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Effect of low-molecular-weight heparin on pregnancy outcomes in Chinese women with recurrent implantation failure undergoing frozen embryo transfer: a double-blind, randomised, placebo-controlled trial

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ABSTRACT

Introduction The incidence of recurrent implantation failure (RIF) can reach up to 10% among patients undergoing in vitro fertilisation and embryo transfer worldwide. However, the clinical efficacy of low-molecular-weight heparin (LMWH) in RIF remains a subject of controversy. Currently, there is a lack of high-quality clinical research validating the effectiveness of LMWH in treating patients with RIF, particularly during frozen embryo transfer (FET) cycles. Therefore, this randomised controlled trial aimed to investigate the impact of LMWH on pregnancy outcomes in women with RIF undergoing FET.

Methods and analysis This prospective, single-centre, double-blind randomised, placebo-controlled clinical trial will be conducted in the Second Hospital of Shandong University, China. A total of 414 women with RIF, aged ≤40 years, who are undergoing FET cycles will be recruited and randomly assigned to the study group (LMWH) or the control group (placebo). Only one blastocyst which is from day 5 or day 6 and has a Gardner morphology score ≥4 BC will be transferred. LMWH 4000–6000 IU per day or placebo will be administered by subcutaneous injection from the day of transplantation. The primary outcome is the live birth rate. The secondary outcomes include the clinical pregnancy rate, biochemical pregnancy rate, embryo implantation rate, early miscarriage rate, ongoing pregnancy rate, ectopic pregnancy rate, pregnancy-related complications, perinatal complications, fetal birth weight, congenital malformations and other adverse reactions.

Ethics and dissemination The protocol received approval from the Ethics Committee of the Second Hospital, Cheeloo College of Medicine, Shandong University (KYLL-2023-442). The findings will be disseminated in peer-reviewed publications.

Trial registration number Chinese Clinical Trial Registry, ChiCTR2400083577.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a single-centre, double-blind, randomised, placebo-controlled clinical trial aiming to include 414 participants.
⇒ Another advantage is that the primary outcome is the live birth rate.
⇒ The design as a single-centre randomised controlled trial is a limitation of the study design.

INTRODUCTION

Background and rationale

A key feature affecting the pregnancy outcomes of in vitro fertilisation and embryo transfer (IVF-ET) is embryo implantation. When multiple attempts at embryo implantation fail, it is referred to as recurrent implantation failure (RIF). However, there is currently no unified definition for RIF.[1 2] The incidence rate of RIF is approximately 10%.[3] The aetiology of RIF is multifactorial and involves maternal, paternal and embryonic factors. However, a significant proportion of patients are still being diagnosed with unexplained RIF, which accounts for up to 50% of all patients with RIF.[4]

Low-molecular-weight heparin (LMWH) is commonly administered empirically to improve pregnancy outcomes. Previous studies have suggested that in addition to its anticoagulant properties, LMWH also has immunomodulatory and other non-anticoagulant benefits, such as promoting the proliferation, invasion and differentiation of trophoblast cells, inhibiting trophoblast apoptosis, protecting vascular endothelium and promoting placental formation.[5 6] Previous studies have demonstrated that LMWH may play a beneficial role during the process of implantation by decreasing the expression of E-cadherin[7] and transforming growth factor-beta 1[8 9] or by upregulating the expression

of insulin-like growth factor-binding protein[10–12] and matrix metalloproteinases.[13] Additionally, LMWH may enhance the effects of several growth factors[14–16] and affect patients' macrovesicles content.[5] Besides, heparin may have anti-inflammatory effects by reducing the levels of inflammation markers[17] or binding to P/L-selectin.[18 19] Inhibiting the cytotoxicity of natural killer cells and the immune response generated by antiphospholipid antibodies (aPLs) by heparin may modulate the immune function during the process of implantation.[20 21]

