Safety and efficacy of allogenic human amniotic epithelial cells transplantation via ovarian artery in patients with premature ovarian failure: a single-arm, phase 1 clinical trial

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Summary

Background Premature ovarian failure (POF) is a prevalent and severe condition that impairs female health but there is currently no effective treatment available to restore ovarian function. Human amniotic epithelial cells (hAECs) exhibit ovarian protection in pre-clinical models. Thus, we conducted a single-arm, phase 1 clinical trial to assess the safety and efficacy of allogenic hAECs in treating POF.

Methods A total of 35 patients received 6×10^7 hAECs via ovarian artery and completed a five-month follow-up from December 30, 2020 to January 31, 2022. The follow-up assessments were conducted at various intervals after hAECs treatment, including one month (Visit-1, V-1), three months (Visit-2, V-2), and five months (Visit-3, V-3) post-treatment. The primary endpoints were incidence of adverse events (AEs), and clinically significant laboratory abnormalities. Secondary endpoints included evaluation of transvaginal ultrasound results, sex hormone levels, Menopausal Quality of Life (MENQOL) questionnaire, as well as reproductive indicators. This trial was registered at www.clinicaltrials.gov as NCT02912104.

Findings No serious AEs were observed throughout the five-month follow-up period. The most common AE was hematoma (7/35, 20.00%), and other AEs include pelvic pain (4/35, 11.43%), fever (2/35, 5.71%), anaphylaxis (2/35, 5.71%), and hepatotoxicity (1/35, 2.86%). After hAECs transplantation (hAECT), significant improvements were observed in the levels of endometrial thickness, left ovarian volume, sex hormones (follicle-stimulating hormone (FSH) and estradiol (E2)), and MENQOL scores in all patients during the five-month follow-up period. Among them, 13 participants (37.14%) experienced spontaneous menstrual bleeding, and 20.00% (7/35) reported more than one regular menstrual bleeding post-hAECT. In this response group, significant improvements were observed in endometrial thickness, left ovarian volume, levels of FSH, E2, anti-Müllerian hormone (AMH), and MENQOL scores one month after hAECT in comparison to pre-hAECT.

Interpretation hAECT via ovarian artery is safe, well-tolerated and temporarily ameliorates endometrial thickness, ovarian size, hormone levels, and menopausal symptoms in POF patients. Further randomized controlled trial of hAECs with longer follow-up period and a larger sample size is warranted.

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Research in context

Evidence before this study

We searched PubMed for English-published clinical studies on cell-based therapies for premature ovarian insufficiency/failure (POI/POF) published up to March 1, 2023, using the search terms "(premature ovarian insufficiency/failure) AND (stem cell)", and "(premature ovarian insufficiency/failure) AND (mesenchymal stem cell)". The research revealed limited clinical reports on stem cell therapy for POI/POF. However, no clinical trials were found on using human amniotic epithelial cells (hAECs) in patients with POF.

Added value of this study

To our knowledge, this is the first clinical trial of human amniotic epithelial cells transplantation (hAECT) through the ovarian artery to treat POF patients. Our results demonstrated that hAECT is safe and well-tolerated in patients with POF, and it appears to temporarily improve endometrial thickness, ovarian size, hormone levels, and menopausal symptoms, indicating that hAECT may be a promising therapeutic option for POI/POF.

Implications of all the available evidence

Our study provide support for the safety and temporary efficacy of hAECs, which warrant further RCT trials of hAECs with longer follow-up period and a larger sample size.

Introduction

Premature ovarian insufficiency/failure (POI/POF) is a condition characterized by a decline in ovarian function and typically diagnosed in women under 40. It manifests as irregular menstruation or early menopause, with elevated serum follicle-stimulating hormone (FSH) and reduced estradiol (E2) levels. This condition affects approximately 3.5% of women globally.[1] Diagnostic criteria include: 1) Oligo/amenorrhea for at least four months; 2) Elevated FSH levels (>25 IU/L) on two occasions over four weeks apart.[2] When FSH levels exceed 40 IU/L on two occasions, it's classified as premature ovarian failure (POF), representing an advanced stage of POI progression.[3] POF brings infertility and endures menopausal symptoms alongside long-term adverse effects, such as cardiovascular disease, osteoporosis, and neurocognitive disorders, potentially leading to premature mortality.[4] Thus, POF signifies not only premature ovarian aging but also premature aging of multiple tissues and organs.

Hormone Replacement Therapy (HRT) is typically recommended to POF patients to alleviate menopausal symptoms resulting from estrogen deficiency, as well as to protect bone health and potentially prevent cardiovascular disease.[2] However, there is limited evidence regarding the optimal type, regimen, and dosage of HRT. Moreover, the side effects associated with HRT have restricted its application in POF patients. Studies have indicated that more than 52% of women with POF either never initiated HRT, commenced it many years after diagnosis, or discontinued it before reaching the age of 45.[5] Therefore, there is an urgent need for a novel therapeutic approach for POF patients.

The rapid advancements in stem cell research have provided a robust foundation for their application in regenerative medicine for damaged or diseased tissues. Stem cell therapy has recently transitioned from preclinical to early clinical trials for a range of incurable diseases.[6] Human amniotic epithelial cells (hAECs), which are abundant in discarded amniotic membrane, retain their stemness. Notably, hAECs exhibit no tumorigenic potential and low immunogenicity as they do not express major histocompatibility protein HLA-DR or co-stimulatory factors CD80, CD86, and CD40 and no acute rejection when immunologically unmatched human amniotic membranes were transplanted under the skin of volunteers.[7] Recent clinical trial involving hAECs has demonstrated that hAECs infusion was safe at low concentrations and low infusion rates.[8]

Our prior research has shown that hAECs significantly enhance ovarian function and promote fertility in chemotherapy-induced POF mice.[9–11] Implanted hAECs migrate to the injured ovary, inhibit the apoptosis of granulosa cells, promote angiogenesis, and improve the ovarian niche.[10,11] Consequently, we initiated this single-arm, phase 1 clinical trial to evaluate the safety and efficacy of allogenic hAECs transplantation (hAECT) via the ovarian artery in patients with POF. This study will likely contribute to assessing the benefit-risk ratio and designing randomized controlled trials (RCTs) in larger POF populations.

Methods

Participants

A single-center, prospective, single-arm clinical trial involving the transplantation of hAECs was conducted at

the International Peace Maternity and Child Health Hospital (IPMCH) in Shanghai, China. Eligible participants were aged between 18 and 45 and diagnosed with POF, defined as experiencing oligo/amenorrhea for a minimum of four months with an elevated FSH level exceeding 40 IU/L on two occasions separated by more than four weeks when they were younger than 40. These participants had not taken part in any other clinical trials for at least three months prior to enrollment. Exclusion criteria included chromosome abnormalities, coagulation abnormalities, abnormal liver function, and a history of severe allergies, cancer, endocrine disorders, thrombosis, or ovarian surgery. Pregnant or lactating women were also excluded.

The clinical trial was registered on ClinicalTrials.gov (NCT02912104). While it was initially registered in 2016, it was temporarily suspended due to a change in China's policy regarding stem cell clinical trials. Following the attainment of the necessary certification for stem cell clinical research institutions, the study was recommenced in 2020.

Ethics

The study protocol was approved by the Institutional Ethics Committee of the IPMCH (GKLW2015-26, GKLW2021-13). Written informed consent was obtained from all participants involved in the study.

Study design

This study represents an early exploratory clinical trial primarily aimed at evaluating the safety and preliminary effectiveness of hAECs in the treatment of POF. Currently, there is a limited number of clinical studies focusing on stem cell therapy for POI/POF, and there is a lack of relevant data regarding the recognized effect size. Based on previous clinical studies, the number of patients included ranges from nine to 61 (Table S5). Additionally, the clinical resources obtained, including research funding and the support of the research team, were taken into account. Therefore, we planned to include 30 participants, considering a dropout rate of about 15%. As a result, a sample size of 36 patients was enrolled in the present study.

