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Transplantation of UC-MSCs in ovary improves ovarian function and intermediate outcomes in IVF/ICSI cycles of POI patients: a clinical cohort study

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Abstract

Background This study aimed to evaluate the clinical outcomes of umbilical cord mesenchymal stem cell (UC-MSC) transplantation on ovarian function in women with premature ovarian insufficiency (POI), and its potential to improve in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) outcomes.

Methods A clinical cohort study was conducted at the Reproductive Medicine Center of Nanjing Drum Tower Hospital from 2019 to 2023. The study included POI patients who underwent either UC-MSC ovarian tissue injection or traditional IVF/ICSI-FET treatment. UC-MSCs were isolated from Wharton's jelly of the umbilical cord, cultured, and transplanted into the ovaries of patients via transvaginal ultrasound-guided injection. The embryo freezing rate, number of oocytes retrieved, and ovarian function indicators (e.g., serum FSH levels, antral follicle count (AFC)) were compared between the UC-MSC-treated group and the traditional IVF/ICSI-FET group.

Results A total of 71 POI patients were included, with 43 in the UC-MSC group and 28 in the IVF/ICSI-FET control group. After treatment, the AFC in the UC-MSC group was significantly higher compared to the control group (3.86 ± 2.93 vs. 2.39 ± 1.66, $p = 0.019$). Furthermore, the number of oocytes retrieved in the initial controlled ovarian hyperstimulation (COS) cycle following treatment was significantly greater in the UC-MSC group (1.88 ± 1.00 vs. 1.39 ± 1.13, $p = 0.034$). Patients undergoing UC-MSC treatment exhibited a substantial increase in the number of oocytes retrieved in their first COS cycle compared to their pre-treatment cycle (1.88 ± 1.00 vs. 1.28 ± 1.01, $p = 0.007$). Additionally, the number of frozen embryos significantly increased (0.74 ± 0.82 vs. 0.14 ± 0.41, $p < 0.001$), and the proportion of cycles resulting in frozen embryos was notably higher (55.81% vs. 11.63%, $p < 0.001$). Further Gene

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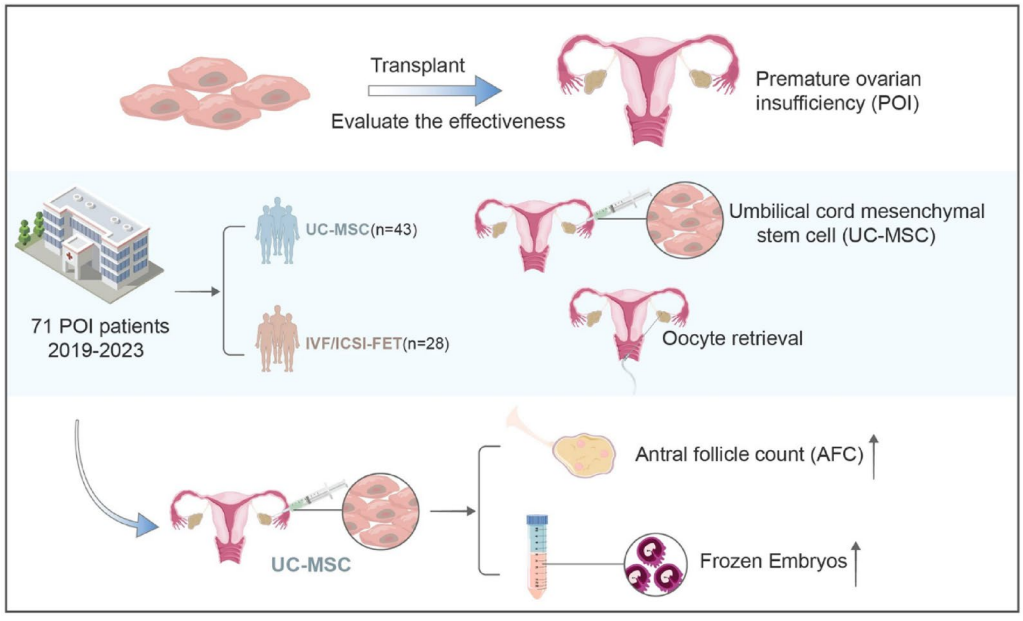
Set Enrichment Analysis (GSEA) revealed that genes expression in ovarian hormones and estrogen related signaling pathways were up-regulated following UC-MSCs therapy.

Conclusion UC-MSC ovarian tissue injection is a promising therapeutic strategy for improving clinical outcomes in IVF/ICSI cycles for women with POI. The use of UC-MSCs may offer a novel and effective alternative to conventional infertility treatments for POI patients, especially those seeking genetic offspring. Further studies are required to explore the long-term safety and efficacy of this treatment in larger, multi-center trials.

Keywords Premature ovarian insufficiency (POI), Umbilical cord mesenchymal stem cells (UC-MSCs), Ovarian transplantation, Stem cell therapy, Embryo freezing rate

Graphical abstract



Background

Premature Ovarian Insufficiency (POI) is characterized by the partial or complete cessation of normal ovarian function before the age of 40 [1, 2]. The classical triad of POI consists of oligomenorrhea or amenorrhea, elevated serum gonadotropin levels, and diminished serum estrogen (E_2) levels. A diagnosis of POI is typically established when oligomenorrhea or amenorrhea persists for more than 4 months, accompanied by serum follicle-stimulating hormone (FSH) levels exceeding 25 IU/L in at least two consecutive tests conducted over a 4-week period in women under 40 years of age [2, 3]. POI is frequently associated with infertility, predominantly due to premature follicular destruction and early ovarian follicle depletion. It affects approximately 1% of women of reproductive age [4], with only 5–10% achieving natural conception and successful live births [5].

