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Comparison the therapeutic effects of bone marrow CD144$^+$ endothelial cells and CD146$^+$ mesenchymal stem cells in POF rats

Nahideh Nazdikbin Yamchi[1], Farhad Amjadi[1], Rahim Beheshti[2], Mehdi Hassanpour[1], Reza Shirazi[3], Amin Tamadon[4,5], Reza Rahbarghazi[6,7], Mahdi Mahdipour[1,8*]

[1]Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Faculty of Veterinary Medicine, Shabestar Islamic Azad University, Shabestar, Iran
[3]Department of Anatomy, School of Medical Sciences, Medicine & Health, UNSW Sydney, Sydney, Australia
[4]Percia Vista R&D Co. Shiraz, Iran
[5]Department for Scientific Work, West Kazakhstan Marat Ospanov Medical University, Aktobe 030012, Kazakhstan
[6]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[7]Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[8]Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

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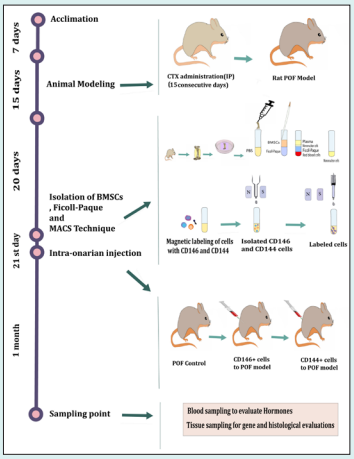
Abstract

Introduction: Premature ovarian insufficiency (POI) is a challenging issue in terms of reproduction biology. In this study, therapeutic properties of bone marrow CD146$^+$ mesenchymal stem cells (MSCs) and CD144$^+$ endothelial cells (ECs) were separately investigated in rats with POI.

Methods: POI rats were classified into control POI, POI + CD146$^+$ MSCs, and POI + CD144$^+$ ECs groups. Enriched CD146$^+$ MSCs and CD144$^+$ ECs were directly injected into ovarian tissue (15×10^4 cells/10 µL) in relevant groups. After 4 weeks, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) levels were measured in blood samples. Ovarian tissues were collected and subjected to Hematoxylin-Eosin and Masson's trichrome staining. The expression of *angp-2, vegfr-2, smad-2, -4, -6,* and *tgf-β1* was studied using qRT-PCR analysis. Histopathological examination indicated an increased pattern of atretic follicles in the POI group related to the control rats (P<0.0001).



Results: Data indicated that injection of POI + CD146$^+$ MSCs and CD144$^+$ ECs in POI rats reduced atretic follicles and increased the number of normal follicles (P<0.01). Along with these changes, the content of blue-colored collagen fibers was diminished after cell transplantation. Besides, cell transplantation in POI rats had the potential to reduce increased FSH, and LH levels (P<0.05). In contrast, E2 content was increased in POI + CD146$^+$ MSCs and POI + CD144$^+$ ECs groups compared to control POI rats, indicating restoration of follicular function. CD144$^+$ (*smad-2*, and *-4*) and CD146$^+$ (*smad-6*) cells altered the activity of genes belonging TGF-β signaling pathway. Unlike POI + CD146$^+$ MSCs, aberrant angiogenesis properties were significantly down-regulated in POI + CD144$^+$ ECs related to the control POI group (P<0.05).

Conclusion: The transplantation of bone marrow CD146$^+$ and CD144$^+$ cells can lead to the restoration of ovarian tissue function in POI rats via modulating different mechanisms associated with angiogenesis and fibrosis

Introduction

The term premature ovarian failure (POF) or premature ovarian insufficiency (POI) is associated with an ovarian dysfunction that coincides with amenorrhea and impaired fertility under the age of 40.[1] Recent statistics support the fact that the prevalence of POI varies due to ethnic differences.[2] In this scenario, the global prevalence of POI reaches about 3.7% of women worldwide[1] From the biochemical aspect, POI is characterized by elevation of follicle-stimulating hormone (FSH) (>40 mIU/mL) with

*Corresponding author: Mahdi Mahdipour Email: Mahdi.Mahdipour@gmail.com, Mahdipourm@tbzmed.ac.ir

an interval of more than 4 weeks, and reduction of anti-Mullerian hormone (AMH) (<1 ng/mL).[3] Along with these conditions, the reduction of estradiol (E_2) can clinically cause menstrual disorders, decreased bone density, and cardiovascular complications.[4] Various predisposing factors such as genetic factors, autoimmune disorders, chemotherapeutic agents, surgical manipulation, radiation, mitochondrial dysfunction, etc. have been proposed in terms of POI features.[5] Traditional treatment strategies include hormone replacement therapy, embryo and egg freezing techniques, and mitochondrial activation,[6-8] however, new emerging therapeutic protocols based on stem cells or cell byproducts have been proposed for POI patients.[1,9]