All eligible patients were required to discontinue medication, especially HRT, for a minimum of one month prior to screening and transplantation. The study was designed using a before-and-after comparison approach, with participant information gathered during the screening period (V-0) serving as the baseline for analysis.

Good manufacturing practice (GMP) manufacturing and characterization of hAECs

The clinical-grade hAECs utilized in this study were manufactured and supplied by iCell Ltd., based in Shanghai, China, and possessed all necessary approvals from regulatory authorities. Detailed procedures and methods are provided in the Supplementary Materials and Methods section.

Intra-arterial catheterization

Using the Seldinger technique and local anesthesia, 4 F femoral artery sheaths were inserted into the right femoral arteries. Subsequently, the ovarian branches of the uterine arteries were superselectively cannulated using a microcatheter (Asahi Intecc Co, WNST150-18PWSFA) and a micro-guide wire (Asahi Intecc Co, STM180-18S). Once the ovarian artery branches and capillary networks were clearly visualized, stem cells were administered. 3×10^7 hAECs (4.5 mL) were slowly infused on each side through the microcatheters, followed by 0.5 mL of sterile saline. The dosage for injection was determined in accordance with our pre-clinical models.[9,11] All angiographic images of the procedures were retained. The femoral artery puncture site was dressed and immobilized for a period of 6 hours (h).

Patient follow-up

Participants were followed up at multiple time points after hAECT to ensure comprehensive data collection. These follow-up visits were scheduled at one month (Visit-1, V-1), three months (Visit-2, V-2), and five months (Visit-3, V-3) post-treatment.

For POF patients, the AMH concentrations is too low to be detected by current commercial assay kits. To solve this problem, we have developed an ultra-sensitive digital AMH detection assay (UD-AMH).[12] The limit of detection (LoD) and limit of quantitation (LoQ) of UD-AMH assay were 0.13 and 0.14 pg/mL, respectively. Employing a SiMoA platform (HD-X), UD-AMH provided systematic and automated analysis.

Further assessments, including blood routine examinations, coagulation, liver/kidney function, and hormonal levels (FSH, LH (luteinizing hormone), E2, T (testosterone), PRL (prolactin)), as well as electrocardiograph (ECG), lung CT scans, and physical exams, were conducted pre- and post-hAECT.

Ultrasound examinations gauged ovarian parameters and endometrial thickness. Follicles within 2–10 mm diameter were considered for AFC. Ovarian volume was calculated using the formula: ovarian volume = L (maximal length) *W (maximal width) *H (maximal height) * (π/6).[13]

Participants completed the Menopause-Specific Quality of Life Questionnaire (MENQOL, Chinese version)[14] surveys online or offline. This questionnaire, tailored for Chinese women, assessed symptoms across vasomotor, physical, psychosocial, and sexual domains. Scores ranged from one to eight, with higher values indicating more discomfort or lower quality of life. The mean score of special domain items was recorded.

To ensure participant well-being, attending gynecologists assessed whether patients should initiate HRT after hAECT. If HRT was administered, subsequent

follow-up data was collected, though statistics was not performed. Data from sex hormone levels and gynecological ultrasounds within five months post-hAECT were utilized for effectiveness analysis.

Outcomes

The primary objectives of this trial were to evaluate the safety and tolerability of ovarian intra-arterial infusion of hAECs. The occurrence of AEs, serious adverse events (SAEs), AEs of special interest (AESI, such as acute allergic reactions, or ectopic mass formation related to treatment), and events graded as Common Terminology Criteria for Adverse Events (CTCAE) $\geq$ III were used to assess the safety of hAECT via the ovarian artery. Clinically significant abnormalities associated with hAECT, including vital signs, physical examinations, blood routine tests, coagulation function, hepatorenal function, chest radiography, and ECG, were also considered.

To preliminarily explore the efficacy of hAECT via the ovarian artery, the following assessments were conducted: 1) Ultrasound measurements of endometrial thickness, ovarian volume, and total AFC were performed at one month (V-1), three months (V-2), and five months (V-3) after hAECT. 2) Fluctuations in hormone levels during the follow-up period, including FSH, LH, E2, T, PRL, and AMH, were tested at V-1 and V-3 after hAECT. 3) Improvement in menopausal symptoms was assessed at V-1, V-2, and V-3 post-hAECT using the MENQOL questionnaire. 4) Embryo number, implantation, pregnancy, and miscarriage during the follow-up period was also documented.

LASSO (Least Absolute Shrinkage and Selection Operator) regression model analysis

The dataset was randomly split (3:1 ratio) for modeling and validation. Relevant features for clinical prediction were selected from pre-hAECT data, covering demographic factors (e.g., age, BMI, age at menarche) and disease indicators (e.g., amenorrhea duration, E2, FSH, AMH, AFC). LASSO regression, known for automated feature selection, was employed. The model underwent ten-fold cross-validation, evaluating Binomial Deviance (classification) or minimum mean square error (regression) on the remaining sample. The hyperparameter λ, minimizing mean square error, was chosen. The area under the receiver operating characteristic curve (AUROC) quantified the classification performance. The contribution of each potential predictor in the prediction models was calculated by: Contribution = ABS (coefficient)/sum (ABS (coefficient)).

Statistics

Data collection was completed on February 10, 2023. IBM-SPSS (Version 26) for Windows (Chicago) was employed for all statistics. Population characteristics were expressed as median (range) and number (%). A combination of visual inspection and formal normality tests were used to verify the normality of data. Paired-samples t-tests were utilized for normally distributed continuous data to assess differences pre- and post-transplantation. Wilcoxon matched-pairs signed-rank tests were applied for non-normally distributed data, and McNemar's test was employed for comparing categorical data. When contrasting baseline information between the response and non-response groups, we utilized independent samples t-tests for normally distributed data with equal variances, otherwise we employed the Mann-Whitney test. Repeated Measures ANOVA and Friedman test were applied as additional analytical methods to account for the multiple repeated measures over time. If the data are normally distributed, Repeated Measures ANOVA method was used for analysis. When Mauchly's Test of Sphericity yielded a p value of less than 0.05, we selected the Greenhouse-Geisser or Huynh-Feldt method for correction based on the epsilon (ε) results. If the data did not meet the criteria for normal distribution, Friedman test was employed.

For all analyses, two-tailed p values were reported, with statistical significance set at $p < 0.05$.

Role of funding source

The funding sources had no influence on the study design, data collection, analysis, verification, and interpretation; the writing of the report; or the decision to submit the paper for publication.

Results

Patient characteristics

Between December 30, 2020 and January 31, 2022, a total of 40 patients were initially screened, of which 36 were ultimately enrolled in the trial. Four patients were excluded due to underlying conditions, including hypothyroidism, hypohepatia, and endometrial polyps. Consequently, a cohort of 36 POF patients successfully underwent hAECT. However, one patient was lost to follow-up following hAECT. She found it inconvenient to attend follow-up visits and refused to undergo further follow-up at local hospitals. This patient was subsequently excluded from the safety and efficacy analysis (Fig. 1). We adhered to the Consolidated Standards of Reporting Trials (CONSORT) statement for reporting clinical trials.

