

BMJ Open Efficacy and safety of autologous adipose tissue-derived stromal vascular fraction in patients with thin endometrium: a protocol for a single-centre, longitudinal, prospective self-control study

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ABSTRACT

Introduction Endometrial thickness is an important parameter to evaluate endometrial receptivity. An appropriate endometrial thickness is necessary for both embryo implantation and maintaining normal pregnancy. Women with thin endometrium are one of the critical challenges in the clinic, and current therapeutic strategies for thin endometrium remain suboptimal. The stromal vascular fraction (SVF) derived from adipose tissue contains a variety of cells, mainly adipose-derived stem/stromal cells and adipose cells. Recently, adipose tissue-derived SVF showed tremendous potential for treating thin endometrium due to its capacity to repair and regenerate tissues. The application of SVF in animal models for treating thin endometrium has been investigated. However, limited evidence has demonstrated the efficacy and safety of autologous SVF in patients with thin endometrium.

Methods and analysis This study is a single-centre, longitudinal, prospective self-control study to investigate the preliminary efficacy and safety of autologous SVF in improving the pregnancy outcome of infertile patients with thin endometrium. Thirty patients diagnosed with thin endometrium will be recruited based on the inclusion and exclusion criteria. The SVF suspension will be transferred into the uterine cavity via an embryo transfer catheter. Then, comparisons between pretreatment and post-treatment will be analysed, and the outcomes, including endometrial thickness, menstrual volume and duration, frequency and severity of adverse events and early pregnancy outcomes, will be measured within a 3-month follow-up, while late pregnancy outcomes and their offspring will be followed up via telephone for 2 years. The proportion of patients with improved symptoms will be calculated and compared.

Ethics and dissemination This study was approved by the Ethics Committee of Peking University Third Hospital (reference number: REC2020-165). Written informed consent will be provided for patients before being included. The results will be presented at academic conferences and a peer-reviewed journal.

Trial registration number ChiCTR2000035126.

Strengths and limitations of this study

- ⇒ This is a single-centre, longitudinal, prospective self-control study.
- ⇒ This study is well developed with follow-ups and procedures.
- ⇒ This study contains a small sample size and lacks a randomised control group.

INTRODUCTION

A favourable good pattern of endometrium is necessary for the establishment and maintenance of pregnancy in women. A thin endometrium is generally defined as an endometrium thickness less than 7 mm under ultrasound in the late follicular or luteal phase, and it is considerably less likely to conceive, even in assisted reproductive technologies and might cause cycle cancellation.¹ Adverse pregnancy outcomes resulting from thin endometrium have imposed a great burden on the family and society. Common causal factors of the occurrence of thin endometrium include repeated curettage, acute or chronic inflammatory diseases, secondary to Asherman syndrome (AS), oral contraceptives, clomiphene citrate and so on.^{2 3} Removal of some factors, such as oral contraceptives and clomiphene citrate, may ameliorate the reduction in endometrial thickness, while other factors, such as secondary to AS, may be difficult to reverse.^{2 3} Numerous studies have shown that alterations in endometrial thickness can affect endometrial receptivity to a certain extent and can be a predictor of adverse pregnancy outcomes in women of reproductive age.^{4 5} A study indicated that better pregnancy outcomes were prone to occur at

an endometrial thickness of 10 mm or more, while 41.7% pregnancy loss was found in women with endometrial thickness of 5 mm or less.⁶ A prospective observational cohort study also suggested that the probability of pregnancy and live birth in patients with thin endometrium was significantly reduced (15.2% vs 29.2%).⁷ Currently, exogenous oestrogen is commonly used for thin endometrium treatment in the clinical setting, but the clinical benefit rate is only 50%, and long-term unopposed exposure to large quantities of oestrogen is a well-defined risk factor for tumourigenesis.^{8,9} Despite a variety of methods explored to treat thin endometrium, including low-dose aspirin, granulocyte colony-stimulating factor and platelet-rich plasma,^{9–12} the effect remains controversial, and the outcomes are still far from ideal. As such, new and more efficient treatments are needed.

