al. Comparison of pregnancy outcomes following preimplantation genetic testing for aneuploidy using a matched propensity score design[J]. Hum Reprod, 2020,35(10):2356-2364.

[9]Adamyan L, Pivazyan L, Obosyan L, et al. Preimplantation genetic testing for aneuploidy in patients of different age: a systematic review and meta-analysis[J]. Obstet Gynecol Sci, 2024,67(4):356-379.

[10]Makhijani R, Bartels CB, Godiwala P, et al. Impact of trophectoderm biopsy on obstetric and perinatal outcomes following frozen-thawed embryo transfer cycles[J]. Hum Reprod, 2021,36(2):340-348.

[11]Morales C. Current Applications and Controversies in Preimplantation Genetic Testing for Aneuploidies (PGT-A) in In Vitro Fertilization[J]. Reprod Sci, 2024,31(1):66-80.

[12]Casper RF. PGT-A: Houston, we have a problem[J]. J Assist Reprod Genet,2023,40(10): 2325-2332.

[13]Kahraman S, Cetinkaya M, Yuksel B, et al. The birth of a baby with mosaicism resulting from a known mosaic embryo transfer: a case report[J]. Hum Reprod, 2020, 35(3): 727-733.

[14]Qin Y, Gong F, Sun X, et al. Live Birth with or without Preimplantation Genetic Testing for Aneuploidy[J]. N Engl J Med, 2021,385 (22):2047-2058.

[15]ESHRE Guideline Group on RPL. ESHRE guideline: recurrent pregnancy loss: an update in 2022[J]. Hum Reprod Open, 2023, 2023(1): hoad002.

[16]Leaver M,Wells D. Non-invasive preimplantation genetic testing (niPGT): the next revolution in reproductive genetics?[J]. Hum Reprod Update,2020,26 (1):16-42.

[17]Tomic M, Vrtacnik Bokal E, Stimpfel M. Non-invasive preimplantation genetic testing for aneuploidy and the mystery of genetic material: a review article[J]. Int J Mol Sci, 2022, 23(7): 3568.

[18]Sun L, Li J, Zeng S, et al. Artificial intelligence system for outcome evaluations of human

in vitro fertilization-derived embryos[J].Chin Med J (Engl), 2024, 137(16): 1939-1949.

[19]Bamford T, Barrie A, Montgomery S, et al. Morphological and morphokinetic associations with aneuploidy: a systematic review and meta- analysis[J]. Hum Reprod Update, 2022,28(5):656-686.

[20]Zhu J, Zou J, Wu L, et al. Total duration of spontaneous blastocyst collapse during the expansion stage is an independent predictor of euploidy and live birth rates[J]. Reprod Biomed Online, 2024,49 (1):103863.

[21]Barnes J, Brendel M, Gao VR, et al. A non-invasive artificial intelligence approach for the prediction of human blastocyst ploidy: a retrospective model development and validation study[J]. Lancet Digit Health, 2023, 5(1):e28-e40.

[22]Xin X, Wu S, Xu H, et al. Non-invasive prediction of human embryonic ploidy using artificial intelligence: a systematic review and meta- analysis[J]. EClinicalMedicine, 2024, 77:102897.

[23]Wang G, Wang K, Gao Y, et al. A generalized AI system for human embryo selection covering the entire IVF cycle via multi-modal contrastive learning[J]. Patterns (N Y), 2024,5(7):100985.

[24]Luong TM, Ho NT, Hwu YM, et al. Beyond black-box models: explainable AI for embryo ploidy prediction and patient-centric consultation[J]. J Assist Reprod Genet, 2024, 41(9): 2349-2358.

[25]Wang S, Zhou C, Zhang D, et al. A deep learning framework design for automatic blastocyst eEvaluation with multifocal images[J].IEEE Access, 2021, 9: 18927-18934.