Some previous randomised controlled trials (RCTs) suggest that LMWH may improve pregnancy outcomes in patients undergoing assisted reproductive technology (ART).[22–24] However, two of these studies were pilot studies, and the differences were not statistically significant. The other two RCT studies, however, reached negative conclusions.[25 26] An RCT study on heparin conducted in 2015 also yielded similar negative results.[27] Two retrospective studies from 2018 and 2023 suggest that LMWH does not significantly improve ART pregnancy outcomes.[28 29] However, studies conducted in 2011 and 2024 arrived at conclusions that contradict these findings.[30 31] Even the results of meta-analyses regarding the impact of LMWH on ART pregnancy outcomes are controversial.[32–36] Only a few of these studies included or focused solely on frozen embryos.[29 31] Considering that oocyte retrieval may affect coagulation function and hormone levels during fresh embryo transfer cycles are relatively higher than those for frozen embryos, we infer that LMWH may have differing effects on pregnancy outcomes for fresh versus frozen embryos. Currently, there is a lack of relevant research on the effects of LMWH on improving pregnancy outcomes in patients with RIF during frozen embryo transfer (FET) cycles, especially randomised controlled studies.

There might be several reasons for the controversial conclusions among these clinical trials. First, the unified definitions of RIF led to population differences among studies. Second, heterogeneity exists among diverse populations with racial disparities across various countries/regions. For example, there are significant racial differences in the genetic factors of the prethrombotic state (PTS). In the Han population, protein C (PC), protein S (PS) and antithrombin deficiencies are commonly observed as hereditary PTS types.[37] Third, in clinical practice, physicians empirically use LMWH with or without a clear indication, as well as different dosages and durations. All of these discrepancies contribute greatly to controversies and conflicts. Therefore, a clinical trial with a defined population and precise design is still needed.

In summary, although doctors often empirically use LMWH to improve pregnancy outcomes in ART, there is currently a lack of high-quality clinical research confirming the effectiveness of LMWH in treating patients with RIF, especially in FET cycles. In accordance with the 2023 ESHRE RIF guidelines, we will include participants with at least three failed transfer cycles. In this study, we will compare the pregnancy outcomes between the LMWH group and the placebo group in FET cycles among patients with RIF through an RCT.

Objectives

This study aims to investigate the efficacy of LMWH as an adjunctive medication for improving FET outcomes in women with RIF by comparing differences in the pregnancy outcomes between the LMWH group and the placebo group. This pragmatic trial holds promise for enhancing pregnancy outcomes in women with RIF undergoing FET cycles.

Trial design

This is a prospective, single-centre, placebo-randomised, double-blind clinical trial to evaluate whether the administration of LMWH could improve pregnancy outcomes in patients with RIF.

METHODS: PARTICIPANTS, INTERVENTIONS AND OUTCOMES

Study setting

Detailed information about the study will be provided to participants who meet the inclusion criteria before FET. All the subjects will be recruited from our own centre. Eligible participants will be randomly assigned to either the LWMH group or the placebo group at a 1:1 ratio. This study will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines and will be approved by the ethics committee of our hospital. The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2400083577, https://www.chictr.org.cn/showproj.html?proj=228678). A flow chart of the study design is shown in figure 1. A schedule of enrolment, interventions and assessment is provided in the Standard Protocol Items: Recommendations for Interventional Trials figure (online supplemental material 1).

Eligibility criteria

Inclusion criteria

The inclusion criteria were as follows:

The participants should take part in clinical research voluntarily and can only participate once. Written informed consent will be obtained from all participants before they are randomly assigned.

1. Age ≤40 years when the oocytes were retrieved.
2. Planning for FET after IVF or Intracytoplasmic Sperm Injection (ICSI) cycle.
3. The participants have at least one good-quality frozen blastocyst and will transfer only one frozen blastocyst. The blastocyst should be from day 5 or day 6 and have a Gardner morphology score ≥4 BC.
4. Participants with a history of at least three failed fresh/frozen high-quality embryo transfer cycles. The criteria for good-quality embryos are shown in table 1.
5. Participants are willing to cooperate with the research plan and sign the informed consent form.