The etiology of POI is multifactorial, encompassing chromosomal abnormalities [2, 6–8], infectious agents [9, 10], autoimmune disorders [11], iatrogenic factors [11, 12], lifestyle influences [11, 13–15], environmental exposures [16–18], and idiopathic origins. At present, egg donation or adoption is considered the most viable option for addressing infertility in POI patients; however, many women with POI still express a strong desire for genetic offspring. Traditional infertility treatments exhibit limited success, with the overall pregnancy rate for ovulation induction therapies around 6.3%. Furthermore, studies comparing gonadotropin-releasing hormone agonist (GnRH-a) suppression to placebo have not demonstrated any statistically significant differences in pregnancy rates [19–22]. A recent retrospective study found that in vitro fertilization (IVF) and frozen-thawed embryo transfer (FET) have yielded some success in POI patients, with cumulative clinical pregnancy and live birth rates comparable to age-matched controls [23]. However, these favorable outcomes are primarily seen in women with a sufficient follicular reserve [11]. Therefore, there is a pressing need to develop innovative infertility treatments for women with POI.

Stem cells (SCs) are undifferentiated or partially differentiated cells derived from both embryonic and adult tissues, known for their capacity for self-renewal and differentiation into various specialized cell types. SCs are

classified into three broad categories: embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs) [24]. Mesenchymal stem cells (MSCs) represent a distinct subgroup of ASCs, originating from multiple tissues, including bone marrow (BM), adipose tissue (AT), menstrual blood, umbilical cord (UC), amniotic fluid, amniotic membrane, placenta, and endometrium [25, 26]. UC-MSCs can be isolated from the entire umbilical cord or specific regions, such as Wharton's jelly, a gel-like connective tissue within the cord, from which Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) can be obtained. Like other MSCs, WJ-MSCs possess multipotent differentiation potential, self-renewal capacity, and the ability to differentiate into all three germ layers. Notably, WJ-MSCs proliferate more rapidly than other adult-derived MSCs, such as adipose-derived stem cells (ADSCs) and bone marrow-derived stem cells (BMSCs) and demonstrate superior expansion abilities [27, 28]. Our previous studies [29] transplanted collagen scaffolds seeded with hUC-MSCs into POI mice. By activating the phosphorylated FOXO3a and FOXO signaling pathways, we successfully induced the formation of mature follicles in the ovaries and successful pregnancies. Furthermore, other research indicates that hUC-MSCs regulate oxidative stress and autophagy through the AMPK/mTOR signaling pathway. This mechanism reduces cisplatin-induced oxidative stress, decreases AMPK activity, thereby suppressing autophagy and reducing follicular apoptosis [30]. Additionally, extracellular vesicles (EVs) derived from hUC-MSCs integrated into granulosa cells (GCs) of cisplatin-induced POI mouse ovaries, enhancing their resistance to cisplatin while providing protective effects on damaged ovarian GCs [31]. Ovarian injection of UC-MSCs has been found to facilitate more rapid functional recovery compared to intravenous administration [32]. Therefore, ovarian tissue injection of UC-MSCs may hold promise as a potential therapeutic strategy for POI; however, further investigation is required to draw definitive conclusions.

In this study, we analyzed the clinical data and outcomes of POI patients who have undergone UC-MSC treatment and traditional IVF-FET treatment at our center over the past four years (2019–2023). Our objective is to preliminarily assess the potential efficacy of UC-MSC ovarian tissue injection in improving IVF / Intracytoplasmic Sperm Injection (ICSI) outcomes in women with POI.

Methods

Design

This study presents a clinical cohort analysis conducted at the Reproductive Medicine Center, Affiliated Drum Tower Hospital, Medical School of Nanjing University. The aim was to assess the outcomes of umbilical mesenchymal stem cell (UC-MSC) ovarian injection transplantation in patients diagnosed with Premature Ovarian Insufficiency (POI), with a placebo treatment group serving as the control. The analysis was initiated in 2019. The inclusion criteria were as follows: patients aged under 40 years, with two consecutive follicle-stimulating hormone (FSH) measurements taken 4–6 weeks apart, ranging between 15 and 40 IU/L, and a history of at least one previous in vitro fertilization (IVF) / Intracytoplasmic Sperm Injection (ICSI) cycle in which no embryos were obtained for transfer. Exclusion criteria included: (1) chromosomal abnormalities; (2) endometriosis or adenomyosis; (3) ovarian borderline or malignant tumors; (4) developmental abnormalities of the reproductive system; (5) autoimmune disorders or other severe systemic conditions; (6) contraindications to hormone replacement therapy; (7) male partners with azoospermia or severe oligospermia; (8) participation in other experimental studies related to POI within the preceding year.

Surgical and transplantation procedure

The isolation, culture, identification, and pre-transplant cytological preparations of umbilical cord mesenchymal stem cells (UC-MSCs) were conducted in accordance with the Standard Operating Procedures (SOPs) established by the Stem Cell Center and the Reproductive Medicine Center of Nanjing Drum Tower Hospital. Comprehensive details of these procedures are outlined in our previously published work [29]. Following the isolation and cultivation of UC-MSCs, UC-MSCs (GMP grade, sourced from the Stem Cell Research Center of the Affiliated Drun Tower Hospital of Nanjing University Medical School, with authorization from the China Food and Drug Administration) were administrated via Transvaginal ultrasound (TVUS)-guided injection into the patient's ovaries. A volume of 800 μL containing UC-MSCs (5 × 10^6 cells in 400 μL per ovary) was prepared, immediately preserved on ice, and subsequently injected. Prior to injection, the surgical area was disinfected, and the transplantation procedure was performed by two senior physicians utilizing the SIEMENS ACUSON ANTANES advanced system (SIEMENS AG, Erlangen, Germany), equipped with a 6–10 MHz probe, for TVUS-guided surgery. A 21-G PTC needle (Hakko Medical, Japan) was employed for precise injection of the solution into the ovary under TVUS guidance. Each patient underwent a single transplant. The transplantation was scheduled 3–7 days post-menstruation, with routine blood and vaginal discharge examinations confirming the absence of any significant abnormalities.