Table 1 displays the baseline demographic characteristics of the remaining 35 subjects. The mean age of the participants was 34.57, with mean amenorrhea duration of 4.71 years. Of the enrolled patients, 32 (91.43%) had been receiving continuous or intermittent HRT prior to screening, while three patients (8.57%) had not received HRT for at least one year. The participants exhibited heterogeneity in ovarian function, as indicated by varying levels of FSH (mean 72.90,

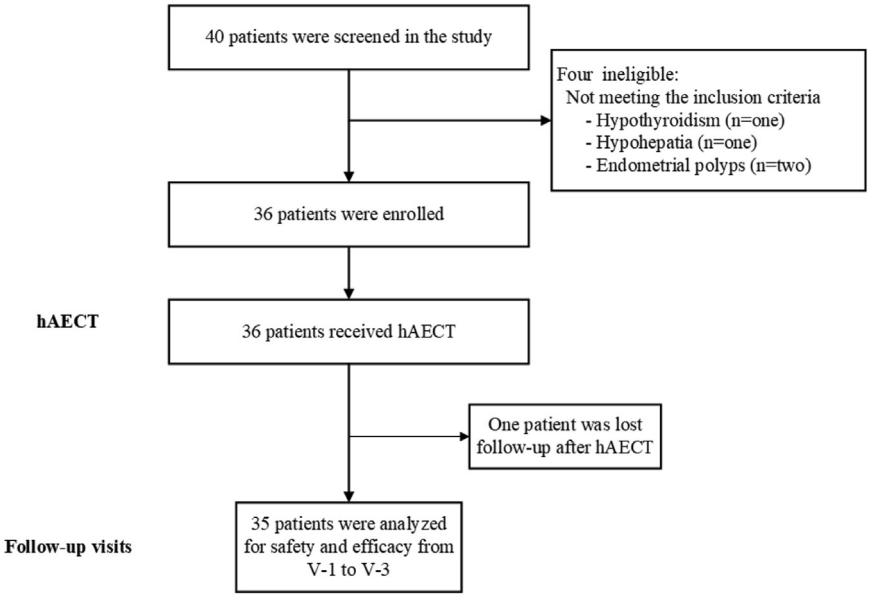

***Fig. 1:* Flow chart of the study**. A total of 40 individuals were screened. 35 patients were enrolled, received hAECT, and completed a five-month follow-up. These 35 patients were included in the final analysis. Note: V-1, one month post-hAECT; V-2, three months post-hAECT; V-3, five months post-hAECT.

STD 24.59 IU/L), E2 (mean 84.21, STD 123.81 pmol/L), AMH (mean 41.10, STD 64.28 pg/mL), and total antral follicle count (AFC) (ranging from 0 to 3) at baseline. Notably, 60.00% (21/35) of patients had non-detectable AFC.

Characteristic	Patients (n = 35)
	No. (%)
Age (years)	34.57 (5.70)
Amenorrhea duration (years)	4.71 (3.64)
Age of menarche (years)	12.57 (1.31)
BMI(kg/m^2)	21.56 (2.65)
Fertility history	
Gravidity (Mean)	1.09 (1.38)
Parity (Mean)	0.40 (0.65)
HRT before enrollment	
Yes	32 (91.43%)
No	3 (8.57%)
FSH (IU/L)	72.90 (24.59)
E2 (pmol/L)	84.21 (123.81)
AMH[a] (pg/mL)	41.10 (64.28)
≥30	13 (37.14%)
<30	22 (62.86%)
Total AFC	
≥1	14 (40.00%)
<1	21 (60.00%)

BMI, Body mass index. HRT, Hormone replacement therapy. AFC, Antral follicle count. Values are given as mean (STD) or as number (%). [a]Tested by self-developed ultrasensitive digital AMH assay (UD-AMH) (Detection of limit was 0.1 pg/mL).

***Table 1:* Characteristics of participants (n = 35).**

The hAECT procedure was successfully carried out in all enrolled participants, as confirmed by typical angiographic images shown in Fig. 2. On average, the duration of the operation was 53.4 min. A significant majority, 94.29% (33/35) of patients, were discharged from the hospital within 24 h post-hAECT. The remaining two patients experienced a delayed discharge, occurring one to two days later, primarily due to symptoms like fever or pelvic pain. In brief, prolonged hospital stay of one patient by 24 h was due to abdominal pain following hAECT. Relief of pain was observed 4–6 h after receiving a 100 mg indomethacin suppository. As a safety precaution, the patient was kept under observation for an additional 24 h. The second patient's hospital stay was prolonged by 48 h due to a postoperative body temperature of 38.1 °C, which returned to normal after 8 h without specific medication. For safety reasons, the patient was observed for 48 h. The patient's overall condition was good, with no reported discomfort, and was subsequently discharged.

Safety

The administration of hAECT was found to be remarkably safe, with no instances of serious adverse event (SAE) associated with the procedure. A total of 16 adverse events (AEs) were reported and are detailed in Table 2. Among the 35 participants, seven (20.00%) developed hematomas at the puncture sites following the transplantations. These hematomas, the most frequently occurring AE, emerged within three days