Recent studies on adipose-derived stromal vascular fraction (SVF) offered a potential therapeutic option for improving thin endometrium. In contrast to the methods mentioned above, SVF is easier and quicker to prepare and is not involved in the process of culturing *in vitro*.¹³ SVF suspensions derived from adipose tissue comprise a variety of cell populations, such as adipose-derived stem/stromal cells (ADSCs), endothelial cells, adipose cells and other cells.¹⁴ ADSCs within the SVF bear the characteristics of regeneration and migration, proliferation, differentiation and paracrine activity.¹⁵ Given its self-renewing and differentiation properties, the efficacy and safety of SVF in regenerative medicine have been widely explored. The application of SVF can facilitate the healing process by enhancing angiogenesis and matrix remodelling in numerous clinical situations, such as wound healing, hernia repair, diabetic foot and multiple sclerosis.^{16,17} These clinical applications of SVF have proven its relatively safety and effectiveness in humans. In regard to the treatment of uterine disorders, the possibility of SVF has also been estimated recently. SVF was transplanted into the uterine cavity to rodent models of AS, and remarkable increases in endometrial thickness and angiogenesis were observed.¹⁸ Six patients with severe AS were treated with autologous adipose tissue-derived SVF. After receiving such treatments, 40% menstrual recovery and a significant increase in endometrial thickness were observed ($p=0.043$).¹⁹ Moreover, the application of SVF for rodent models with thin endometrium showed a nonsignificant trend for improvement in endometrial area or gland density.²⁰ However, powerful evidence to demonstrate the effectiveness of SVF in treating patients with thin endometrium is still insufficient. Hence, this study was designed to assess the efficacy and safety of SVF in humans, which has never been reported before.

MATERIAL AND METHODS

Study design and setting

This study will be a single-centre, open, prospective self-control study with patients acting as their own controls. This study will be carried out at Peking University Third

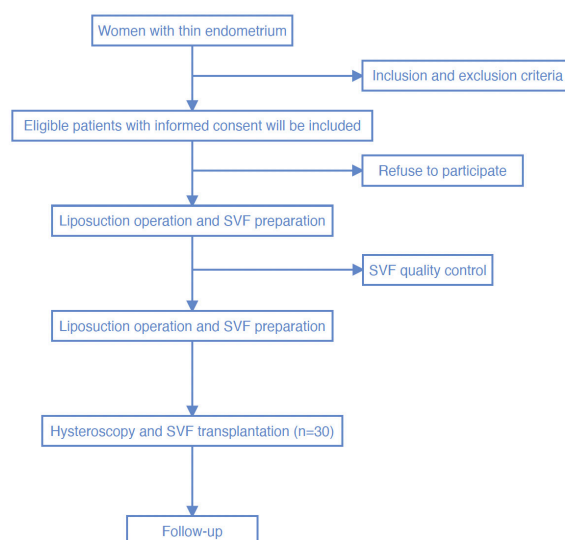


Figure 1 Flowchart of enrolment and recruitment. SVF, stromal vascular fraction.

Hospital from January 2022 to December 2024. The assumption of better uterine situation and pregnancy outcomes for application of autologous SVF in infertile patients with thin endometrium will be evaluated in this study. All included patients will be examined and followed up (figure 1). Before, during and after the operation, any adverse events, including local and systemic reactions, will be recorded. The outcomes, including the change in menstrual volume and endometrial thickness, will be measured, and the pregnancy outcomes and offspring will be followed up via telephone every 6 months for 2 years. If necessary, hysteroscopy will be rechecked.

Patients recruitment

This study was approved by the Ethics Committee of Peking University Third Hospital (reference number: 2020-165). Patients will be enrolled and recruited according to the inclusion and exclusion criteria, and informed consent will be signed from each participant. Patients relating to the following will be included: (1) infertile patients diagnosed with thin endometrium (less than 7 mm on the day of ovulation or on the day of endometrial transformation in hormone replacement cycles); (2) 20–45 years old; (3) body mass index with a range of 22–29 kg/m²; (4) patients with FSH < 15 IU/L or frozen embryos. The following patients will be excluded: (1) patients who were diagnosed with moderate or severe adhesion (patients with intrauterine adhesion can be included after correcting the morphology of the uterine cavity under hysteroscopy); (2) infection of the genitourinary system; (3) uncontrolled systematic diseases; (4) suspicious or underlying malignancy in both patients and their immediate family and (5) alcohol or drug abuse.