Mesenchymal stem cells (MSCs) exist in most connective tissues with high differentiation capacity, lower immunogenicity, and the ability to restore the function of injured tissues.[10-13] The regenerative potential of MSCs has been confirmed in the treatment of various deficiencies in several preclinical and clinical studies.[14] Emerging data suggest that MSCs are a suitable alternative therapy for ovarian regeneration and follicular growth in POI patients.[15] Several datasets have revealed that MSCs encompass a heterogeneous cell population with surface markers emphasizing their different biological properties, therefore, enrichment strategies based on their markers will be the future path for accurate stem cell treatment.[16] Among the surface markers of MSCs, CD146 MSCs exhibit significant therapeutic capacities such as proper paracrine, angiogenesis, and migration properties.[9,17] CD146 is touted as an early mesenchymal marker of MSCs in several tissues such as bone marrow, the dental pulp, uterine, adipose tissue, etc.[18]

To increase the therapeutic efficiency of MSCs, combined transplantation of MSCs with other lineages such as endothelial lineage can lead to promising outcomes.[19] Co-administration of MSCs with endothelial lineage can promote *de novo* vascularization and tissue restoration via engaging specific signaling pathways.[19,20] Endothelial cells (ECs) are key role cells in the promotion of angiogenesis during pathological and physiological conditions.[21] These cells can express CD144, known also as VE-Cadherin or Cadherin-5, which is essential for EC-to-EC adhesion and the formation of the endothelial layer. Various studies have shown that CD144 plays a key role in regulating EC permeability, migration, and accumulation within nascent blood vessels.[22]

However, there is a limited number of studies related to the application of varied cell types for the restoration of ovarian tissue in POI patients and animals. Which cell type and mechanism can restore the function of ovarian tissue in POI patients is at the center of attention in cell-based therapy approaches. Here, the regenerative potential of both $CD146^+$ MSCs and $CD144^+$ ECs were compared in a rat model of POI. Regarding the fact that the promotion of angiogenesis is an efficient strategy to reduce and/or inhibit progressive pathological changes in chronic diseases, thus the angiogenic properties of both $CD146^+$ MSCs and $CD144^+$ ECs were examined in POI rats.

Materials and Methods

Animals

In this study, female Wistar rats of 7-8 weeks old, weighing between 150 to 180 g were obtained from Med Zist Company, Tehran. Animals were kept in standard cages in a room with normal light conditions (12 hours of light and darkness), a temperature of (22 ± 2 ˚C) with *ad libitum* access to water and chewing pellets.

Induction of rat model for POI

Thirty rats were assigned randomly into two groups; Control and POI rats. To induce POI-like condition, rats were subjected to intraperitoneal (i.p.) injection of cyclophosphamide (CTX; Cat no: RHRI404, Supelco), 200 mg/kg in the first day and 8 mg/kg/day for the 2nd to 14th day.[23] In the control group, rats did not receive any injections.[23] To confirm the POI condition, rats of different groups (each in 3) were selected 20 days after the last day of CTX administration, and both serum and ovaries were sampled.[24] After POI confirmation, POI rats were randomly allocated into three groups; POI, POI + CD146, and POI + CD144.

Isolation of mononuclear cells (MNCs)

In this study, 10 rats were assigned as a source for MNCs. The isolation of marrow cells was performed according to the previously published protocol.[25] In short, rats were humanly sacrificed and femur bones were dissected and rinsed with sterile phosphate-buffered saline (PBS). After cutting the extremities, the medullary content was aspirated using a syringe and needle under sterile conditions.[26] Further, the Ficoll-Paque density gradient method (Sigma-Aldrich, GE17-1440-02) was implemented to enrich MNCs according to the protocol.[27]

Enrichment of $CD146^+$ MSCs and $CD144^+$ ECs

To purify $CD144^+$ or $CD146^+$ cells, the magnetic activated cell sorting method was used as previously described.[28,29] Freshly enriched MNCs were blocked in PBS containing 1% fetal bovine serum (FBS) for 20 minutes and incubated in solutions containing CD146 (Cat No: 130-093-596, Miltenyi Biotec) or CD144 (Cat No: 130-097-857, Miltenyi Biotec) microbeads at 4°C according to the manufacturer's instruction. Following several washes using PBS, samples were passed through the medium-sized LS columns and samples containing $CD146^+$ and $CD144^+$ cells were kept in separate tubes. Finally, the cells were counted using the Neubauer chamber and transferred to the animal lab for transplantation.