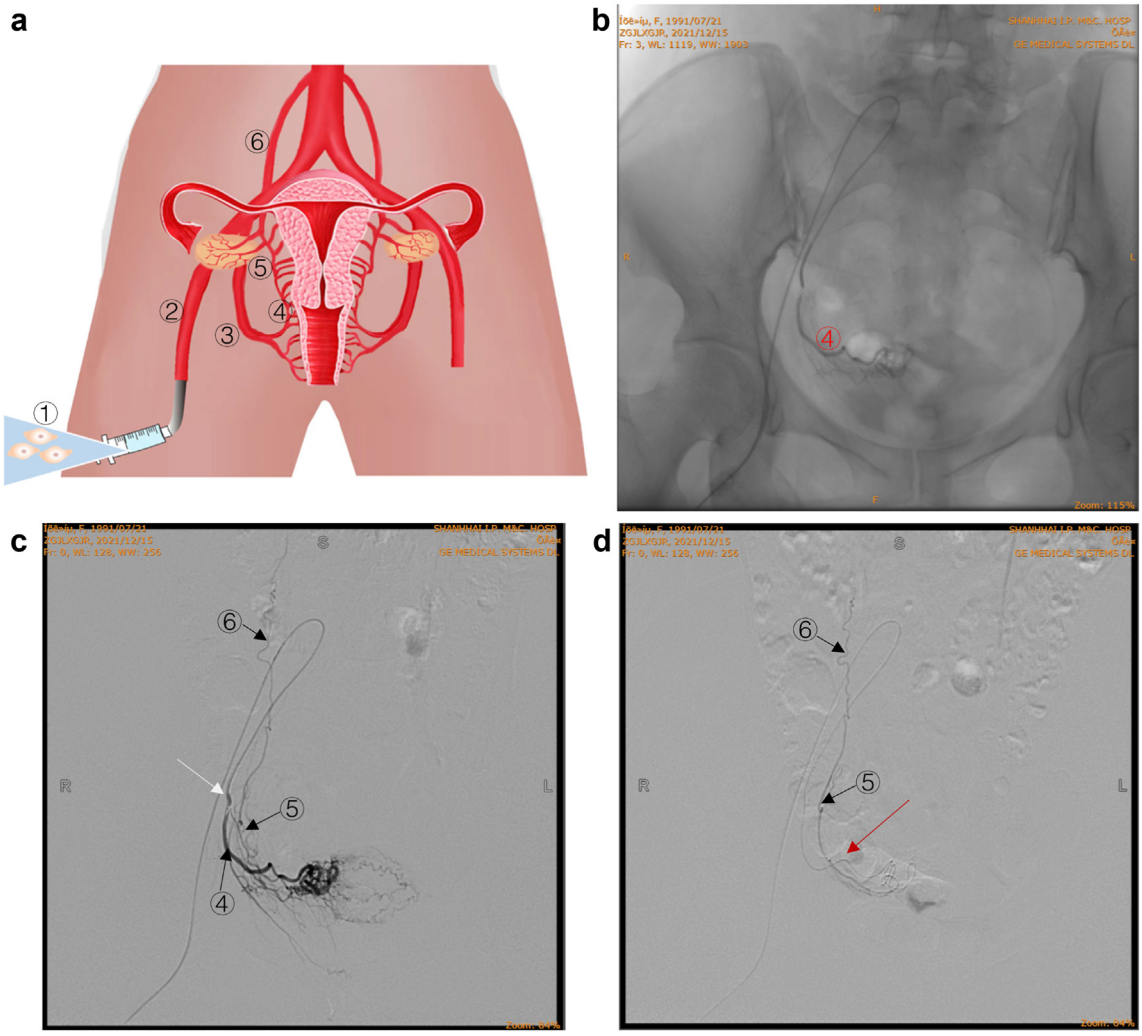

Fig. 2: **The precise infusion of hAECs through the ovarian artery. a.** Diagram illustrates the hAECs infusion procedure through the ovarian artery. It begins with an arterial puncture made in the right femoral artery. Subsequently, a catheter is introduced to access the uterine artery. The next step involves the superselective cannulation of the ovarian branch of the uterine artery, which is a process facilitated by contrast imaging. This is marked by the clear visualization of both the ovarian microvasculature and the ovarian artery branch stemming from the abdominal aorta. Finally, the hAECs are injected slowly along the microcatheter, ensuring precise delivery. **b–d.** Representative ovarian artery during angiography. The white arrow points the primary catheters and the red arrow points the microcatheter. The arterial branches supplying the ovary have been infused with hAECs through the microcatheters. (①hAECs; ②Femoral artery; ③Internal iliac artery; ④Uterine artery; ⑤Ovarian artery branch of uterine artery; ⑥Ovarian artery branch stemming from abdominal aorta).

post-hAECT and resolved spontaneously. Vascular complications at the puncture site are common after femoral artery puncture. Studies reported that the incidence of hematoma is usually not higher than 17% in angiography or endovascular interventional therapies.[15] In this study, it was observed that 20.00% of participants developed hematomas at the puncture site after hAECT. The occurrence of hematomas may be primarily associated with the puncture, and there is a possibility of underlying associations with cell therapy or the POF disease itself, which is worth to be further verified.

Systemic AEs accounted for nine out of the 16 reported AEs (56.25%). These included incidents of pelvic pain, fever, anaphylaxis, and hepatotoxicity. Intraoperative or postoperative pelvic pain was experienced by four subjects (11.43%), which subsided within 4–6 h, which may be associated with vasospasm or uterine contractions induced by the surgical procedure. Two participants (5.71%) had a mild episode of fever (Grade 1) that resolved within 4–8 h. Anaphylactic reactions were documented in two patients (5.71%) who exhibited a red rash with itching after treatment. They were promptly administered antihistamine medication

Adverse events (AEs)	Patients (n = 35)		Grade 1		Recovery time
	No.	(%)	No.	(%)	
Puncture side AEs					
Hematoma	7	20.00%	7	20.00%	3–7 days
Systemic AEs					
Pelvic pain	4	11.43%	4	11.43%	4–6 h
Fever	2	5.71%	2	5.71%	4–8 h
Anaphylaxis	2	5.71%	2	5.71%	7–10 days
Hepatotoxicity	1	2.86%	1	2.86%	a

Data are n (%). All adverse events were assessed according to CTCAE (Version 5.0). [a]One patient displayed an abnormal liver function indicators of alanine transaminase (ALT) and aspartate transaminase (AST) at V-1 without experiencing any discomfort. The values returned to normal after one month of follow-up examination.

Table 2: **Local and systemic adverse events (AEs) post-hAECT.**

and recovered within seven to ten days. Additionally, one patient displayed an abnormal liver function test after a one-month follow-up without experiencing any discomfort. Notably, all reported AEs occurred in close proximity to the time of cell infusion, which could not be ruled out as related to cell therapy. However, all of them were of Grade 1 severity, and were transient, leaving no lasting effects.

Furthermore, there were no notable abnormalities observed in various parameters before and after hAECT (Table S1). These encompassed blood routine parameters (including red blood cell count, hemoglobin, platelet count, white blood cell count, neutrophil count and ratio, lymphocyte count and ratio), liver function indicators (total bilirubin, direct bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)), kidney function metrics (urea and creatinine), coagulation markers (activated partial prothrombin time, thrombin time, prothrombin time, fibrinogen concentration, D-dimer), and thyroid function tests (free triiodothyronine, free thyroxine, thyroid stimulating hormone). Additionally, both lung CT scanning and ECG results showed no noteworthy abnormalities. Importantly, no participants experienced hAECT-related infections, embolism, or the development of ovarian cysts/tumors throughout the follow-up period.

Recognizing the importance of evaluating immune parameters in safety assessments, we conducted plasma immune proteomics testing before (V-0) and one month after (V-1) hAECT. Plasma proteomics were assessed using the Olink proteomics targeted 96 inflammation panel. This panel includes markers for T cell-mediated rejection (CD28, IL5, IFNLR1, IL12RB1),[16] antibody-mediated rejection (CLEC4D, IL6),[17] and chemokines/chemokine receptors (CCL11, CXCL12).[18] No significant differences in the concentrations of these factors in plasma were observed before and one month after hAECT (Table S6).

Efficacy

Follow-up assessments were conducted at various intervals after hAECs treatment, including one month (V-1), three months (V-2), and five months (V-3) post-treatment. Among the patients who underwent hAECT, 13 out of 35 (37.14%) experienced spontaneous menstrual bleeding at least once (Fig. S1a), and seven patients (20.00%) reported more than one regular menstrual bleeding post-hAECT (Fig. S1b). Notably, hAECT led to a significant improvement in the resumption of menstrual bleeding (p = 0.001) (Table S2a). In the five months prior to hAECT, the average number of spontaneous menstrual bleeding periods in the 35 patients exhibited a significant increase compared to the period following hAECT (p = 0.001) (Table S2b).

Observations revealed a significant increase in endometrial thickness at V-1 (p = 0.021) and V-3 (p = 0.014) following hAECT (Fig. 3a). Significant enlargement of the left ovary was observed in V-1 (p = 0.041) (Fig. 3b). However, right ovarian volumes and total AFC showed no significant changes compared to baseline from V-1 to V-3 for all subjects (Fig. 3c and d). The FSH concentration showed a declining trend, with a statistically significant difference observed at V-3 (p = 0.036) (Fig. 3e). The E2 concentration exhibited a significant increase after hAECT treatment at V-1 (p = 0.047) and V-3 (p = 0.016) (Fig. 3g). Levels of LH and AMH exhibited non-significant changes from baseline to five months post-hAECT in the 35 subjects (Fig. 3f and h). Evaluation of the impact of hAECT on menopausal symptoms showed significant decreases in total MENQOL scores at V-1 (p = 0.024, 95% CI, 7.57–10.42), V-2 (p = 0.006, 95% CI, 7.28–11.46), and V-3 (p = 0.016, 95% CI, 6.85–9.58) post-hAECT compared to baseline (95% CI, 8.74–13.48) (Fig. 3i). Additionally, lower psychosocial symptomatology was observed at one-month follow-up visit (V-1) (p = 0.012, 95% CI, 1.70–2.87) compared to baseline (95% CI, 1.83–3.44) (Fig. 3k). Physical symptoms were significantly alleviated at V-1 (p = 0.002, 95% CI, 1.45–2.27), V-2 (p = 0.002, 95% CI, 1.30–2.31), and V-3 (p = 0.026, 95% CI, 1.31–1.98) post-hAECT compared to pre-hAECT (95% CI, 1.63–2.82) (Fig. 3l). Significant improvements in sexual domains were observed at V-2 (p = 0.022, 95% CI, 1.90–3.97) and V-3 (p = 0.004, 95% CI, 1.47–3.32) compared to baseline (95% CI, 2.77–4.75) (Fig. 3m). The scores of vasomotor items showed a declining trend, but without statistically significant differences (Fig. 3j). To enhance the reliability and accuracy of the results, we investigated the utilization of alternative analytical methods to address the multiple repeated measures over time in the study (Table S7). Through these statistical approaches, the findings revealed significant improvements over time in levels of FSH and E2, endometrial thickness, as well as