Controlled ovarian stimulation (COS) protocol

All patients included in this study received the routine controlled ovarian stimulation protocol of our center [33, 34].

GnRH-antagonist protocol: 150–300 IU recombinant FSH (rFSH, Gonal-F, Merck Sereno) or 225–300 IU human menopausal gonadotropin (HMG, Menotropins for.

Injection, Livzon Pharm) was initiated on Day 2 or 3 of the menstrual cycle until trigger day. The dosage of rFSH or HMG was adjusted, and HMG or recombinant LH (rLH, Luveris, Merck Sereno) was added according to the ovarian response evaluated by trans-vaginal ultrasonically and serum hormone levels. 0.25 mg cetrorelix (Cetrotide, Merck Serono, France) was used daily when the leading follicles reached a mean diameter of 14 mm until trigger day.

Gentle Stimulation Protocol: Initiate oral administration of Clomiphene Citrate (CC, Fertilan, CODAL SYNTO LTD) at a dose of 25–100 mg, commencing on the second to third day of the menstrual cycle. HMG is subsequently introduced at a dosage of 75–150 IU, adjusted as necessary. The dosage of both medications is tailored according to ovarian response, with monitoring of serum hormone levels (estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P)), to guide further adjustments.

For both protocols, when two to three dominant follicles reached a diameter of 18 mm, a single dose of 250 μg recombinant human chorionic gonadotropin (rhCG, Ovitrelle, Merck Serono, France) was administered. Oocyte retrieval was performed under transvaginal ultrasound guidance 36 to 38 h after hCG administration. The retrieved oocytes were subsequently fertilized either through conventional IVF or ICSI. Normal fertilization was confirmed by the presence of two pronuclei or two polar bodies, typically observed 16 to 18 h post-insemination. Embryos were cultured in G1/G2 sequential media (Vitrolife, Goteborg, Sweden) at 37 °C in an incubator maintained with 6% CO_2, 5% O_2, and 89% N_2 under high humidity. Embryo morphology was assessed using the Gardner scoring system [35]. Frozen embryos were cryopreserved by vitrification and endometrial preparation was carried out by means of artificial cycles at appropriate timings.

Sample collection and Preparation

Granulosa cells were isolated from follicular fluid obtained from both POI patients and control subjects with normal ovarian function. All participants provided informed consent, and the study protocol was approved by the institutional review board. Follicular fluid was collected following standard clinical procedures during routine oocyte retrieval.

RNA extraction and sequencing

Total RNA was extracted from tissue samples, and its concentration and purity were assessed using a NanoDrop 2000 spectrophotometer. The integrity of the RNA was analyzed by agarose gel electrophoresis, and the RNA integrity number (RIN) was determined using an Agilent 2100 Bioanalyzer. RNA sequencing libraries were prepared using the Illumina TruSeq Stranded mRNA Library Prep Kit, according to the manufacturer's instructions. The libraries were sequenced on an Illumina NovaSeq 6000 platform, generating paired end reads of 150 base pairs in length. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1243731. The SRA records can be accessed via https://www.ncbi.nlm.nih.gov/sra/PRJNA1243731.

Statistical analysis

Most of the data in this study were directly extracted from the patients' medical record systems, while a minor portion was manually recorded by dedicated data entry personnel and subsequently verified by the responsible clinical physicians. The data collection process was standardized, ensuring the authenticity and reliability of the sources. The primary outcome of this study was the embryo freezing rate, while the secondary outcome was the number of oocytes retrieved. The study population was divided into two groups based on the utilization of UC-MSCs ovarian transplantation: Group A (control group, $n = 28$) and Group B (stem cell treatment group, $n = 43$). Data of Group A and B were respectively reclassified into two groups according to before or after treatment (Group A1/B1: before treatment and Group A2/B2: after treatment). Baseline data and IVF/ICSI cycle outcomes were analyzed both pre- and post-treatment for Group A. For Group B, baseline data and IVF/ICSI cycle outcomes were similarly compared before and after the stem cell ovarian transplantation procedure. The Kolmogorov-Smirnov test was employed to assess the normality of variable distributions. For normally distributed variables, independent t-tests were used, while for non-normally distributed variables, the Mann-Whitney U test was applied. Statistical analysis of categorical variables was performed using the chi-square test, assuming the following conditions: theoretical frequency (T) > 5 and sample size (n) > 40. Continuous variables are expressed as means ± standard deviations (SD). All statistical analyses were conducted using R software (http://www.R-project.org) and EmpowerStats software (www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). A p-value < 0.05 was considered statistically significant.

Ethics approval and consent to participate

This cohort study received ethical approval from the ethics committee of Nanjing Drum Tower Hospital. All methods were carried out in accordance with relevant guidelines and regulations.

