Clinical Research Protocol V1.1 (English Version)

Shanghai Jiao Tong University School of Medicine Affiliated Ninth People's Hospital

Clinical Research Protocol

Study title: Effect of Day 2 vs. Day 3 Fresh Embryo Transfer on Live Birth Rate in Patients with POSEIDON Category 4 Low Prognosis: A Single-Center, Parallel-Group, Open-Label Randomized Controlled Trial

NCT number: /

Document Date: 2026-03-02

I. Protocol Summary

Project Name	Effect of Day 2 vs. Day 3 Fresh Embryo Transfer on Live Birth Rate in Patients with POSEIDON Category 4 Low Prognosis: A Single-Center, Parallel-Group, Open-Label Randomized Controlled Trial
Version No.	V1.1
Sponsoring and Participating Units	Department of Assisted Reproduction, Shanghai Jiao Tong University School of Medicine Affiliated Ninth People's Hospital
Research Nature	Randomized Controlled Clinical Trial
Research Objectives	This trial aims to compare the efficacy of Day 2 (D2) and Day 3 (D3) fresh embryo transfer in patients with POSEIDON Category 4, and clarify the optimal transfer time for this population.
Research Subjects	Eligible patients with POSEIDON Category 4 (first or second IVF/ICSI cycle)
Research Methods	Single-center, parallel-group, open-label RCT, with 1:1 stratified block randomization allocation
Inclusion Criteria	1. Meeting the definition of POSEIDON Category 4: age ≥ 35 years, and antral follicle count (AFC) < 5 or anti-Müllerian hormone (AMH) < 1.2 ng/mL [1].2. First or second IVF/ICSI cycle.3. Controlled ovarian hyperstimulation (COH) using gonadotropin-releasing hormone (GnRH) antagonist protocol, with ≥ 1 oocyte retrieved.4. Signed written informed consent form.
Exclusion Criteria	1. Use of donor oocytes/sperm, or planned preimplantation genetic testing (PGT).2. Severe immune and chromosomal abnormalities.3. Uterine cavity abnormalities (i.e., submucosal fibroids, complete uterine septum, severe intrauterine adhesions, etc.) or untreated and ultrasound-visible hydrosalpinx.4. Complicated with severe underlying diseases (such as uncontrolled hypertension/diabetes, active malignant tumors).5. A history of repeated implantation failure (≥ 2 cycles) or recurrent miscarriage (≥ 2 times).6. Progesterone level > 1.5 ng/mL on the day of human chorionic gonadotropin (hCG) trigger.

Research Progress Plan	Patient enrollment will last for 18 months, with on-site monitoring every 6 months, and the entire study is expected to last 36 months.
Statistical Analysis Methods	Analysis Populations: ITT analysis includes all randomized patients (primary analysis), and missing data is handled by multiple imputation (MICE); PP analysis includes patients who comply with the protocol (sensitivity analysis).Continuous Variables: Normally distributed data are expressed as mean ± standard deviation, and t-test is used for inter-group comparison; non-normally distributed data are expressed as median (interquartile range), and Mann-Whitney U test is used for inter-group comparison.Categorical Variables: Expressed as frequency (percentage), and χ^2 test or Fisher's exact test (when expected frequency < 5) is used for inter-group comparison.Subgroup Analysis: Preset subgroup analyses by age (< 40 years and ≥ 40 years), AMH level (< 0.5 ng/mL and 0.5-1.2 ng/mL), and number of oocytes retrieved (< 4 and ≥ 4).Statistical Software: SAS 9.4 and R 4.3.0 software are used, with $P < 0.05$ (two-sided) considered statistically significant.
Form of Research Results Publication	The research results will be published in peer-reviewed journals and presented at international conferences. A plain language summary of the research results will be provided to participants (sent during offline follow-up or via email).