Patients and public involvement statement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Sample size estimation

An increase of 38% in the clinical pregnancy rate in the oestrogen treatment group for patients with thin endometrium was reported.⁹ Our pretest data reported a 70% effective rate of SVF treatment, stipulating $\alpha=0.025$, one sided and power=90%, and a total of N=24 patients was obtained. Given the missing data less than 20%, the final sample size required in this study was N=30. Statistically significant differences at $p<0.05$.

SVF preparation and surgery procedure

After liposuction operation, SVF will be prepared in a laboratory with good manufacturing practice-approved facilities immediately. As described in a previous study,¹³ adipose tissue was cut into pieces, and the same volume of 0.1% collagenase I was added to digest the adipose tissue. After 40 min, the digestion will be terminated, followed by isolation, filtration, centrifugation, resuspension and dilution to obtain an SVF suspension. The test items, including bacteria, fungi, viruses and endotoxin, will be tested, and the expression of SVF surface markers, including CD45, CD34, CD31, CD11b, CD44, CD73, CD105 and CD90, will be detected by flow cytometry to ensure the quality of the SVF suspension. Routine treatment, including hysteroscopic examination and endometrial biopsy, will be performed at Peking University Third Hospital to evaluate the morphology of the uterine cavity and the thickness of the endometrium. In addition to conventional therapies, SVF with a total amount of 10^7 – 10^8 cells from autologous fat tissue will be then transplanted into the uterine cavity with an embryo transfer catheter after all relevant examinations (PRODIMED, 1321600).

Outcome measurements

Primary outcome

Morphology and the thickest part in the longitudinal axis of the uterus (endometrial thickness) will be assessed via transvaginal ultrasound by the same physician at any of the follow-ups pretreatment or post-treatment in a blinded fashion. That is, the physician assigned to evaluate the endometrial thickness of the included patients will never be told about this project during this study. The primary outcome refers to the proportion of patients whose endometrial thickness increases to 7 mm. Efficacy rate (%) = The number of patients with endometrial thickness increases after treatment/total number of included patients $\times 100\%$.

Secondary outcomes

The menstrual status and preliminary pregnancy outcomes, including biochemical pregnancy rate and clinical pregnancy rate, will be followed up for 3 months after treatment, while ongoing pregnancy, miscarriage and live birth outcomes will be followed up via telephone for 2 years.

- Menstrual status will be assessed by calculating the proportion of patients whose volume or duration of

menses increase after treatment. Menstrual recovery rate (%) = The number of patients with menstrual recovery after treatment/total number of included patients $\times 100\%$.

- Biochemical pregnancy was defined as a positive pregnancy test (a level of serum human chorionic gonadotropin higher than 25 mIU/ml). Biochemical pregnancy rate (%) = The number of patients with biochemical pregnancy after treatment/total number of included patients $\times 100\%$.
- Clinical pregnancy: defined as the status with at least one viable gestational sac detected by ultrasound 28–30 days after embryo transfer. Clinical pregnancy rate (%) = The number of patients with clinical pregnancy after treatment/total number of included patients $\times 100\%$.
- Ongoing pregnancy was defined as at least one viable fetus detected at 12 weeks or more.
- Miscarriage: defined as a loss of pregnancy before 24 weeks gestation. Ongoing pregnancy rate (%) = The number of patients with ongoing pregnancy after treatment/total number of included patients $\times 100\%$.
- Live birth rate: defined as the delivery of at least one viable fetus after 24 weeks gestation. Live birth rate (%) = The number of patients with live births after treatment/total number of included patients $\times 100\%$.
- The frequency, date of onset, duration and severity of any local and systemic adverse reactions will be observed and recorded during the entire follow-up period. Incidence rate of adverse reactions (%) = The number of patients with adverse reactions after treatment/total number of included patients $\times 100\%$.

Follow-up strategy

To collect the outcomes, patients were required to return to the hospital for 6 visits within 3 months, and follow-up was continued via telephone for 2 years. The evaluation of endometrial thickness will be performed by physicians in a blinded fashion. The schedule of follow-ups is shown in table 1.