Exclusion criteria

Any of the following will result in exclusion:

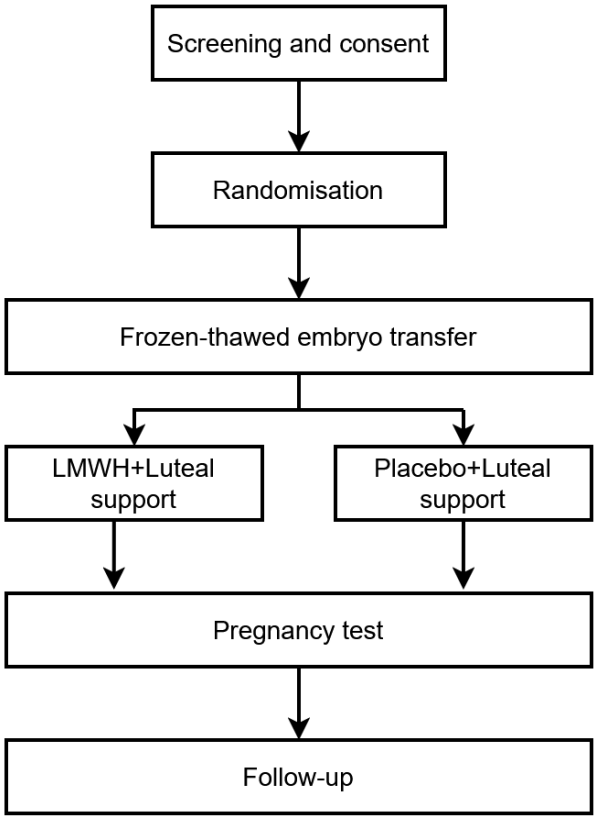

Figure 1 Flow chart of the study design. LWMH, low-molecular-weight heparin.

1. One of the couples has chromosomal abnormalities.
2. Untreated submucosal uterine fibroids or endometrial polyps.
3. History of autoimmune diseases such as antiphospholipid syndrome, Sjogren's syndrome systemic lupus erythematosus and rheumatoid arthritis.
4. Thin endometrium (<7 mm) before transfer.
5. Medical contraindications to LMWH or ART and/or pregnancy.
6. Women who are currently receiving any anticoagulant drugs, such as aspirin.
7. History of intrauterine adhesions.
8. Untreated hydrosalpinx visible via ultrasound.
9. Embryos planned for thawing and transfer are from cryopreserved oocyte sources or have undergone secondary cryopreservation
10. History of recurrent spontaneous abortion.
11. FET after preimplantation genetic testing.

Table 1 Criteria for good-quality embryos

Embryo	Scoring system	Criteria of good quality
Blastocyst	Gardner	≥4 BC
Cleavage (day 3)	Puissant	7–10 cell 3; 4 or compact
	Peter	7–10 cell I; II or compact

Who will provide informed consent?

Detailed information about the study will be provided by the Clinical Research Coordinator (CRC) to the subjects who are enrolled in this study. Investigators will fully inform the participants of any safety or possible adverse effects associated with the interventions. After a detailed explanation, the couples who agreed to participate in the trial signed an informed consent form. Refusal to participate or withdrawal midway has no impact on their clinical treatment. Details of the informed consent form are provided in online supplemental material 2.

Additional consent provisions for the collection and use of participant data and biological specimens

For further information regarding the consent required for the collection and utilisation of participant data, refer to online supplemental material 2.

INTERVENTIONS

Explanation for the choice of comparators

The primary outcome for this trial is the live birth rate, and the secondary outcomes include the clinical pregnancy rate, biochemical pregnancy rate, embryo implantation rate, early miscarriage rate, ongoing pregnancy rate, ectopic pregnancy rate, pregnancy-related complications, perinatal complications, fetal birth weight, congenital malformations and other adverse reactions.