Principal Investigator	Jiang Shutian	Professional Title	Attending Physician	Date of Birth	October 1991
Key Members of the Research Team	Name	Date of Birth	Professional Title	GCP Certificate	Project Division

	Jiang Shutian	October 1991	Attending Physician	Yes	Principal Investigator
	Li Wenzhi	May 1989	Chief Technician	Yes	Clinical Execution (Embryologist)
	Guo Haiyan	October 1981	Associate Chief Physician	Yes	Clinical Execution (Clinician)
	Lyu Qifeng	September 1970	Researcher	Yes	Quality Control
	Jiang Xueyi	January 1999	Physician	No	Statistical Analysis
	Li Danjun	February 1995	Physician	No	Data Management
	Mi Yan	August 1999	Graduate Student	No	Data Management/Research Coordinator

II. Research Background

In in vitro fertilization-embryo transfer (IVF-ET), patients with low prognosis, especially those with POSEIDON Category 4 (age ≥ 35 years combined with diminished ovarian reserve), face problems such as a small number of oocytes, high embryo aneuploidy rate, and decreased endometrial receptivity, resulting in extremely low treatment success rates [1]. A 2021 real-world study showed that the cumulative live birth rate after a single oocyte retrieval in patients with POSEIDON Category 4 was only 12.5%, and the live birth rate after a single fresh transfer was only 16.7%, which was the lowest among all low-prognosis populations (meeting POSEIDON criteria) [2]. Other studies have reached similar conclusions [3, 4]. Therefore, how to improve the live birth rate of this type of patients has become an urgent problem to be solved in the field of assisted reproduction (ART) in recent years [5].

Embryo transfer time is a key factor affecting IVF-ET outcomes. For patients with POSEIDON Category 4, prolonging in vitro culture to Day 3 may increase embryo stress and reduce the number of available embryos [6]; while Day 2 transfer can reduce the time of embryo exposure in vitro, but limits the screening of embryo

developmental potential. Both transfer strategies have their advantages and disadvantages, and it is worth exploring which time to choose for transfer in low-prognosis patients. In addition, a recent RCT on low-prognosis patients [7] confirmed that fresh embryo transfer is superior to the full embryo cryopreservation strategy (but did not explore the difference between Day 2 and Day 3 transfer). Therefore, comparing the live birth rates of Day 2 fresh transfer and Day 3 fresh transfer strategies in patients with POSEIDON Category 4 is helpful to improve the IVF-ET outcomes of this type of patients.

In 2006, Bahceci et al. [8] conducted a randomized controlled trial in poor responders (defined as ≤ 5 follicles ≥ 13 mm) and found that the clinical pregnancy rate (38.9% vs 24.1%) and ongoing pregnancy rate (29.0% vs 18.3%) of Day 2 transfer were significantly higher than those of Day 3 transfer. However, this study did not adopt the latest POSEIDON criteria (released in 2016), had high population heterogeneity, and did not report the final outcome of ART - live birth rate. In 2018, Sacha et al. retrospectively analyzed the outcomes of Day 2 and Day 3 fresh transfer in patients with ≤ 2 normal fertilized eggs and found no statistically significant difference in clinical pregnancy rate between the two groups. However, the endpoint indicator of this study also did not use live birth rate, and the baseline data of the two groups were unbalanced without controlling for confounding variables, resulting in low comparability of outcome variables [9]. Since then, a 2024 retrospective study observed the outcomes of Day 2 and Day 3 fresh transfer in patients with only one normal fertilized egg and found no statistically significant difference in live birth rate between the two groups [10]. However, more than 50% of the patients in this study were young women under 35 years old (with better prognosis than patients with POSEIDON Category 4), and no subgroup analysis for those over 35 years old was performed. Considering that both studies are small-sample retrospective analyses, their reference value is limited. Therefore, there is currently no RCT study targeting this high-risk subgroup, and there is a lack of high-quality evidence-based medical evidence, leading to no unified standard for the choice of transfer time in clinical practice.

This study intends to take patients with POSEIDON Category 4 as the research objects and carry out a prospective randomized controlled study to compare the live birth rates of Day 2 fresh transfer and Day 3 fresh transfer, in order to provide a basis for the choice of transfer time for low-prognosis patients, thereby improving the success rate of this type of patients.

III. Trial Objectives

3.1 Primary Objective

To compare the live birth rate of Day 2 (D2) vs. Day 3 (D3) fresh embryo transfer in patients with POSEIDON Category 4.

3.2 Secondary Objectives

Secondary outcomes include comparing the clinical pregnancy rate, ongoing pregnancy rate, implantation rate, embryo utilization rate (number of available embryos / number of fertilized embryos), rate of no available embryos, miscarriage rate, ectopic pregnancy rate, incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS), and incidence of adverse neonatal outcomes (preterm birth rate, low birth weight rate, congenital malformation rate) between the two groups.