Adverse events monitoring

Adverse reactions, including rashes, bleeding, infection or any complications related to anaesthesia during or after liposuction, abdominal pain, allergy reactions or any other adverse events after SVF transplantation, uterine perforation, haemorrhage, adhesion or infection after hysteroscopy and any other emerging gynaecological symptoms, will be closely monitored and recorded in detail.

Data collection and management

Each selected participant will cooperate with the researchers to fill in the records, and then each participant will obtain a coded ID number. After the completed case records are reviewed by the clinical prosecutor, the data will be submitted to a data importer for data entry and management. During this period, all case records will

Table 1 Time schedule and assessment items of participant enrolment and follow-ups

Time	Items assessment
Pretreatment	
The first visit: the preceding 4 weeks	<ol style="list-style-type: none"> 1. Informed consent, basic information, medical history physical examination and routine preoperative investigations of each participant will be obtained. 2. The conditions of uterine cavity including endometrial thickness and endometrial pattern will be assessed via transvaginal ultrasound.
The day of treatment	
The second visit: the day of operation	Lower abdominal liposuction for preparation of SVF and hysteroscopy will be performed at first. Then, 2–3 mL SVF will be transplanted into uterine cavity and routine postoperative care will be received. Intrauterine fluid was aspirated 1 day after operation. All side effects or adverse events will be observed and recorded during this procedure.
Post-treatment	
The third visit: 7–10 days	<ol style="list-style-type: none"> 1. Basic information, medical history (ongoing health problems and drug history) and physical examination of each participant will be reviewed. 2. All side effects or adverse events will be recorded. 3. Endometrial thickness and morphology will be evaluated via transvaginal ultrasound. 4. 4 mL venous blood will be taken and C reactive protein, alanine aminotransferase and phosphocreatine kinase will be detected to confirm the presence of inflammatory condition.
The fourth visit and the fifth visit: 1 month \pm 3 days and 2 weeks after the second menstrual cycle (Both in follicular phase of menstrual cycle)	<ol style="list-style-type: none"> 1. Medical history (ongoing health problems and drug history) and physical examination of each participant will be reviewed. 2. All side effects or adverse events will be recorded. 3. The changes of the volume or duration of the menses will be recorded. 4. Endometrial thickness and morphology will be evaluated via transvaginal ultrasound. 5. Implantation and clinical pregnancy outcomes will be assessed and recorded if necessary.
The sixth visit: 1 week after the third menstrual cycle (Follicular phase)	<ol style="list-style-type: none"> 1. The evaluation items are the same as those in the previous two visits. If there are clinical indications in this visit, hysteroscopy will be performed again to observe the morphological changes of uterine cavity and endometrium, and all the imaging data will be retained for comparison with those before treatment.
Telephone follow-up: Every 6 months for 2 years (Follow-up phone call at 6 months, 1 year, one and half year, 2 years after treatment)	<ol style="list-style-type: none"> 1. Ongoing health problems and drug history will be reviewed. 2. All side effects or adverse events will be recorded. 3. The changes of the volume or duration of the menses will be recorded. 4. Pregnancy outcomes including implantation, clinical pregnancy, ongoing pregnancy, miscarriage and live birth will be followed up.
SVF, stromal vascular fraction.	

be stored safely, and all patient information will be kept confidential.

Statistics analysis

Relevant data will be collected and analysed for all patients receiving treatment. Quantitative data with a normal distribution are expressed as the mean and standard deviation (mean \pm SD), and data with a skewed distribution are expressed as the median and IQR. Student's t-test or the Mann-Whitney test was used for comparison of quantitative data. The qualitative data are expressed as the proportion (%), and the χ^2 test was used for comparisons between the groups. The difference was considered to be statistically significant at $p < 0.05$.

Ethics and dissemination

This study was approved by the Ethics Committee of Peking University Third Hospital (reference number: REC2020-165). Written informed consent will be signed in a specific and quiet room for included patients before

treatment. Before the recruitment of the first participant, any modifications of the protocol and informed consent needs to be approved by the ethics committee. The results will be presented at academic conferences and a peer-reviewed journal.

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Correction notice This article has been corrected since it first published. Author name 'Rong Li' has been updated.

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