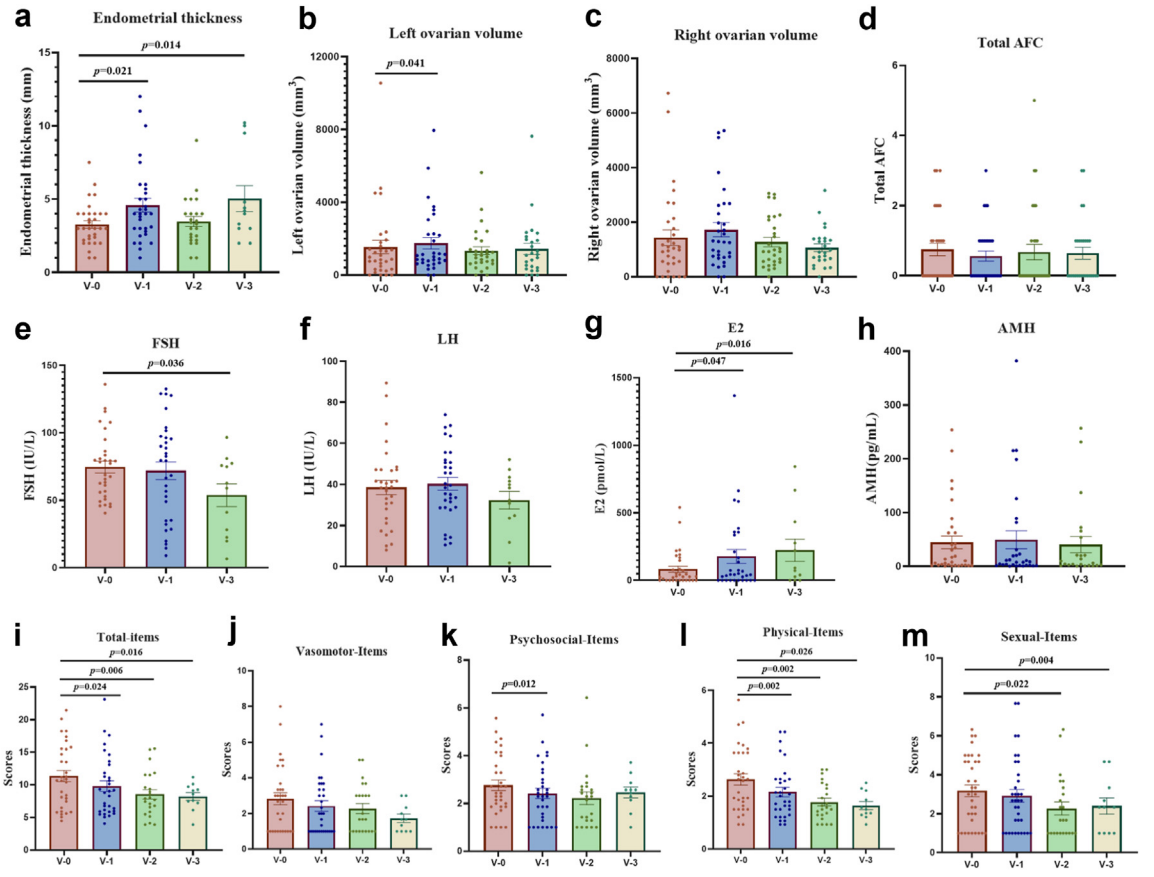

***Fig. 3*: Effect of hAECT in total POF patients at V-1, V-2, and V-3. a–d.** Changes of endometrial thickness, bilateral ovarian volume, and total AFC. **e–h.** Changes of sex hormone concentrations (FSH, LH, E2, and AMH). **I–m.** Changes of total MENQOL scores and four domains' scores including vasomotor, physical, psychosocial, and sexual items. Note: V-0 represents baseline pre-hAECT; V-1, V-2, and V-3 represent one month, three months, and five months post-hAECT. Data represent means ± SEM and was subjected to paired tests with the baseline.

MENQOL scores (total items, physical items, and sexual items).

Based on the recovery of menstrual bleeding, patients were categorized into a response group (n = 13, 37.14%) and a non-response group (n = 22, 62.86%). The response group exhibited thickened endometrial thickness, larger left ovarian volumes, lower level of FSH, higher level of E2 and AMH post-hAECT. Specifically, a significant thickening of the endometrium was observed at V-1 ($p = 0.003$), V-2 ($p = 0.010$), and V-3 ($p = 0.018$) compared to baseline in the response group (Fig. 4a). A larger volume of the left ovary was observed at V-1 post-hAECT ($p = 0.013$) (Fig. 4b), but no significant change was noted in the volume of the right ovary and total AFC between these two groups (Fig. 4c and d). The mean FSH level in V-1 decreased from 70.4 to 38.3 (IU/L) in the response group, and the difference was significant at V-1 ($p = 0.004$) and V-3 ($p = 0.007$) (Fig. 4e). Consistent with the thickened endometrial thickness in the response group, results also showed a significantly increased E2 levels at V-1 ($p = 0.011$) and V-3 ($p = 0.015$) (Fig. 4g). Additionally, a significant difference was observed in AMH levels at V-1 in the response group ($p = 0.047$) (Fig. 4h). In accordance with MENQOL scores of patients from the response group, a significant decrease in total items was observed at V-1 ($p = 0.021$, 95% CI, 7.81–10.91), V-2 ($p = 0.017$, 95% CI, 7.65–12.35), and V-3 ($p = 0.037$, 95% CI, 7.67–10.27) compared to baseline (95% CI, 9.71–14.78) (Fig. 4i) in the response group. There was a significant decrease in vasomotor symptoms of responders at V-1 ($p = 0.039$, 95% CI, 0.98–2.94) compared to baseline (95% CI, 1.78–3.89) (Fig. 4j). Psychosocial symptoms were not statistically significantly relieved (Fig. 4k). Scores of physical symptoms were significantly decreased in the response group at V-1 ($p = 0.0496$, 95% CI, 1.44–2.42) and V-2 ($p = 0.048$, 95% CI, 1.19–2.49) post-hAECT compared with pre-hAECT (95% CI, 1.53–3.11) (Fig. 4l). hAECT had a significant ameliorating effect on sexual dysfunction in the response group at V-2 ($p = 0.022$, 95% CI, 1.99–4.67 vs 3.06–5.44 baseline) and V-3 ($p = 0.003$, 95% CI, 1.56–3.94) (Fig. 4m). The levels