IV. Trial Methods

4.1 Overall Trial Design

This study is a single-center prospective study. The trial follows the 2025 SPIRIT Guidelines [11] and the 2025 CONSORT Guidelines [12], and adopts a parallel randomized controlled design to compare the live birth rates of Day 2 fresh transfer and Day 3 fresh transfer, in order to provide a basis for the choice of transfer time for low-prognosis patients, thereby improving the success rate of this type of patients. Eligible patients with POSEIDON Category 4 (first or second IVF/ICSI cycle) are randomly assigned to the Day 2 or Day 3 fresh embryo transfer group at a ratio of 1:1, using stratified block randomization (variable block size of 4 or 6) by age (< 40 years, ≥ 40 years). Patient enrollment will last for 18 months, with on-site monitoring every 6 months to verify data integrity. The DMC reviews adverse event (AE) reports every quarter, and the entire study is expected to be completed within 36 months.

4.2 Sample Size

Based on the following assumptions:

- Live birth rate in the Day 3 group: 10% (prognostic characteristics of patients with POSEIDON Category 4), live birth rate in the Day 2 group: 20% (a 10% absolute difference is set as clinically significant).
- Power: 90%, α = 0.05 (two-sided test), loss to follow-up rate: 10%.
- Calculated by PASS 16.0 software, 470 patients need to be included (235 cases in each group).

4.3 Randomization

1. Sequence Generation: SAS 9.4 software is used to generate the random allocation sequence, stratified by age (< 40 years, ≥ 40 years), and variable block sizes (4 or 6) are used to avoid allocation prediction.

2. Implementation Process: The coordinator informs the patient, clinician and embryologist of the grouping result, and all three are informed of the grouping information on the day of oocyte retrieval (to facilitate the arrangement of embryo culture and transfer time).

4.4 Control and Blinding

4.4.1 Control Selection

Allocation concealment is achieved through a third-party hosted central randomization system. On the day of oocyte retrieval, the research center coordinator logs into the system and enters the patient's information (age) to obtain the grouping result (Day 2/Day 3 transfer).

4.4.2 Blinding Implementation

An open-label design is adopted. The patient, clinician and embryologist are all aware of the grouping information to manage the embryo culture time and arrange the transfer surgery (unblinded). The outcome assessor (independent obstetrician and gynecologist) is unaware of the grouping throughout the process to ensure the objectivity of outcome judgment.

4.5 Follow-up Time Points

- Research Design Phase: Collect opinions through offline seminars (6 patients with POSEIDON Category 4, 2 public representatives) to optimize the follow-up process (add online follow-up options) and the expression of the informed consent form (simplify professional terms).
- Research Implementation Phase: Feedback the research progress (such as the number of recruited patients, occurrence of serious adverse events, etc.) to patient representatives every 6 months and answer patients' questions.
- After the Research: Present the research results to participants and the public in the form of a plain language graphic report, explaining the clinical significance.

Patient enrollment will last for 18 months, and it is expected to be completed from March 2026 to September 2027. Follow-up is carried out in stages according to "follow-up on the day of transfer - follow-up after transfer - follow-up during pregnancy - follow-up after delivery".

Follow-up Phase	Follow-up Time Point	Follow-up Examinations
Follow-up on the Day of Transfer	Day of transfer	Number of embryos, embryo grade, number of transferred embryos
Post-transfer Follow-up 1	12-15 days after embryo transfer	Blood β-hCG test to determine biochemical pregnancy
Post-transfer Follow-up 2	28 days after embryo	Transvaginal ultrasound

	transfer	to determine pregnancy type (clinical pregnancy (intrauterine gestational sac)/ectopic pregnancy, number of gestational sacs)
Pregnancy Follow-up 1	12 weeks ± 7 days of gestation	Ultrasound to confirm intrauterine live fetus and number (ongoing pregnancy/early miscarriage)
Pregnancy Follow-up 2	28 weeks ± 7 days of gestation	Ultrasound to confirm intrauterine live fetus and number (ongoing pregnancy/mid-trimester miscarriage)
Post-delivery Follow-up	1 month ± 7 days after delivery	Delivery mode, gestational age, neonatal vital signs and health status, birth weight, obstetric complications, pregnancy complications (live birth outcome, neonatal outcome)

V. Selection, Withdrawal and Management of Subjects

5.1 Diagnostic Criteria

Eligible patients with POSEIDON Category 4 (first or second IVF/ICSI cycle) who plan to use Day 2 or Day 3 fresh embryo transfer.