Frozen-thawed embryo transfer

The protocols used to prepare the endometrium include the natural cycle protocol, ovulation induction protocol and hormone replacement therapy. For the total natural cycle protocol, luteal phase support begins on the day of ovulation (or expected ovulation) or the day after ovulation; for the modified natural cycle protocol, medication begins 2 days after human chorionic gonadotropin (HCG) injection. Oral administration of 10 mg dydrogesterone (Duphaston, Abbott) two times daily or a combination of 100–200 mg progesterone soft capsules (Qining, Zhejiang Aisheng Pharmaceutical Co, Ltd) is given for luteal phase support. For the ovulation induction protocol, luteal phase support begins 2 days after HCG injection. Oral administration of 10 mg dydrogesterone (Duphaston, Abbott) two times daily combined with 200–400 mg of progesterone soft capsules daily is given for luteal phase support. In both the natural cycle protocol and ovulation induction protocol, the administration of luteal support medication before ovulation or expected ovulation is not allowed. In hormone replacement therapy, participants will be given estradiol valerate (Progynova, Delpharm Lille SAS, France) and/or estradiol tablets (Femoston, Abbott Biologicals B.V., The Netherlands) at a dosage of 2–8 mg/day from days 2 to 5 of the menstrual cycle or 28 to 35 days after long-acting Gonadotropin-Releasing Hormone (GnRH) agonists. When the endometrial thickness reaches ≥7 mm and P is ≤1.5 ng/mL, progesterone will be administered. The luteal phase support

plan includes a combination of 10 mg dydrogesterone (Duphaston, Abbott) two times daily and 90 mg vaginal progesterone gel (Crinone, Merck Serono) daily. Only one blastocyst will be transferred from each participant in this trial. All the transplanted embryos will be required to be of good quality, and on the basis of the embryonic Gardner morphology score, the highest-grade embryo will be selected for transfer. In cases of an ongoing pregnancy, the administration of oestrogen gradually ceases and will be tapered off on the seventh to eighth week of gestation. Regardless of which protocol is used to prepare the endometrium, luteal support is gradually stopped at 12 weeks of pregnancy. In addition, if biochemical pregnancy loss, ectopic pregnancy or embryonic arrest occurs, both luteal support and LMWH/placebo will be discontinued.

Intervention description

After the process of IVF, the embryos were frozen and preserved in the form of blastocysts. Before FET, a total of 414 eligible participants will be enrolled and equally randomised into the experimental (LMWH) group and the control (placebo) group. Frozen embryos will be transferred on the fifth day of luteal transformation. Participants will receive subcutaneous injections of 4000–6000 IU LMWH or a placebo daily from the day of FET. The study medications are manufactured by Nanjing King-Friend Biochemical Pharmaceutical Co, Ltd in China. The packaging of LWMH and placebo are in the same appearance and cannot be distinguished. The primary component of the placebo is normal saline infusion. In addition to the primary component, the remaining excipients in both LMWH and the placebo are identical. The quality of the placebo, encompassing its composition and potential bacterial contamination, will be meticulously regulated in accordance with the standards set forth by Good Manufacturing Practice.

Criteria for discontinuing or modifying allocated interventions

The reasons for discontinuing the research methods include but are not limited to the following. (1) The participant self-administered other anticoagulant and/or immunosuppressive drugs without authorisation. (2) The participant has severe complications or adverse reactions to the treatments. (3) Before embryo transfer, the thickness of the endometrium is less than 7 mm. (4) The participants request to withdraw from the study before completion.

Strategies to improve adherence to interventions

Participants can consult the attending physician about the treatment regimen and possible complications at each visit to our centre. In addition, online hospital services and WeChat platform services can eliminate problems associated with trial-related questions or discomfort in a timely manner. Adherence will be assessed by counting the number of prefilled injection syringes returned by the participants or during the telephone interview. The total number of medications taken is calculated by subtracting the number of prefilled injection syringes remaining from the number of syringes dispensed. Adherence is considered good if the reported use of prefilled injection syringes is 85% or more of the total number that the participants are expected to have been given.