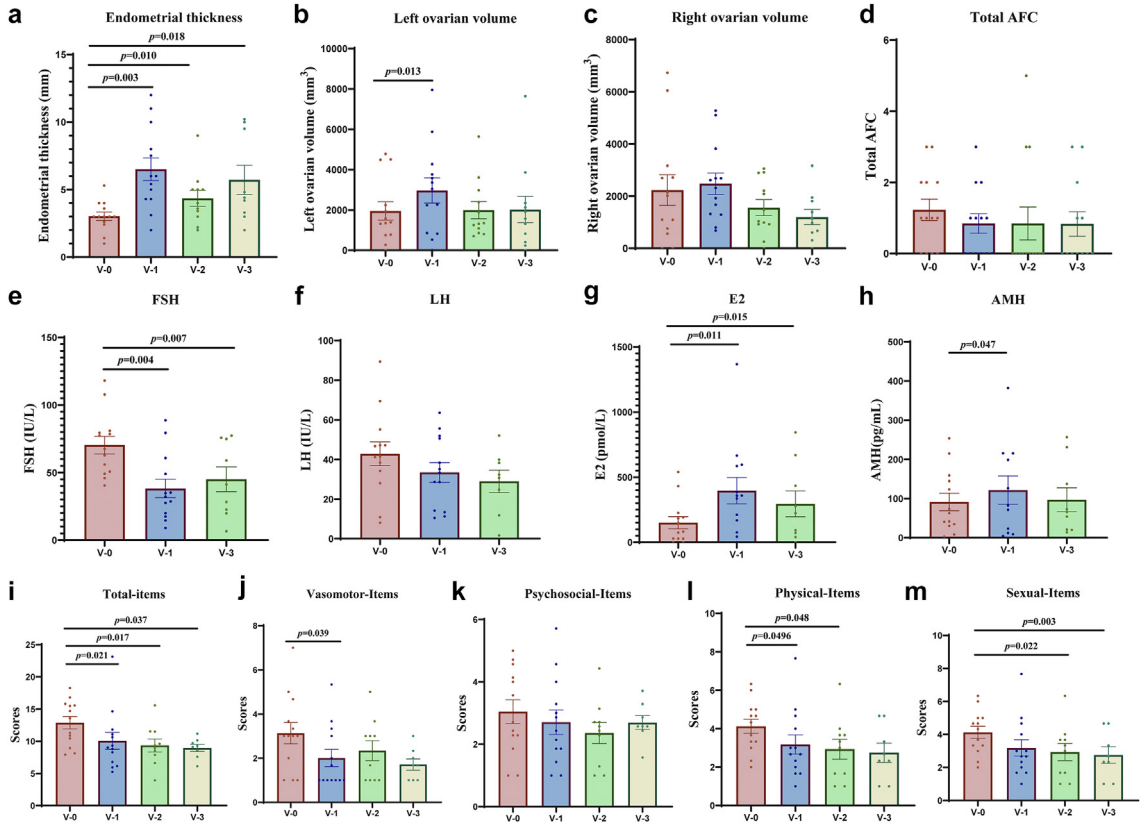

Fig. 4: **Changes in ovarian function after hAECT in response group from V-0 to V-3. a–d.** Changes of endometrial thickness, bilateral ovarian volume, and total AFC. **e–h.** Fluctuating levels of sex hormone (FSH, LH, E2, and AMH). **i–m.** Scores of total MENQOL and four domains (including vasomotor, physical, psychosocial and sexual items). Note: V-0 represents baseline pre-hAECT; V-1, V-2, and V-3 represent one month, three months, and five months post-hAECT. Data represent means ± SEM and was subjected to paired tests with the baseline.

of FSH and E2, endometrial thickness, and MENQOL scores (total items and sexual items) showed significant improvements over time in the response group (Table S8).

Six patients who had previously experienced failed IVF-ET procedures underwent oocyte retrieval after hAECT. Among them, one patient (16.67%) successfully retrieved two oocytes. This patient achieved natural menstruation, subsequently retrieved oocytes two months after hAECT, and she did not undergo any hormonal therapy or other drug treatments. Subsequently, two viable frozen embryos were obtained. As of now, these embryos have not yet been transplanted.

Factors affecting patients' response to hAECT

To elucidate the factors influencing the response to hAECT, we conducted an analysis of the baseline characteristics between the non-response group (n = 22) and the response group (n = 13). These two groups did not show any difference in age ($p = 0.229$) (Fig. 5a). However, compared to non-responders, those in the response group exhibited a shorter duration of amenorrhea ($p = 0.012$) (Fig. 5b). Additionally, patients in the response group had significantly higher baseline values for total AFC ($p = 0.026$) (Fig. 5c), E2 levels ($p = 0.020$) (Fig. 5e), and AMH levels ($p = 0.000$) (Fig. 5f) compared to those in the non-response group.

Furthermore, we employed a ten-fold cross-validated LASSO model to predict the therapeutic effect after hAECT. This approach involves adding L1 regularization to the loss function through penalized regression. In essence, the coefficients of unimportant features are shrunk to zero, while the coefficients of important features remain non-zero (Fig. 6a). We utilized the training set to fit a LASSO classification model with cross-validation errors (Fig. 6b). The optimal lambda values are indicated between the two vertical dashed lines. The left line represents the smallest mean square error, while the right line represents the smallest number of features. This model revealed that the duration of amenorrhea and the baseline level of AMH are pivotal in predicting the response to hAECT (Fig. 6c). The Area Under

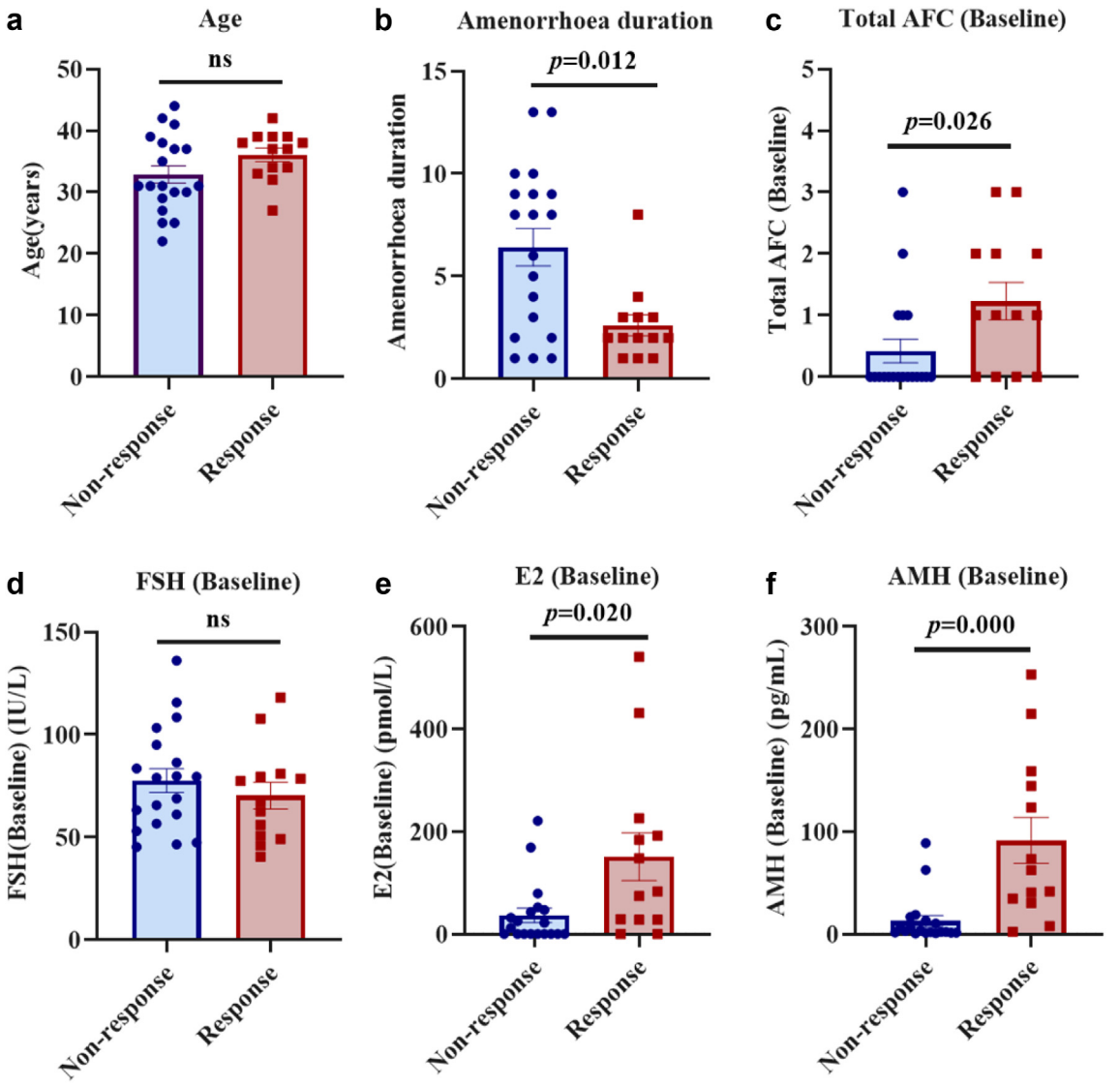

Fig. 5: **The differences of baseline clinical characteristics, including age, amenorrhea duration, total AFC, and levels of FSH, E2, AMH of patients of response group and non-response group**. **a.** There is no difference in age of two groups. **b.** Amenorrhea duration of response group is significantly shorter than that of non-response group. **c.** Number of total AFC of POF patients in response group is significantly higher than non-response group. **d.** There is no difference of baseline FSH levels in two groups. **e and f.** Baseline levels of E2 and AMH of response group are significantly higher than non-response group, respectively. Note: Non-response group, n = 22; response group, n = 13. Data represent means ± SEM; The FSH level was analyzed using independent samples t-tests, while other parameters were analyzed using the Mann–Whitney test.

the Receiver Operating Characteristics (AUROC) for the discovery set and the verification set were 0.9353 and 0.9333 (Fig. 6d) respectively, suggesting a relatively robust predictive capability. Combining all available data in this model, the AUROC was found to be 0.9406 (Fig. 6d), further emphasizing its strong predictive potential.