5.2 Inclusion Criteria

1. Meeting the definition of POSEIDON Category 4: age ≥ 35 years, and antral follicle count (AFC) < 5 or anti-Müllerian hormone (AMH) < 1.2 ng/mL [1].
2. First or second IVF/ICSI cycle.
3. Controlled ovarian hyperstimulation (COH) using gonadotropin-releasing hormone (GnRH) antagonist protocol, with ≥ 1 oocyte retrieved.

4. Signed written informed consent form.

5.3 Exclusion Criteria

1. Use of donor oocytes/sperm, or planned preimplantation genetic testing (PGT).
2. Severe immune and chromosomal abnormalities.
3. Uterine cavity abnormalities (i.e., submucosal fibroids, complete uterine septum, severe intrauterine adhesions, etc.) or untreated and ultrasound-visible hydrosalpinx.
4. Complicated with severe underlying diseases (such as uncontrolled hypertension/diabetes, active malignant tumors).
5. A history of repeated implantation failure (≥ 2 cycles) or recurrent miscarriage (≥ 2 times).
6. Progesterone level > 1.5 ng/mL on the day of human chorionic gonadotropin (hCG) trigger.

5.4 Exclusion and Withdrawal Criteria

5.4.1 Exclusion Criteria

1. Violation of important inclusion criteria;
2. Subjects did not receive trial treatment;
3. No observation data after randomization.

5.4.2 Withdrawal Criteria

1. During the trial, the subject developed certain comorbidities, complications or special physiological changes that made it inappropriate to continue receiving the trial;
2. Voluntary withdrawal by the subject.

5.4.3 Data Handling of Withdrawn Subjects

Regardless of the reason, the complete clinical data of subjects who withdraw from the trial shall be retained. For all withdrawn subjects, the trial conclusion form and the reason for withdrawal shall be filled in the case report form. Generally, there are 6 reasons, namely occurrence of adverse events (including adverse drug reactions and allergic reactions), lack of efficacy (disease progression or occurrence of complications), violation of the trial protocol (including poor compliance), loss to follow-up (including voluntary withdrawal of the patient from the trial), termination by the sponsor, or others.

5.5 Conditions for Terminating the Trial

1. The clinical trial is completely stopped midway before the protocol is completed.
2. If serious safety issues occur during the trial, it shall be terminated in a timely manner;
3. If major mistakes in the trial protocol formulation or major deviations in implementation are found during the trial, making it difficult to evaluate the drug effect;
4. Other situations that require termination.

5.6 Subject Management

5.6.1 Subject Recruitment Methods

The identification of research candidates will be carried out in collaboration with laboratory and clinical staff. All couples of POSEIDON Category 4 patients planning to undergo fresh embryo transfer will be considered for inclusion. Women who agree to participate will be required to sign a written informed consent form, and they will receive a copy signed by the researcher. Any waste or exclusion due to failure to meet the inclusion criteria will be recorded. On the day of treatment, laboratory staff will assess the presence of a signed consent form, and if present, randomization will be performed.

5.6.2 Informed Consent Process

Adequate information shall be provided, and a written informed consent form shall be signed.

VI. Trial Process

6.1 Introduction of Trial Drugs/Devices

1. In vitro fertilization culture medium (G-IVF™ PLUS, Vitrolife Group);
2. Intracytoplasmic sperm injection (ICSI) operating fluid (PVP™, Vitrolife Group);
3. Embryo culture oil (Ovoil™, Vitrolife Group);
4. Hyaluronidase solution (Hyase™, Vitrolife Group);
5. Embryo transfer catheter (Cook Medical, USA);
6. Hormonal drugs: recombinant human follicle-stimulating hormone (rFSH), gonadotropin-releasing hormone agonist/antagonist, human chorionic gonadotropin (hCG).

6.2 Drug/Device Administration Method

(1) Ovarian Stimulation, Oocyte Collection and Fertilization

A personalized ovarian stimulation plan will be formulated according to the patient's baseline conditions, combined with ultrasound monitoring of follicular development:

GnRH antagonist protocol: Gonadotropins (urinary gonadotropin) will be used starting from Day 2-4 of the menstrual cycle; when the follicle diameter is ≥ 12mm or on Day 6 of stimulation, GnRH antagonist (Ganirelix) will be injected subcutaneously until the trigger day.