Relevant concomitant care permitted or prohibited during the trial

The simultaneous application of other anticoagulant or immunomodulatory drugs is not allowed. The medications listed below will be authorised for use throughout the duration of this study, and it is imperative that any concomitant medications are documented: (1) folic acid supplement; (2) for individuals experiencing chronic illnesses or complications related to pregnancy, standard clinical care protocols will be implemented and (3) for individuals experiencing abnormal bleeding and/or extended amenorrhea, the administration of micronized progesterone, dydrogesterone or progestin are permitted.

Provisions for post-trial care

The study medications in this clinical research (including LMWH and placebo) are provided free of charge. Participants could communicate face-to-face with their attending physician about their condition and treatment plan during each follow-up visit. The use of the WeChat platform and online hospital follow-up platforms will ensure participants can communicate with their attending physician and receive proper treatment in a timely manner once adverse events (AEs) occur.

Outcomes

Primary outcome

The primary outcome is the live birth rate. Live birth is defined as the delivery of any number of neonates born at ≥24 weeks gestation with signs of life. The live birth rate per FET cycle=number of live births/numbers of FET cycles×100%.

Secondary outcomes

Clinical pregnancy is defined as the presence of a gestational sac visible on ultrasound within the uterus or outside the uterus. The clinical pregnancy rate per FET cycle is calculated as the number of clinical pregnancy cycles/number of FET cycles×100%. Biochemical pregnancy is defined as a serum β-HCG level ≥10 IU/L 12–14 days after embryo transfer. The biochemical pregnancy rate is calculated as the number of biochemical pregnancy cycles/number of FET cycles×100%. The embryo implantation rate is the ratio of the number of observed gestational sacs on ultrasound to the number of embryos transferred (the number of gestational sacs in single embryo transfer cycles is counted as 1). The early miscarriage rate is calculated as the number of natural miscarriages within 12 weeks of pregnancy/the number of clinical pregnancy cycles×100%. Ongoing pregnancy is defined as the presence of a fetal heartbeat detected by ultrasound after 12

weeks of gestation. Ongoing pregnancy rate=number of ongoing pregnancy cycles/numbers of FET cycles×100%.

Participant timeline

A total of 36 months will be required to complete the study after initiation, which include a 24-month enrolment period and a 1-month treatment period, with an 11-month additional observation period to determine pregnancy outcomes.

Sample size

According to previously reported papers, in women with three or more failed assisted reproduction treatment cycles, the live birth rate ranged from 16% to 23.5%, and the application of LWMH could improve the live birth rate by 8%–13%.[23 26 32] In this study, we planned to test the main hypothesis that included a 13% difference in the live birth rate between the two randomisation groups. We assumed a live birth rate of 20% in the RIF group and 33% in the LWMH group. In the sample size calculation, a two-sided significance level of α=0.05 was set, and the statistical power was calculated as 1 − β=0.80. The ratio between the two groups was 1:1. The minimum sample size for each group was 180, for a total of 360 participants. Considering a dropout rate of 15%, we expected a final enrolment of approximately 414 participants.

Recruitment

Women with RIF who undergo FET at our centre will be screened for eligibility. According to the detailed information, the potential subjects who meet the enrolment criteria will be included in the study.

ASSIGNMENT OF INTERVENTIONS: ALLOCATION

Sequence generation

The randomisation sequence will be generated by a computer at the Data Coordination Center (DCC) at hospital's clinical trial research centre. The eligible participants will be recruited and randomly assigned to the experimental (LMWH) group or the control (placebo) group at a 1:1 ratio according to the randomisation sequence. Participants, researchers, physicians and follow-up staff are prohibited from influencing the assignment of individuals to specific groups. The randomisation scheme is maintained with strict confidentiality by the DCC personnel and will not be revealed to any patients, staff members or investigators, including the principal investigator (PI).

Concealment mechanism

Once the randomisation sequence is generated by computer in the DCC, the study medication (prednisone/placebo) will be prepared in accordance with the established randomisation scheme. Consequently, randomisation will occur following the distribution of the study medication.