Table 1 Comparison of POI patients before treatment in group A and B

Group	A	B	*P*-value
N	28	43	
Female age (years)	32.11 ± 4.10	32.70 ± 3.94	0.546
Male age (years)	32.82 ± 5.11	32.67 ± 4.66	0.901
Basal E_2 (pg/mL)	53.57 ± 68.07	44.08 ± 34.66	0.441
Basal FSH (IU/L)	12.38 ± 6.16	13.55 ± 5.83	0.421
Basal LH (IU/L)	5.99 ± 5.03	6.11 ± 3.36	0.899
Basal P (ng/mL)	0.37 ± 0.33	0.33 ± 0.31	0.694
AFC (n)	3.32 ± 1.81	4.05 ± 2.08	0.136
AMH (ng/mL)	0.48 ± 0.37	0.43 ± 0.38	0.669
Number of previous cycles (n)	0.68 ± 1.36	0.42 ± 1.13	0.429
Number of canceled cycles (n)	0.40 ± 0.71	0.28 ± 0.45	0.387
Number of cycles (n)	2.04 ± 1.10	2.05 ± 1.04	0.971
E_2 on hCG day (pg/mL)	734.70 ± 451.90	598.00 ± 346.30	0.164
Dominant follicle number on hCG day (n)	1.39 ± 0.74	1.67 ± 1.30	0.303
Number of retrieved oocytes (n)	1.36 ± 0.91	1.28 ± 1.01	0.742
Number of frozen embryos (n)	0.07 ± 0.26	0.14 ± 0.41	0.441
Embryo cryopreservation cycle rate	7.14% (2/28)	11.63% (5/43)	0.676

Table 2 Comparison of POI patients after treatment in group A and B

GROUP	A	B	*P*-value
N	28	43	
Basal E_2 (pg/mL)	36.57 ± 26.13	39.87 ± 27.77	0.622
Basal FSH (IU/L)	13.57 ± 6.13	13.81 ± 6.62	0.877
Basal LH (IU/L)	5.53 ± 2.65	6.52 ± 4.07	0.268
Basal P (ng/mL)	0.31 ± 0.38	0.21 ± 0.28	0.212
AFC (n)	2.39 ± 1.66	3.86 ± 2.93	0.019
E_2 on hCG day (pg/mL)	679.16 ± 418.84	717.37 ± 381.48	0.696
Dominant follicle number on hCG day (n)	1.57 ± 0.88	1.86 ± 1.04	0.228
Number of retrieved oocytes (n)	1.39 ± 1.13	1.88 ± 1.00	0.034
Number of frozen embryos (n)	0.50 ± 0.69	0.74 ± 0.82	0.197
Embryo cryopreservation cycle rate	39.29% (11/28)	55.81% (24/43)	0.381
Cumulative number of frozen embryos (n)	1.11 ± 1.47	1.30 ± 1.34	0.566
Cumulative embryo cryopreservation cycle rate	53.57% (15/28)	69.77% (30/43)	0.605

Results

Comparison of POI patients before treatment in group A and B

A total of 71 participants were included in the study, with 28 individuals in Group A and 43 in Group B. No adverse events occurred in all participants during our 1-year follow-up period. The demographic and hormonal characteristics of the participants are summarized in Table 1. The mean age of female and male participants in Group A was 32.11 ± 4.10 years and 32.82 ± 5.11 years, respectively, while in Group B, it was 32.70 ± 3.94 years and 32.67 ± 4.66 years, respectively, with no significant difference observed. Basal E_2 levels, FSH levels, LH levels, P levels, Anti-Müllerian hormone (AMH) levels, and antral follicle count (AFC) were similar in both Group A and Group B. Both groups showed a similar number of total cycles (2.04 ± 1.10 in Group A vs. 2.05 ± 1.04 in Group B; $p = 0.971$).

Estradiol levels on the day of hCG administration were higher in Group A (734.70 ± 451.90 pg/mL) compared to Group B (598.00 ± 346.30 pg/mL), but this difference was not statistically significant ($p = 0.164$). The dominant follicle count on hCG day, the number of retrieved oocytes, and the number of frozen embryos were similar between the groups. The embryo cryopreservation cycle rate was lower in Group A (7.14%, 2 out of 28 cycles) than in Group B (11.63%, 5 out of 43 cycles), with a p-value of 0.676, indicating no significant difference. Overall, no statistically significant differences were observed in the reproductive and hormonal parameters between Group A and Group B.

Comparison of POI patients after treatment in group A and B

No significant differences were observed in baseline hormone levels (such as E_2, FSH, LH, and P) between the two groups of participants after treatment (Table 2). However, group B exhibited a significantly higher AFC compared to group A (Table 2, 3.86 ± 2.93 vs. 2.39 ± 1.66, $p = 0.019$). Additionally, there were no significant differences in E_2 levels and the number of dominant follicles on the hCG day of the first controlled ovarian hyperstimulation cycle after treatment. However, group B had a higher number of retrieved oocytes compared to group A, showing statistical significance (Table 2, 1.88 ± 1.00 vs. 1.39 ± 1.13, $p = 0.034$). At the same time, group B performed better in terms of the number of frozen embryos (Table 2, 1.30 ± 1.34 vs. 1.11 ± 1.47, $p = 0.566$) and the rate of cycles with embryo freezing (Tables 2 and 69.77% vs. 53.57%, $p = 0.605$), although these indicators did not reach statistical significance.

Comparison of POI patients before and after treatment in group A

Furthermore, a comparison of baseline hormone levels between the two subgroups from Group A (A1: before treatment and A2: after treatment, Table 3) revealed no significant differences in the levels of baseline E_2, FSH, LH, and P. The AFC for Group A1 was 3.32 ± 1.81,

marginally higher than that of Group A2, which was 2.39 ± 1.66; however, this did not attain statistical significance ($p = 0.056$). The levels of E_2 and the number of dominant follicles on the day of hCG, as well as the final number of retrieved oocytes, also did not exhibit significant differences. Notably, significant differences were observed in the outcomes of embryo cryopreservation. The number of embryos cryopreserved in Group A1 was significantly lower than in Group A2 (0.07 ± 0.26 vs. 0.50 ± 0.69, $p = 0.004$). Moreover, the embryo cryopreservation cycle rate was 7.14% (2/28) in Group A1 and 39.28% (11/28) in Group A2, and this difference was also statistically significant ($p = 0.017$).