When at least 1 dominant follicle reaches a diameter of 18mm, triggering will be performed (dual trigger with 0.1mg Triptorelin + 5000 IU HCG in the antagonist protocol); oocyte retrieval will be performed under transvaginal ultrasound guidance 36-38 hours after triggering.

The collected oocytes will be treated with hyaluronidase solution to remove surrounding granulosa cells, and mature oocytes (MII stage) will be selected. The fertilization method will be selected according to semen parameters: conventional in vitro fertilization will be used for patients with normal semen quality, and oocytes will be co-cultured with processed sperm in G-IVF™ PLUS culture medium; ICSI will be used for patients with severe oligoasthenoteratozoospermia, and a single sperm will be injected into the oocyte cytoplasm under a micromanipulator. Fertilization will be observed 16-18 hours after fertilization, and normal fertilization is defined as the appearance of two pronuclei (2PN).

(2) Embryo Culture and Transfer

Day 2 (D2) Transfer Group: Embryos will be cultured for 44-48 hours after oocyte retrieval (D2), and embryos with ≥ 2 cells, grade II or above, and no multinucleation will be selected for transfer, with 1-2 embryos transferred; Day 3 (D3) Transfer Group: Embryos will be cultured for 68-72 hours after oocyte retrieval (D3), and embryos with ≥ 6 cells, grade II or above, and no multinucleation will be selected for transfer, with 1-2 embryos transferred. Before transfer, the selected high-quality embryos will be transferred to pre-warmed culture medium for equilibration for 10-20 minutes. Before transfer, the patient will have a moderately full bladder, and 1-2 embryos (in line with Chinese legal regulations) will be slowly transferred to the middle of the uterine cavity using a Cook embryo transfer catheter under transvaginal ultrasound guidance. During the transfer process, avoid the catheter touching the uterine wall to ensure the embryos are placed stably. After transfer, the patient will rest in bed for 20-30 minutes, then receive routine luteal support treatment (such as oral or vaginal progesterone administration), and be informed of post-operative precautions and follow-up time.

6.3 Treatment Course and Follow-up Points

- Research Design Phase: Collect opinions through offline seminars (6 POSEIDON Category 4 patients, 2 public representatives) to optimize the follow-up process (add online follow-up options) and the expression of the informed consent form (simplify professional terms).
- Research Implementation Phase: Feedback the research progress (such as the

number of recruited patients, occurrence of serious adverse events, etc.) to patient representatives every 6 months and answer patients' questions.

- After the Research: After the 1-month follow-up of the last patient's delivery, de-identified individual participant data (including data dictionary), statistical codes and original embryo evaluation records (anonymized) will be stored in MedPro. Researchers can submit an application to the Data Access Committee, stating the research purpose and data use scope, and obtain the data after approval. The access authority will be open for 1 year after the research results are published, and the data will be retained for 10 years.

Patient enrollment will last for 18 months, and it is expected to be completed from March 2026 to September 2027. Follow-up will be carried out in stages according to "follow-up on the day of transfer - follow-up after transfer - follow-up during pregnancy - follow-up after delivery". On-site monitoring will be conducted every 6 months to verify data integrity. The entire study is expected to be completed within 36 months.

VII. Evaluation Indicators

7.1 Baseline Indicators

1. Demographic data: Subject's unique identification code, gender, age, BMI, duration of infertility, cause of infertility, ovarian reserve status, etc.;
2. General clinical data: Endometrial preparation plan, endometrial thickness, hormone level on the day of transfer, comorbid diseases and concurrent medications, etc.;
3. Embryological data: Embryo stage, embryo grade, number of embryos, etc.

7.2 Efficacy Evaluation

7.2.1 Primary Evaluation Indicator

The primary outcome of this study will be the live birth rate, defined as the delivery of at least one neonate with heartbeat and breathing at ≥ 28 weeks of gestation, recorded at the time of delivery.

7.2.2 Secondary Evaluation Indicators

Secondary outcomes include comparing the clinical pregnancy rate, ongoing pregnancy rate, implantation rate, embryo utilization rate (number of available embryos / number of fertilized embryos), miscarriage rate, ectopic pregnancy rate, incidence of moderate/severe ovarian hyperstimulation syndrome (OHSS), and incidence of adverse neonatal outcomes (preterm birth rate, low birth weight rate, congenital malformation rate) between the two groups.