Implementation

The attending physician will enrol eligible participants and refer them to the CRCs. After the recruitment period, the researchers will distribute the first batch of corresponding medications (14 prefilled injection syringes of LMWH/placebo). Patients will be instructed to administer a subcutaneous injection daily after the day of FET. If pregnancy is confirmed, a second batch of corresponding medications (20 prefilled injection syringes) will be distributed on the day of the biochemical pregnancy test and the medication will continue to be administered until 8–10 weeks. If pregnancy is not confirmed or biochemical pregnancy loss, ectopic pregnancy or missed abortion occurs, then the medication will be stopped. The remaining medications will be returned to the researchers.

Study agent storage

The investigational medications will be stored in a secure location in compliance with GCP standards. The dispensation of the study medication will be conducted by personnel who are both authorised and adequately trained. The record logs must encompass the following elements: (1) the quantity of the study medications received and presently stored; (2) the dispensing log of the study drug and (3) signatures of the study personnel responsible for the study drug.

Who will be blinded?

In light of the double-blind design of this clinical trial, given that LMWH and placebo exhibit identical appearances and packaging, the participants, clinicians and research staff will be blinded to the treatment allocation throughout the duration of the study.

Procedure for unblinding if needed

Routine unblinding is prohibited to avoid bias. Unblinding in the process of clinical trials may occur only in medical emergencies, serious AEs (SAEs) or ethical requests. Apart from that, the unblinding will occur only after all participants have provided and reported their outcomes to the DCC. The unblinding procedures include confirming that the authorised clinical doctors have received approval from PI, querying grouping through the electronic randomisation system, completing the unblinding log and reporting to the Data and Safety Monitoring Board (DSMB).

DATA COLLECTION AND MANAGEMENT

Plans for assessment and collection of outcomes

Data from the start of enrolment to the end of the study, including demographic characteristics, detailed medical history, laboratory test reports, ultrasound parameters, embryo status, pregnancy status, etc, will be first accurately filled out by the designated staff in the case report form (CRF) (paper version), and then the data will be

recorded in Resman Clinical Trial Public Management Platform.

Before data collection, all personnel involved in the study, including attending physicians, nurses, researchers and research assistants, will undergo uniform training and will be provided with a standard protocol and standard operating procedure to ensure the accuracy of the data and experimental results.

Plans to promote participant retention and complete follow-up

The study endpoints include biochemical pregnancy visits, clinical pregnancy visits and follow-up visits. Professional follow-up personnel will make regular and timely telephone return visits to ensure that participant outcomes are tracked.

Data management

Two distinct tiers of quality assurance are implemented to maintain data integrity. Initially, research coordinators and data entry personnel are tasked with verifying the accuracy of the data. Real-time range checks within the web-based data entry system will play a supportive role. The subsequent level of quality evaluation involves DCC ensuring consistency between the electronic database and the original source documents. Any discrepancies identified will be addressed collaboratively between the DCC and the clinical centre.

Access to data

PI will have access to the final trial dataset. Before the end of the study, any investigators are forbidden to access the research data and results.

Confidentiality

The complete procedure will rigorously ensure data quality and participant protection, including the security and privacy of participants. Computer-generated audit trails will be used to monitor modifications made to electronic source documentation. In conjunction with the internal safeguards integrated within the computerised system, external protective measures will also be used. The data will be securely stored at the Department of Reproductive Medicine at the Second Hospital of Shandong University. Regular backups of records will be conducted to mitigate the risk of catastrophic data loss. Besides data entry and modification, trace records will be systematically maintained to ensure the quality and integrity of the data.

Plans for collection, laboratory evaluation and storage of biological samples for genetic or molecular analysis in this trial and in future use

All participants enrolled will undergo the following examinations before corresponding intervention measures, including D-Dimer, anti-ds DNA antibody, aPLs, antinuclear antibodies, antithrombin activity, coagulation function (activated partial thromboplastin time, prothrombin time, fibrinogen, thrombin time), PC, PS, homocysteine (HCY), levels of the tumour necrosis factor-α, interferon (IFN)-γ, interleukin (IL)-2, IL-4, IL-6 and IL-10. Approximately 7–8 days after FET, a venous blood sample of approximately 3 mL will be obtained for the purpose of assessing cytokine levels.