Comparison of POI patients before and after UC-MSCs treatment in group B

The comparison of baseline hormone levels between the two subgroups from Group B (B1: before treatment and B2: after treatment, Table 4) revealed no significant differences in the levels of E_2, FSH, LH, and P. The AFC in Group B1 was 4.05 ± 2.08, whereas in Group B2 it was 3.86 ± 2.93, with no significant difference ($p = 0.735$). The E_2 level on the day of hCG was higher in Group B2 than in Group B1 (717.37 ± 381.48 vs. 598.00 ± 346.30 pg/mL), but it did not reach statistical significance ($p = 0.135$). Furthermore, there was no significant difference in the number of dominant follicles on the day of hCG between the two subgroups. Significant differences were observed in the number of oocytes retrieved and the number of frozen embryos. The number of oocytes retrieved in Group B1 was 1.28 ± 1.01, significantly lower than that in Group B2, which was 1.88 ± 1.00 ($p = 0.007$). In terms of the number of embryos frozen, Group B1 was 0.14 ± 0.41, while Group B2 was 0.74 ± 0.82, with a statistically significant difference ($p < 0.001$). Additionally, the proportion of cycles with embryo freezing in Group B1 was 11.63% (5/43), compared to 55.81% (24/43) in Group B2, with a significant difference ($p < 0.001$).

Transcriptome high-throughput sequence of POI patients before and after UC-MSCs treatment

Our study utilized transcriptome high-throughput sequencing to investigate the molecular mechanisms underlying the therapeutic effects of UC-MSCs ovarian injection on POI. The analysis uncovered significant differences in gene expression profiles between granulosa cells from POI patients and those from a control group. A total of 10,861 differentially expressed genes were identified, with 6,399 upregulated and 4,462 downregulated (Fig. S1). Gene Ontology (GO) enrichment analysis indicated that upregulated genes in POI patients with UC-MSCs ovarian injection are primarily involved in proteasome-mediated, ubiquitin-dependent protein catabolic processes, regulation of intracellular transport, and regulation of protein stability (Fig. 1). Conversely, downregulated genes were predominantly associated with RNA methylation and DNA modification (Fig. 2). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis further indicated significant involvement of upregulated genes in pathways related to glutathione metabolism, autophagy, insulin resistance, MAPK signaling, and ErbB signaling pathways (Fig. S2). Further Gene Set Enrichment Analysis (GSEA) revealing pathways up-regulated following stem cell therapy were shown in Figure S3 and S4, such as ovarian hormones and estrogen related signaling pathways.

Table 3 Comparison of POI patients before and after treatment in group A

Group	A1	A2	*P*-value
N	28	28	
Basal E_2 (pg/mL)	53.57 ± 68.07	36.57 ± 26.13	0.23
Basal FSH (IU/L)	12.38 ± 6.16	13.57 ± 6.13	0.476
Basal LH (IU/L)	5.99 ± 5.03	5.53 ± 2.65	0.676
Basal P (ng/mL)	0.37 ± 0.33	0.31 ± 0.38	0.616
AFC (n)	3.32 ± 1.81	2.39 ± 1.66	0.056
E_2 on hCG day (pg/mL)	734.70 ± 451.90	679.16 ± 418.84	0.644
Dominant follicle number on hCG day (n)	1.39 ± 0.74	1.57 ± 0.88	0.414
Number of retrieved oocytes (n)	1.36 ± 0.91	1.39 ± 1.13	0.897
Number of frozen embryos (n)	0.07 ± 0.26	0.50 ± 0.69	0.004
Embryo cryopreservation cycle rate	7.14% (2/28)	39.28% (11/28)	0.017

Table 4 Comparison of POI patients before and after UC-MSCs treatment in group B

Group	B1	B2	*P*-value
N	43	43	
Basal E_2 (pg/mL)	44.08 ± 34.66	39.87 ± 27.77	0.535
Basal FSH (IU/L)	13.55 ± 5.83	13.81 ± 6.62	0.845
Basal LH (IU/L)	6.11 ± 3.36	6.52 ± 4.07	0.618
Basal P (ng/mL)	0.33 ± 0.31	0.21 ± 0.28	0.102
AFC (n)	4.05 ± 2.08	3.86 ± 2.93	0.735
E_2 on hCG day (pg/mL)	598.00 ± 346.30	717.37 ± 381.48	0.135
Dominant follicle number on hCG day (n)	1.67 ± 1.30	1.86 ± 1.04	0.466
Number of retrieved oocytes (n)	1.28 ± 1.01	1.88 ± 1.00	0.007
Number of frozen embryos (n)	0.14 ± 0.41	0.74 ± 0.82	< 0.001
Embryo cryopreservation cycle rate	11.63% (5/43)	55.81% (24/43)	< 0.001

Discussion

This study aimed to investigate the potential benefits of UC-MSC ovarian transplantation in enhancing clinical outcomes in IVF/ICSI cycles for patients with POI. The findings indicate that UC-MSCs therapy significantly