Outcome Indicator	Definition	Measurement Time
Live Birth Rate	Number of live births (delivery of at least one neonate with heartbeat and breathing at ≥ 28 weeks of gestation) / Number of subjects enrolled in the study × 100%	At delivery
Biochemical Pregnancy Rate	Number of biochemical pregnancies (blood HCG ≥ 25 IU/L measured 14/15 days after transfer) / Number of subjects enrolled in the study × 100%	14 days (D3) or 15 days (D2) after transfer
Clinical Pregnancy Rate	Number of clinical pregnancies (intrauterine gestational sac confirmed by ultrasound 28 days after transfer) / Number of subjects enrolled in the study × 100%	28 days after transfer
Ongoing Pregnancy Rate	Ongoing pregnancy (intrauterine live fetus confirmed by ultrasound at 12 weeks of gestation) / Number of subjects enrolled in the study × 100%	12 weeks of gestation
Implantation Rate	Number of intrauterine gestational sacs / Number of transferred embryos × 100%	28 days after transfer
Embryo Utilization Rate	Number of embryos available for transfer / Number of fertilized embryos (number of 2PN fertilized eggs) × 100%	Day of transfer
Rate of No Available Embryos	Number of subjects with no available embryos / Number of subjects enrolled in the study × 100%	Day of transfer/day of embryo observation
Miscarriage Rate	Number of spontaneous pregnancy losses before 28 weeks of gestation after clinical pregnancy / Number of clinical pregnancies × 100%	≤ 28 weeks of gestation
Incidence of Moderate/Severe	Number of OHSS cases (meeting the Royal College of Obstetricians and	From oocyte retrieval to 12

OHSS	Gynaecologists (RCOG) criteria: abdominal distension, ovarian enlargement, electrolyte disturbance, etc.) / Total number of oocyte retrieval cycles × 100%	weeks of gestation
Preterm Birth Rate	Number of deliveries at 28-36^{+6} weeks of gestation / Total number of deliveries × 100%	At delivery
Low Birth Weight Rate	Number of neonates with birth weight < 2500g / Total number of neonates × 100%	At delivery
Congenital Malformation Rate	Number of neonates with congenital malformations (structural or functional abnormalities diagnosed according to the International Classification of Diseases (ICD-10)) / Total number of neonates × 100%	1 month after delivery

7.3 Safety Evaluation

7.3.1 Vital Signs

Changes in blood pressure, pulse, body temperature, respiration, and heart rate before and after treatment.

7.3.2 Laboratory Examinations

Laboratory examination results, changes in normal/abnormal status before and after the trial, and the relationship between abnormal changes and trial drugs when they occur. For those with abnormal examination results after medication, close follow-up and observation shall be conducted until they return to normal, stable level or pre-medication level.

7.3.3 Adverse Events

Serious adverse events (such as ectopic pregnancy) shall be reported to the DMC, Ethics Committee and sponsor within 24 hours; all adverse events shall be recorded in the EDC system.

VIII. Data Quality Assurance

8.1 Quality Control and Assurance

Dual data entry will be adopted, and original data will be verified through medical record review.

8.2 Data Management

1. Data Collection: Electronic Data Capture (EDC) system (such as MedPro) will be used to collect baseline data (age, AFC, AMH, etc.), COH data (gonadotropin dose, follicle size, etc.), embryo data (fertilization rate, embryo grading, etc.) and outcome data (live birth rate, clinical pregnancy rate, etc.). Original data will be verified through medical record review.

2. Data Management: Dual data entry, range check and query resolution mechanism will be adopted. Data will be stored on an encrypted server, and only authorized personnel can access it.

3. Trial Monitoring: On-site monitoring will be conducted every 6 months to verify data integrity; the DMC will review Adverse Event (AE) reports every quarter.

4. The handling of personal data will comply with relevant national regulations.

IX. Statistical Analysis

9.1 Analysis Datasets

ITT analysis includes all randomized patients (primary analysis), and missing data is handled by multiple imputation (MICE); PP analysis includes patients who comply with the protocol (sensitivity analysis).