STATISTICAL METHODS

Statistical methods for primary and secondary outcomes

The data used in this study will be collected and analysed by nonclinical biostatisticians who are responsible for data management. The main analysis will be conducted according to the intention-to-treat (ITT) principle, meaning that all randomised subjects will be analysed according to the original randomisation groups. Sensitivity analysis will be performed on the basis of the actual treatment received by the participants. Baseline characteristics such as age, type of infertility, cause of infertility, duration of infertility, body mass index (BMI), baseline hormone levels, anti-Mullerian hormone (AMH), antral follicle count, number of previous embryos transferred and proportion of embryos derived from ICSI will be recorded and compared. Baseline characteristics will be declared through descriptive analysis. Categorical variables like live birth and clinical pregnancy will be described via frequencies and percentages, and intergroup comparisons will be conducted via Pearson's χ^2 test. For expected frequencies <5, Fisher's exact test will be used. Normally distributed continuous variables will be described via means±SD, and intergroup comparisons will be conducted via t-tests. Continuous variables that do not follow a normal distribution will be characterised by their median value along with the IQR (25th percentile–75th percentile), and intergroup comparisons will be made via the Wilcoxon rank-sum test. For binary outcome measures, the relative risk and corresponding 95% CI will be calculated. Statistical analysis will be performed via SPSS V.29 (SPSS Inc., Chicago, IL, USA) software. $P<0.05$ will be considered to indicate statistical significance.

Interim analyses

There are no interim analyses planned for this trial.

Methods for additional analyses (eg, subgroup analyses)

Stratified analysis will be conducted on the basis of factors such as age, BMI, endometrial thickness, number of previous embryos transferred, AMH and D-dimer. Besides, subgroup analyses will be applied to the different endometrial preparation regimen and abnormal blood test result groups such as patients with abnormal cytokine levels. As a secondary analysis, we will employ a generalised linear mixed-effects model using a logit link to compare the pregnancy outcomes in the treatment groups.

Analysis methods to handle protocol nonadherence and any statistical methods to handle missing data

Nonadherence to the protocol will be addressed by conducting analyses in accordance with the ITT principle.

If the participant decides to withdraw from the study, the data collected prior to withdrawal will be preserved, and the follow-up work will not be interrupted. The reasons for withdrawal will be documented for future analysis in the context of interpretation of the results. Concurrently, a per-protocol (PP) analysis will be performed for participants who adhered to the trial protocol. This clinical study will employ both ITT and PP analyses simultaneously to assess the efficacy and safety of the treatment from distinct perspectives. Given the anticipated low rate of missing data, no specific strategies for handling missing data are planned.

Plans to provide the full protocol, participant-level data and statistical code

CRFs will be created in a WORD format and will be subsequently integrated into Resman Clinical Trial Public Management Platform. The web-based data entry forms will be designed to closely resemble the WORD (paper) forms, maintaining consistency in the questions presented. Metadata will be shared through the Resman Clinical Trial Public Management Platform. The protocol can be found in the Chinese Clinical Trial Registry.

OVERSIGHT AND MONITORING

Composition of the coordinating centre and trial steering committee

The project will be overseen by a trial steering committee. The key stakeholders oversee the project from start to finish and provide guidance and support throughout the project life cycle.

Our institution will hire dedicated CRCs to form the DCC, which is responsible for coordinating and managing the clinical trial process. The primary responsibilities of the DCC include but are not restricted to providing support for ethical review processes, facilitating subject recruitment and screening, obtaining informed consent, collecting and recording data, managing experimental pharmaceuticals, aiding in the oversight of experimental procedures and monitoring safety protocols.

Composition of the data monitoring committee, its role and reporting structure

This study will establish a DSMB. The DSMB, composed of a group of impartial, external experts, will be responsible for evaluating the progress and safety of the research subjects. The DSMB will review the safety of the trials; the progress of the experiment; and the authenticity, completeness and accuracy of the data to improve the final data quality and will provide advice on study conduct via regular conference calls biweekly. The DSMB will be composed of five voting members. This membership will include three clinical experts, one biostatistician and one advocate for the protection of human subjects.