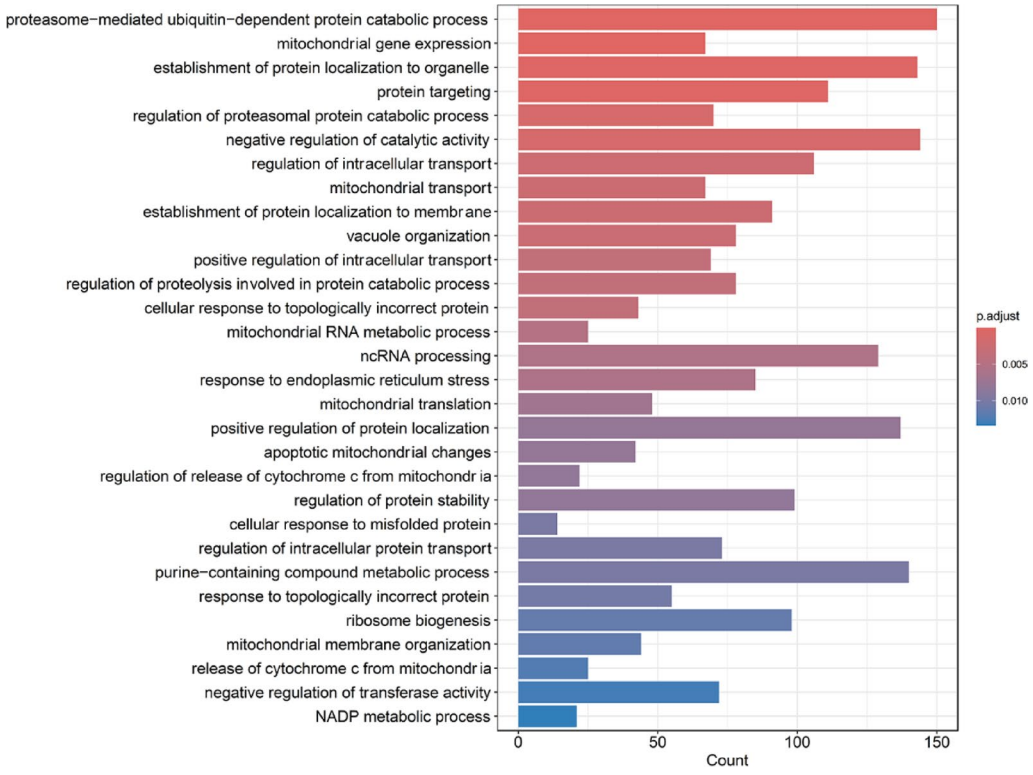


Fig. 1 Up-regulated gene expression in the treatment group of GO term Enrichment analysis

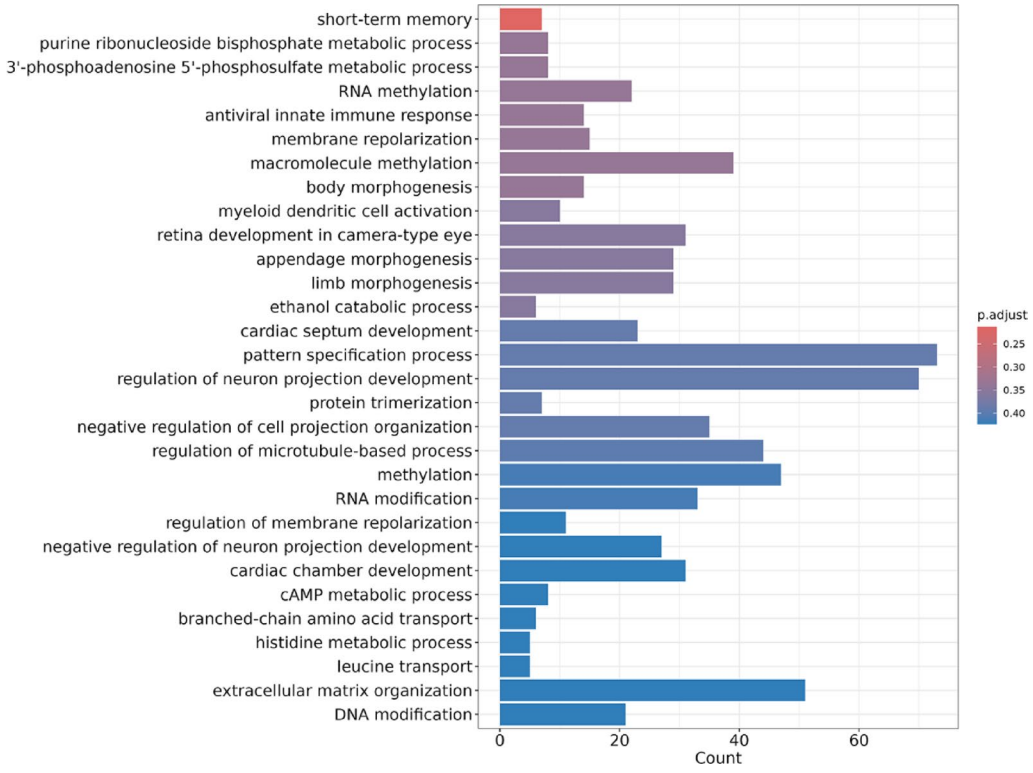


Fig. 2 Down-regulated gene expression in the treatment group of GO term Enrichment analysis

improves the embryo cryopreservation rate in POI patients, thereby increasing the efficiency of embryo accumulation across multiple IVF/ICSI cycles. This, in turn, may contribute to an enhanced cumulative pregnancy rate and a reduced time to achieve a singleton live birth.

Stem cells can be classified into two primary categories: ESCs and ASCs. ESCs exhibit pluripotency, which theoretically enables them to differentiate into a wide range of cell types within the body. However, their clinical application remains restricted due to several limitations, including their restricted availability, ethical concerns, tumorigenic potential, and the risk of immune rejection. In contrast, ASCs are undifferentiated cells found within differentiated tissues. Notable examples include MSCs, hematopoietic stem cells, neural stem cells, and epidermal stem cells [36]. ASCs are abundant, readily accessible, exhibit low tumorigenicity, and present minimal immunogenicity, making them particularly promising for clinical applications. Among these, MSCs derived from various tissues have garnered significant attention in recent research. Evidence indicates that transplanted MSCs can migrate to injured ovarian tissues, undergo direct differentiation, and secrete key cytokines via exosomes. These activities contribute to various therapeutic effects, including the promotion of cell proliferation, inhibition of apoptosis, anti-fibrotic and anti-inflammatory actions, immune modulation, antioxidant effects, angiogenesis, and regulation of autophagy. As such, MSCs show considerable potential as a therapeutic strategy for restoring ovarian function in patients with POI [37].