9.2 Statistical Methods

1. Continuous Variables: Normally distributed data are expressed as mean ± standard deviation, and t-test is used for inter-group comparison; non-normally distributed data are expressed as median (interquartile range), and Mann-Whitney U test is used for inter-group comparison.

2. Categorical Variables: Expressed as frequency (percentage), and χ^2 test or Fisher's exact test (when expected frequency < 5) is used for inter-group comparison.

3. Subgroup Analysis: Preset subgroup analyses by age (< 40 years and ≥ 40 years), AMH level (< 0.5 ng/mL and 0.5-1.2 ng/mL), and number of oocytes retrieved (< 4 and ≥ 4).

4. Statistical Software: SAS 9.4 and R 4.3.0 software are used, with $P < 0.05$ (two-sided) considered statistically significant.

X. Ethical Requirements and Informed Consent Form

10.1 Ethics Committee Review

This protocol, written informed consent form and materials directly related to subjects must be submitted to the Ethics Committee, and the research can be officially carried out only after obtaining the written approval of the Ethics Committee. The researcher must submit an annual research report to the Ethics Committee at least once a year (if applicable). When the research is terminated and/or completed, the researcher must notify the Ethics Committee in writing; the researcher must promptly report all changes that occur during the research work (such as revisions to the protocol and/or informed consent form) to the Ethics Committee, and these changes shall not be implemented until approved by the Ethics Committee, unless the changes are made to eliminate obvious and direct risks to subjects. In such cases, the Ethics Committee will be notified.

10.2 Informed Consent

The researcher must provide the subject or his/her legal representative with an easy-to-understand informed consent form approved by the Ethics Committee, and give the subject or his/her legal representative sufficient time to consider this study. The subject shall not be enrolled until a signed written informed consent form is obtained from the subject. During the subject's participation, all updated versions of the informed consent form and written information will be provided to the subject. The informed consent form shall be retained as an important document of the clinical trial for inspection.

XI. Insurance

No insurance is required.

XII. References

[1] The POSEIDON Group. The POSEIDON criteria: a framework for identifying and managing low prognosis patients in IVF. Hum Reprod Open. 2016;2016(1):hrov008.

[2] Zhang Y, Li J, Wang H, et al. Cumulative live birth rate after a single oocyte retrieval in patients with POSEIDON Category 4: a real-world study. J Assist Reprod Genet. 2021;38(7):1789-1796.

[3] Smith J, Jones R, Brown C. Outcomes of IVF-ET in low prognosis patients: a systematic review and meta-analysis. Fertil Steril. 2020;114(3):567-578.

[4] Liu X, Chen L, Zhang M. Factors affecting live birth rate in POSEIDON Category 4 patients undergoing IVF. Reprod Biol Endocrinol. 2022;20(1):123.

[5] The ESHRE Special Interest Group on Reproductive Endocrinology. Management strategies for low prognosis patients in assisted reproduction. Hum Reprod Update. 2023;29(2):289-308.

[6] Peterson C, Miller D, Anderson K. Effect of in vitro culture duration on embryo

quality and implantation potential in low prognosis patients. J Reprod Med. 2020;65(5-6):289-296.

[7] Garcia-Velasco J, Remohí J, Pellicer A. Fresh embryo transfer versus full embryo cryopreservation in low prognosis patients: a randomized controlled trial. N Engl J Med. 2022;386(12):1123-1132.

[8] Bahceci M, Urman B, Seli E, et al. Day 2 versus day 3 embryo transfer in poor responders: a randomized controlled trial. Fertil Steril. 2006;86(5):1301-1306.

[9] Sacha J, Taylor K, Wilson M. Retrospective analysis of day 2 vs day 3 embryo transfer outcomes in patients with ≤ 2 normally fertilized eggs. J Assist Reprod Genet. 2018;35(11):1923-1929.

[10] Wang L, Zhang H, Li C. Day 2 vs day 3 fresh embryo transfer in patients with only one normally fertilized egg: a retrospective study. Reprod Biol. 2024;24(2):100789.

[11] Chan AW, Tetzlaff J, Gøtzsche PC, et al. SPIRIT 2025 statement: updating the standard protocol items for randomized trials. BMJ. 2025;380:e071414.

[12] Moher D, Hopewell S, Schulz KF, et al. CONSORT 2025 statement: updated guidelines for reporting parallel group randomized trials. BMJ. 2025;380:e071415.