Discussion

In our previous work, we conducted preclinical studies involving the delivery of hAECs to treat mouse models of POF induced by chemotherapy or immune injury.[9–11] In this pilot clinical trial, we found that the application of clinical-grade hAECs is both safe and temporarily effective in patients with POF. Importantly, the effectiveness of the treatment appears to be linked to the underlying ovarian reserve of the patients, as indicated by their baseline AMH levels.

The primary objective of our trial was met, as we did not observe any SAEs or significant abnormalities in clinical or biochemical indicators related to heart, lung, coagulation, liver, and kidney function. Furthermore,

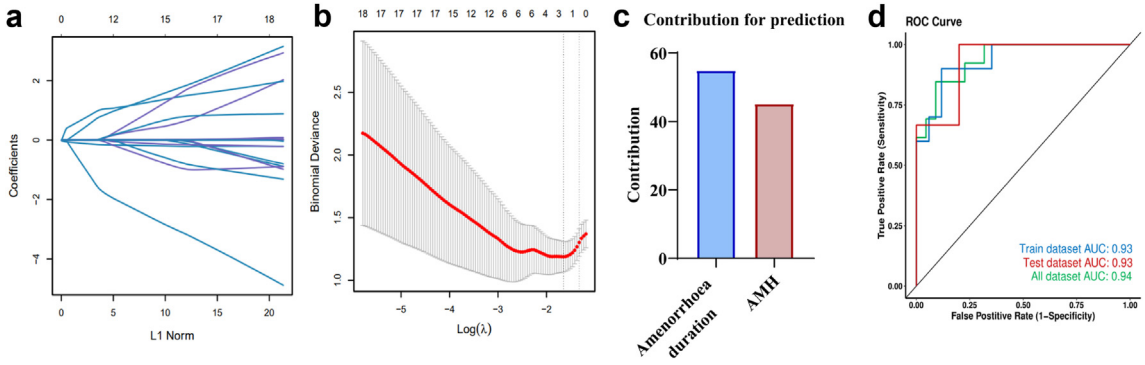

Fig. 6: **LASSO model constructed based on clinical information**. **a.** Coefficient path diagram of classification LASSO model. **b.** LASSO analyzes the cross–validation graph, and the dashed line on the left indicates that the log of the lambda's optimal value is approximately 2. **c.** Two important contributors to the classification model are amenorrhea duration and baseline level of AMH. **d.** AUROC in the training set, test set, and all dataset.

there were no instances of infection complications or tumor development observed either during the hAECT procedure or in the subsequent follow-up period. While some other AEs were reported, such as hematoma, pelvic pain, fever, anaphylaxis, and abnormal liver function tests after hAECT, and these events were all mild in nature, temporary, and ultimately resolved on their own.

We did observe significant improvements in the endometrial thickness, hormone levels, ovarian size, and menopausal symptoms of POF patients after hAECT (Fig. 3). And the changes are more pronounced in the response group (Fig. 4). According to the results, improvements associated with E2-related outcomes were more significant, such as E2, FSH, endometrial thickness, menopausal symptoms, and others. These improvements may be related to the mechanism involving hAECs homing and migration to the ovaries, and differentiation into granulosa cells, as well as the immunomodulatory function and growth factor secretion characteristics of hAECs.[9–11] As with any clinical trial with predefined strict inclusion and exclusion criteria, its generalisability has certain limitations. However, the participants in this study were drawn from patients in daily clinical practice and this inclusion cohort is representative.

This is the first study focusing on postmenopausal symptoms and quality of life after treatment for POI/POF, revealing that hAECT significantly improves patients' quality of life. POF patients often experience menopausal symptoms mainly due to low E2 levels, which can significantly impact their quality of life.[19] Furthermore, 13 patients (37.14%) were able to get off HRT for more than five months after hAECT, indicating that hAECT restored partial ovarian function and improved their quality of life.

Currently, it is widely accepted that the number of primordial follicles in females does not increase after birth and gradually decreases over time. The decline in ovarian function in females is a progressive process. However, POF is a devastating occurrence that results from a premature depletion of the ovarian pool of primordial follicles. AMH is commonly used as ovarian reserve markers for assessing ovarian function.[20] In the present study, 94.29% (33/35) of patients could not be detected using commercially available AMH tests (lower than 0.06 ng/mL), indicating a near depletion of ovarian reserve in these patients. The mechanism of stem cell therapy is to rescue the residual follicle in POF patients rather than inducing them to differentiate into oocytes in vivo. The lack of alterations in AMH levels in all patients before and after hAECT may be attributed to the limited number of residual follicles in patients, making them insufficient for restoration. Additionally, a singular stem cell transplantation might not be substantial enough to induce significant changes in AMH levels among them. Similar to other stem cell therapies,[6] the repeated hAECT is necessary to maintain long-lasting effects.

With the application of our self-developed UD-AMH,[12] all AMH levels pre- and post-hAECT could be accurately evaluated. The novel LASSO model integrates AMH levels detected by UD-AMH with the duration of patient amenorrhea and can be utilized to predict the responsiveness of patients to hAECT. This provides valuable insights for further clinical research in terms of precise evaluation of therapeutic effects.

POF patients frequently grapple with infertility issues attributed to aging ovaries. Prior studies have documented the positive impact of stem cell therapy on achieving successful natural pregnancies or improving outcomes in IVF procedures.[21,22] In our study, although six participants had previously attempted IVF-ET without success, we did not observe any spontaneous pregnancies. Only one patient successfully retrieved two oocytes, resulting in the acquisition of two viable frozen

embryos two months after hAECT. When compared to the notably low ovarian reserve with POF patients, those classified as poor responders exhibited a comparatively higher ovarian reserve.[21] This indicates that the baseline ovarian reserve plays a critical role in determining the effectiveness of stem cell therapy.

We have summarized previous completed clinical studies of stem cell therapy for POI/POF in Table S5. The primary methods for transplanting stem cells into the ovaries typically encompass in situ procedures performed via laparoscopic surgery or transvaginal ultrasound surgery.[22–24] Studies have demonstrated that intra-arterial delivery in cell-based therapy leads to higher and sustained cell presence at the target organ compared to intravenous infusion.[25] Herein, we provide an innovative technique for stem cell transplantation into the ovaries. This method bypasses the challenges posed by fibrotic, atrophic, and small ovaries in POF patients. The success of this approach is attributed to superselective cannulation of the ovarian branches from the uterine artery, enabling direct stem cell transplantation into the ovary. Supplementary Video S1 and S2 provides two representative records of the hAECT process in POF patients. We suggest that stem cell transplantation via the ovarian artery is a minimally invasive and easily replicable procedure.