AE reporting and harm

On the basis of current research findings, it is believed that the use of LMWH for prevention or treatment during pregnancy is safe for both mothers and fetuses.[38] The most common adverse reaction of LMWH is bleeding, whereas other common adverse reactions include allergies, drug-induced liver injury, itching, hives and rare reactions such as hypersensitivity and heparin-induced thrombocytopenia. AEs will be documented systematically via the Medical Dictionary for Regulatory Activities. We will document and disseminate all recorded AEs. AEs will be adequately assessed to determine whether they are related to the study drugs. Each event will be monitored until it reaches a resolution or stability.

SAEs refer to events such as death, life-threatening situations, permanent or severe disabilities or functional loss, the need for hospitalisation or prolonged hospital stay and congenital abnormalities or birth defects that occur in participants subsequent to the administration of investigational drugs. The SAE should be reported to the PI within 24 hours, which includes whether the SAE is unanticipated or anticipated. The PI will decide whether to send the SAE record form to the DCC. Once it is reported to the DCC, the DSMB will review the event and return a report to the DCC. The DSMB will provide a report detailing the evaluation of the information reviewed, along with any recommendations back to the DCC. If a serious AE occurred, the trial treatment strategy may have been altered with the full informed consent of the participants and their families. Those who suffer harm from trial participation will receive corresponding compensation on the basis of the extent of their harm.

Frequency and plans for auditing trial conduct

An independent auditor will perform an annual assessment during the duration of the project.

Plans for communicating important protocol amendments to relevant parties (eg, trial participants and ethical committees)

Any suggested modifications to the protocol will be evaluated and will require approval from the ethics committee prior to implementation. Participants will be notified of these modifications by a member of the research team via email or telephone communication.

Dissemination plans

The results of the study will be disseminated in peer-reviewed academic journals. The funders will not exert any influence or impose any limitations on the publication decisions.

DISCUSSION

The publication of the guidelines on RIF by the ESHRE and the Chinese Medical Association in 2023 signifies the growing scholarly focus on RIF by the international level. RIF places significant economic and psychological

pressure on women undergoing ART. Poor fertility outcomes in women with RIF present significant challenges in assisted reproduction. Improving pregnancy outcomes for women with RIF is crucial.

Previous pharmacological studies have suggested that the application of LMWH may be beneficial.[5 6] However, there is still controversy surrounding the clinical research results. More research and explorations are urgently needed, especially RCT research concerning FET cycles. Our retrospective cohort study revealed that the clinical pregnancy rate in patients undergoing FET for RIF was significantly higher in the LMWH group (supplemented with LMWH from the day of transplantation) than in the control group (unpublished). We hypothesise that LMWH may improve pregnancy outcomes in patients with RIF. Therefore, we designed this randomised controlled, prospective study to evaluate the impact of LMWH on pregnancy outcomes during FET cycles, including the live birth rate, clinical pregnancy rate, early miscarriage rate, etc. This study plans to conduct further stratified analyses and subgroup analyses to clarify the effects of LMWH in different populations and thus provide a theoretical basis for the more precise and effective use of LMWH in clinical practice.

The single-centre design is one of the shortcomings of this clinical trial. In the future, more rigorous, scientific and multicentre studies on RIF are needed to provide powerful proof for clinical drug guidance to improve pregnancy outcomes in ART.

Trial status

The trial enrolment has not yet started at the time of submission of the manuscript.

Protocol version

The trial protocol is version 2.0, dated 1 April 2025.

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Contributors LC and SG conceived the study idea. WWang, MC, WWan and YanZ participated in the design of the study, recruitment of participants and drafting of the manuscript. SG, WWang, MC and WWan supervised participant diagnosis. MC, WWan, YaxZ and YanZ coordinated the data collection. WWang, SG and AS designed the statistical analysis plan and oversaw statistical analysis. All authors critically reviewed the article and approved the final manuscript. WWang is the guarantor of the content.

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