While autologous bone marrow mesenchymal stem cell (BMSC) transplantation has demonstrated partial efficacy in premature ovarian failure (POF) treatment, its clinical scalability remains constrained by donor site morbidity, quality variability, and immunogenic risks [38]. These limitations underscore the need for alternative stem cell sources with improved safety and accessibility profiles. UC-MSCs emerge as a compelling candidate due to their unique biological advantages: (1) non-invasive procurement from discarded umbilical tissue, (2) inherently low immunogenicity (characterized by low expression of major histocompatibility complex I (MHC I) and the absence of MHC II expression), and (3) documented tissue regenerative capacity without evidence of hemolysis, toxicity, or tumorigenesis [39–41]. Particularly noteworthy is their superior safety profile compared to BMSCs, as established in preclinical toxicology studies. Our selection of ovarian local transplantation as the delivery route is supported by comparative efficacy data showing significant functional restoration over systemic administration methods [42]. This approach aligns with the pathophysiological rationale of targeted niche repopulation in ovarian tissue. By leveraging UC-MSCs' trophic effects rather than direct differentiation, we hypothesize they may modulate the ovarian microenvironment to enhance follicular recruitment and maturation-a mechanism particularly relevant for improving IVF/ICSI outcomes in POI patients.

The emerging therapeutic potential of UC-MSCs for POI warrants nuanced interpretation. While current evidence demonstrates varied administration approaches (e.g., direct intra-ovarian injection vs. scaffold-assisted delivery) and source comparisons (umbilical artery-derived cells vs. WJ-MSCs), the field lacks consensus on optimal protocols. Notably, the YB1113 compound study highlights the importance of pharmacological characterization in UC-MSC derivatives. Clinically, the pregnancy outcomes (4/61 cases) after UC-MSC transplantation suggest two critical insights: First, the intervention appears most beneficial for patients with shorter amenorrhea duration, implying a potential therapeutic window. Second, the technical feasibility of ultrasound-guided ovarian injection was confirmed, though efficacy may depend on treatment frequency. These findings position UC-MSC therapy as a promising - yet still evolving - option that requires standardized delivery systems and patient stratification criteria in future trials [43]. Our study primarily investigates the frozen embryo cycle rate, with the ultimate goal of achieving a live birth. However, for POI patients, the timely generation of viable embryos is of equal importance. Given the progressive decline in ovarian reserve function, maximizing time efficiency is crucial. Once a sufficient number of transplantable embryos are obtained, subsequent evaluation of the uterine environment and maternal health is essential to optimize clinical pregnancy outcomes. Consequently, we did not include final clinical pregnancy outcomes as a primary endpoint in our analysis. Nevertheless, our findings suggest that UC-MSCs may offer significant therapeutic potential for POI patients.

At present, one may naturally inquire: How do UC-MSCs contribute to the improvement of POI? Existing research indicates that UC-MSCs may augment the functional reserve of aging ovaries via paracrine signaling mechanisms. Microvesicles and exosomes, which are secreted by stem cells, are present in their conditioned medium (CM). When UC-MSC-conditioned medium (UC-MSC-CM) is intraperitoneally administered to a cisplatin-induced POI mouse model, it has been observed that UC-MSC-CM can mitigate cisplatin-induced follicular depletion, reduce granulosa cell apoptosis, and preserve fertility. Moreover, it has been suggested that granulocyte colony-stimulating factor plays a pivotal role in protecting granulosa cells from apoptosis [44].

Existing studies have revealed the molecular blueprint of how UC-MSCs improve ovarian function through