POF is a refractory disease and HRT is strongly recommended to POF women to prevent the long-term adverse effects of estrogen deficiency. However, lifelong HRT carries risks such as increased risks of breast cancer,[26] ovarian cancer,[27] thromboembolic events,[28] and dementia.[29] In addition, HRT may lead to side effects and discomfort, including breast tenderness, headaches, nausea, and endometrial hyperplasia, which may persistently affect patients' quality of life. hAECT offers a novel and potentially safe alternative to lifelong HRT. Unlike HRT, which involves the administration of exogenous hormones, this approach aims to stimulate endogenous ovarian function by delivering hAECs. In this study, along with other clinical studies involving hAECs,[30] it has been preliminarily found that hAECT is safe and well-tolerable in patients. Given the risks and limitations associated with HRT, cell therapy may be a potential alternative therapy to help patients improve ovarian function, alleviate menopausal symptoms, and enhance quality of life. It is important to note that further research is needed to compare the efficacy and safety of hAECT with lifelong HRT.

The study still has some limitations. Conducted at a single center, the present study may introduce bias and limit the external validity of the results. The follow-up period in the study was limited to a specific duration, which may not capture the long-term effects of hAECT on reproductive outcomes. The study design involved a single-arm clinical trial without a control group, making it challenging to attribute observed outcomes solely to hAECT. Incorporating control groups, such as placebo or standard treatment arms, in future trials would allow for better comparison and interpretation of treatment effects. Larger-scale, well-controlled prospective clinical trials involving a more extensive patient cohort with extended follow-up periods are needed to confirm the efficacy and safety of hAECT for POF.

In conclusion, hAECT via the ovarian artery, a potential new treatment avenue for patients with ovarian functional decline, is shown to be safe, well-tolerated, and temporarily effective in treating POF patients. Predictors of hAECT outcomes include basic plasma AMH levels and the duration of amenorrhea. It warrants further investigation.

Contributors

Conception and design: DL, ZQ, and QZ. Data access and analysis: LW, LW, BW, and DL. Verification of underlying data: LW, LW, QZ, and BW. Data collection: All authors. Data interpretation: LW, LW, BW, and DL. Manuscript writing and review: All authors. Final approval of manuscript: All authors. All authors read and approved the final version of the manuscript and DL, ZQ, and BW had final responsibility for the decision to submit for publication.

Data sharing statement

The datasets generated and/or analyzed in the current study can be obtained from the corresponding author upon reasonable request.

Declaration of interests

All authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102744.

References

1 Li M, Zhu Y, Wei J, Chen L, Chen S, Lai D. The global prevalence of premature ovarian insufficiency: a systematic review and meta-analysis. *Climacteric*. 2023;26(2):95–102.
2 Webber L, Davies M, Anderson R, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. *Hum Reprod*. 2016;31(5):926–937.
3 De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. *Lancet*. 2010;376(9744):911.
4 Christ JP, Gunning MN, Palla G, et al. Estrogen deprivation and cardiovascular disease risk in primary ovarian insufficiency. *Fertil Steril*. 2018;109(4):594–600.e1.
5 Sullivan SD, Sarrel PM, Nelson LM. Hormone replacement therapy in young women with primary ovarian insufficiency and early menopause. *Fertil Steril*. 2016;106(7):1588–1599.
6 Hoang DM, Pham PT, Bach TQ, et al. Stem cell-based therapy for human diseases. *Signal Transduct Target Ther*. 2022;7(1):272.
7 Akle CA, Welsh KI, Adinolfi M, Leibowitz S, Mccoll I. Immunogenicity of human amniotic epithelial cells after transplantation into volunteers. *Lancet*. 1981;318(8254):1003–1005.
8 Lim R, Malhotra A, Tan J, et al. First-in-human administration of allogeneic amnion cells in premature infants with

bronchopulmonary dysplasia: a safety study. *Stem Cells Transl Med*. 2018;7(9):628–635.
9 Wang F, Wang L, Yao X, Lai D, Gu L. Human amniotic epithelial cells can differentiate into granulosa cells and restore folliculogenesis in a mouse model of chemotherapy-induced premature ovarian failure. *Stem Cell Res Ther*. 2013;4(5):124.
10 Zhang Q, Bu S, Sun J, et al. Paracrine effects of human amniotic epithelial cells protect against chemotherapy-induced ovarian damage. *Stem Cell Res Ther*. 2017;8(1):270.
11 Zhang Q, Huang Y, Sun J, Gu T, Shao X, Lai D. Immunomodulatory effect of human amniotic epithelial cells on restoration of ovarian function in mice with autoimmune ovarian disease. *Acta Biochim Biophys Sin*. 2019;51(8):845–855.
12 Kuang X, Wei L, Huang Y, et al. Development of a digital anti-Müllerian hormone immunoassay: ultrasensitive, accurate and practical strategy for reduced ovarian reserve monitoring and assessment. *Talanta*. 2023;253:123970.
13 Campbell S, Goessens L, Goswamy RK, Whitehead MI. Real-time ultrasonography for determination of ovarian morphology and volume. A possible early screening test for ovarian cancer? *Lancet*. 1982;319(8269):425–426.
14 Yang H, Chen F, Wang X, Wen Z, Zhang C. On clinical application and assessment of menopause-specific quality of life(Chinese Version). *Chin J Epidemiol*. 2005;26(1):47–50.
15 Casadaban LC, Mandell JC, Epelboym Y. Genicular artery embolization for osteoarthritis related knee pain: a systematic review and qualitative analysis of clinical outcomes. *Cardiovasc Intervent Radiol*. 2020;44(1):1–9.
16 Yu X, Carpenter P, Anasetti C. Advances in transplantation tolerance. *Lancet*. 2001;357(9272):1959–1963.
17 Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med*. 2018;379(12):1150–1160.
18 Nelson PJ, Krensky AM. Chemokines, chemokine receptors, and allograft rejection. *Immunity*. 2001;14(4):377–386.
19 Daly E, Gray A, Barlow D, Mcpherson K, Roche M, Vessey M. Measuring the impact of menopausal symptoms on quality of life. *BMJ*. 1993;307(6908):836–840.
20 Tal R, Seifer DB. Ovarian reserve testing: a user's guide. *Am J Obstet Gynecol*. 2017;217(2):129–140.
21 Herraiz S, Mónica R, Buigues A, et al. Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders. *Fertil Steril*. 2018;110(3):496–505.e1.
22 Ding L, Yan G, Wang B, et al. Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF patients with long history of infertility. *Sci China Life Sci*. 2018;61(12):1554–1565.
23 Mashayekhi M, Mirzadeh E, Chekini Z, et al. Evaluation of safety, feasibility and efficacy of intra-ovarian transplantation of autologous adipose derived mesenchymal stromal cells in idiopathic premature ovarian failure patients: non-randomized clinical trial, phase I, first in human. *J Ovarian Res*. 2021;14(1):5.
24 Yan L, Wu Y, Li L, et al. Clinical analysis of human umbilical cord mesenchymal stem cell allotransplantation in patients with premature ovarian insufficiency. *Cell Prolif*. 2020;53(12):e12938.
25 Cherkashova E, Namestnikova D, Leonov G, et al. Comparative study of the efficacy of intra-arterial and intravenous transplantation of human induced pluripotent stem cells-derived neural progenitor cells in experimental stroke. *PeerJ*. 2023;11:e16358.
26 Vinogradova Y, Coupland C, Hippisley-Cox J. Use of hormone replacement therapy and risk of breast cancer: nested case-control studies using the QResearch and CPRD databases. *BMJ*. 2020;371:m3873.
27 Greiser CM, Greiser EM, Dören M. Menopausal hormone therapy and risk of ovarian cancer: systematic review and meta-analysis. *Hum Reprod Update*. 2007;13(5):453–463.
28 Lekovic D, Miljic P, Dmitrovic A, Thachil J. How do you decide on hormone replacement therapy in women with risk of venous thromboembolism. *Blood Rev*. 2017;31(3):151–157.
29 Hershey L, Tarawneh R. Women need to be advised about the risks of long-term hormone replacement therapy. *Neurology*. 2022;99(17):733–734.
30 Zhang Q, Lai D. Application of human amniotic epithelial cells in regenerative medicine: a systematic review. *Stem Cell Res Ther*. 2020;11(1):439.