multi-pathway synergy. Research by Yin et al. [45] and Lu et al. [30] jointly established a stress-response-autophagy regulatory axis: The HO-1/JNK/Bcl-2 pathway maintains immune microenvironment homeostasis by regulating CD8 + CD28-T cell circulation, while the AMPK/mTOR pathway coordinates oxidative stress and autophagy balance through metabolic reprogramming. This dual-pathway synergy may explain the FOXO3a-mediated primordial follicle activation phenomenon observed in our team's 2018 study [29], as a core transcription factor in stress responses, FOXO3a is likely a downstream integration node in these pathways. In vitro co-culture experiments have confirmed the critical role of the Hippo signaling pathway in granule cell function reconstruction [46], while in vivo transplantation studies highlight the systemic regulatory advantages of miR-17-5p delivered via exosomes [47]. These differences suggest that early intervention may rely more on intercellular contact-dependent paracrine mechanisms, whereas late-stage treatment should focus on systematic epigenetic regulation. Through comprehensive transcriptome high-throughput sequencing of granulosa cells, we have identified significant differential gene expression patterns between POI patients and control subjects with normal ovarian function, highlighting both upregulated and downregulated genes associated with distinct biological processes and pathways. The upregulation of genes involved in proteasome-mediated ubiquitin-dependent protein catabolic processes, intracellular transport regulation, and protein stability suggest that these processes may play critical roles in the cellular response to MSC therapy in POI patients. These pathways could be integral to improving ovarian function and may represent potential targets for enhancing the efficacy of MSC treatment. Conversely, the downregulation of genes related to RNA methylation and DNA modification highlights potential areas of concern, indicating that these processes might be disrupted in POI and warrant further investigation. These modifications are crucial for maintaining genomic stability and regulating gene expression, and their dysregulation could contribute to the pathophysiology of POI. KEGG pathway analysis provided further elucidation of the pathways affected by the upregulated genes, implicating glutathione metabolism, autophagy, insulin resistance, MAPK signaling, and ErbB signaling pathways. These pathways are known to be involved in cell survival, oxidative stress response, and metabolic regulation, all of which may be relevant to restoring ovarian function [48, 49]. Our transcriptomic analysis has identified three pivotal regulatory pathways: (1) The activation of the proteasome-ubiquitin system may improve ovarian microenvironment by eliminating misfolded proteins; (2) Abnormal RNA methylation patterns suggest promising potential for combined application of epigenetic modifiers; (3) The co-regulation between glutathione metabolism and the MAPK/ErbB signaling pathway provides a novel theoretical basis for developing targeted antioxidant-proliferative agents. Regarding transcriptomic sequencing data, further Gene Set Enrichment Analysis (GSEA) revealed pathways up-regulated following stem cell therapy (Figure S3&S4). Notably, ovarian hormone synthesis and estrogen signaling pathways may be closely associated with improved ovarian function pf POI patient after UC-MSCs treatment. To further advance this line of research, we intend to collect additional patient samples in future studies. By expanding the cohort size, we aim to conduct a more comprehensive analysis of alterations in the relevant signaling pathways. The integration of both cellular and animal models will facilitate a deeper understanding of the molecular mechanisms underlying the therapeutic effects of stem cell treatment on POI. Understanding these molecular mechanisms could facilitate the development of more effective, targeted interventions and advance personalized treatment strategies for POI.

This study has several limitations that warrant consideration. First, our study did not involve the clinical pregnancy outcomes (e.g., clinical pregnancy rate, miscarriage rate and live birth rate) of the two groups of patients after embryo transfer, but only the primary outcomes related to frozen embryos (number of frozen embryos and cycle rate with frozen embryos). Moreover, due to constraints within the data system, certain baseline and clinical outcome data were incomplete. Previous research indicates that multiple weekly tail vein injections of UC-MSCs result in superior ovarian function recovery in chemotherapy-induced POI mouse models compared to a single tail vein injection [50]. However, our study was confined to a single stem cell ovarian transplantation in POI patients, which may have influenced the final evaluation of therapeutic efficacy. Furthermore, in the process of our study grouping, the lack of strict randomization may introduce bias into the final results. The primary drawbacks of this study include its small sample size and non randomized controlled trial design. To further elucidate the role of UC-MSCs in improving clinical outcomes in IVF/ICSI cycles for POI patients, larger-scale, high-quality randomized controlled trials are essential. Future studies should aim to rigorously assess the efficacy of UC-MSC ovarian transplantation in POI patients through well-designed randomized controlled trials.

Conclusions

Our study finds that UC-MSCs therapy significantly improves the embryo cryopreservation rate in POI patients, thereby increasing the efficiency of embryo accumulation across multiple IVF/ICSI cycles, which may contribute to an enhanced cumulative pregnancy

rate and a reduced time to achieve a singleton live birth of POI patients.

Abbreviations

POI	Premature Ovarian Insufficiency
E_2	Estrogen
FSH	Follicle-stimulating hormone
GnRH-a	Gonadotropin-releasing hormone agonist
IVF	In vitro fertilization
FET	frozen-thawed embryo transfer
SC	Stem cell
ESCs	Embryonic stem cells, ASCs: adult stem cells, iPSCs: induced pluripotent stem cells
MSCs	Mesenchymal stem cells
BM	Bone marrow
AT	Adipose tissue
UC	Umbilical cord
WJ-MSCs	Wharton's jelly-derived mesenchymal stem cells
ICSI	Intracytoplasmic Sperm Injection
SOP	Standard Operating Procedure
TVUS	Transvaginal ultrasound
COS	Controlled ovarian stimulation
HMG	Human menopausal gonadotropin
CC	Clomiphene Citrate
LH	Luteinizing hormone
P	Progesterone
hCG	Human chorionic gonadotropin
RIN	RNA integrity number
SD	Standard deviations
AMH	Anti-Müllerian hormone
AFC	Antral follicle count
KEGG	Kyoto Encyclopedia of Genes and Genomes
POF	Premature ovarian failure
MHC	Major histocompatibility complex
CM	Conditioned medium
GCs	Granulosa cells
CTX	Cyclophosphamide

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

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The authors declare that they have not use AI-generated work in this manuscript.

Author contributions

C.Y.H., N.K. J.M. and H.X.S. contributed to study design, execution, acquisition, analysis and interpretation of data, manuscript drafting and critical discussion. P.P.X., H.Z., L.J.D., L.J.W., J.J.Z. and J.X. contributed to execution and acquisition of data. All authors read and approved the final manuscript.

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Data availability

The clinical datasets generated and analysed during the current study are not publicly available due to the special requirements of our hospital and our reproductive medicine center for the disclosure of patients' clinical data but are available from the corresponding author on reasonable request. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1243731. The SRA records can be accessed via https://www.ncbi.nlm.nih.gov/sra/PRJNA1243731.

Declarations

Ethics approval and consent to participate

Title of the approved project: Clinical Study on the Treatment of Premature Ovarian Insufficiency with Umbilical Cord Mesenchymal Stem Cells. Name of the institutional approval committee: the ethics committee of Nanjing Drum Tower Hospital. Approval number: SC2018-001-05. Date of approval: October 27, 2020. All methods followed the relevant guidelines and regulations, strictly adhering to the Declaration of Helsinki. The patients provided written informed consent for participation in the study and/or the use of samples.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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