Cell transplantation

The next day after the last CTX injection, cell

transplantation was performed under deep anesthesia using ketamine (90 mg/kg) and xylazine (10 mg/kg). In brief, a 3-cm incision size was induced in the supra flank anatomical site, and about 15×10^4 cells/10 µL were injected directly into the right and left ovaries.[24,30] Four weeks post-transplantation, rats were euthanized and ovaries were sampled for different analyses.

Histopathological evaluation

To investigate the histopathological changes after cell transplantation, left ovarian tissues were rinsed in 10% formalin (Merck). After dehydration in ascending alcohol series, 5 µm slices were prepared using a microtome instrument (Leica). The structure and average healthy follicle number and corpus luteum (CL) were calculated at different developmental stages using H & E staining.[31] The fibrosis rate was monitored using Masson's trichrome staining under the Olympus BX-51 light microscope.[32]

Hormonal analysis

Serum levels of FSH, luteinizing hormone (LH), and E2 were detected in blood samples taken from the hearts of rats under deep anesthesia according to our previous work.[33] Serum samples were then collected after centrifugation at 400*g* for 20 minutes. Enzyme-linked immunosorbent assay (ELISA) was used for measuring FSH (334-096-4, Monobind), LH (0234-96, Monobind), and E2 (4925-300A, Monobind) levels.

qRT-PCR analysis

The expression of angiopoietin 2 (*Angpt2*), *KDR (vegfr-2),* and *Smad2*, *4*, *6,* and *Tgf-β1* genes were measured to assess angiogenesis response and fibrosis rate.[34,35] To this end, the right ovaries were subjected to total RNA isolation using Trizol Reagent (MaxZol Cat No: 0000124). Subsequently, defined concentrations of RNA were reverse-transcribed using a cDNA synthesis kit (Cat No: YT4500, Yekta Tajhiz Azma). Primers targeting target genes were designed using Primer-Blast online software (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 1). qRT-PCR reactions were performed using cDNA and SYBR Green 2X (Cat No: YT2551, Yekta Tajhiz Azma) and Roche Light Cycler 96 system in three stages of denaturation, annealing, and expansion for 15 seconds at 95, 60, and 72 °C, respectively, for 45 cycles. The expression rate was calculated using the $2^{-\Delta\Delta CT}$ method related to internal β-actin. Finally, the specificity of PCR reactions was evaluated by melting curves.

Statistical analysis

All results are shown as mean ± standard deviation. The results were analyzed using one-way analysis of variance (ANOVA) with LSD as a post hoc test (Fisher's least significance difference) using GraphPad Prism 8. Statistical significance was set at $P<0.05$.

Results

POI rat model

Twenty days after the last dose of CTX administration, sampling was performed. Histopathological evaluations revealed the general follicular atresia at all stages of follicular growth ($P<0.0001$), including primordial ($P<0.001$), primary ($P<0.0001$), secondary ($P<0.001$), and antral follicles ($P<0.0001$) (Fig. 1A), whereas healthy follicles were significantly reduced at different development stages ($P<0.01$) (Fig. 1B). Our findings confirmed that CTX administration induced pathological changes and atretic signs at all stages of follicular development (Fig. 1C) coincided with tissue shrinkage, granulosa cells disintegration, disassociation of follicles, and deposition of collagen fibers indicated by Masson's trichrome staining (Fig. 1D). Data revealed a significant reduction of the CL population in the CTX group when compared to the control rats ($P<0.01$) (Fig. 1E).

Table 1. List of primers used for qRT-PCR

Genes	Sequence (5'→3')	Annealing temperature (°C)	Product length
Rn-ANGPT2 NCBI # NM_134454.1	F: GCAGCGTTGACTTCCAGAGA R: ATACAGAGAGTGTGCCTCGC	60	199
Rn-KDR (VEGFR2) NCBI # NM_013062.1	F: AGATGCGGGAAACTACACGG R: GGGAGGGTTGGCATAGACTG	60	184
Rn-Smad2 NCBI # NM_001277450.1	F: TCCATCGAACTCGGAGAGGT R: ATACAAGCGCACTCCCCTTC	60	106
Rn-Smad4 NCBI # NM_019275.3	F: CCACCAAGTAATCGCGCATC R: AAGCCACAGGAATGTTGGGG	60	109
Rn-Smad6 NCBI # NM_001109002.2	F: CGCCTCTATGCGGTGTATGA R: AGCAGGATGCCAAAACCGAT	60	146
Rn-TGF-β1 NCBI # NM_021578.2	F: TCCATGACATGAACCGACCC R: TGCCGTACACAGCAGTTCTT	60	142
Rn-β-actin NCBI # NM_031144.3	F: TGACAGGATGCAGAAGGAGA R: TAGAGCCACCAATCCACACA	60	104

Abbreviations: NCBI: National Center for Biotechnology Information; F: Forward strand; R: Reverse strand.

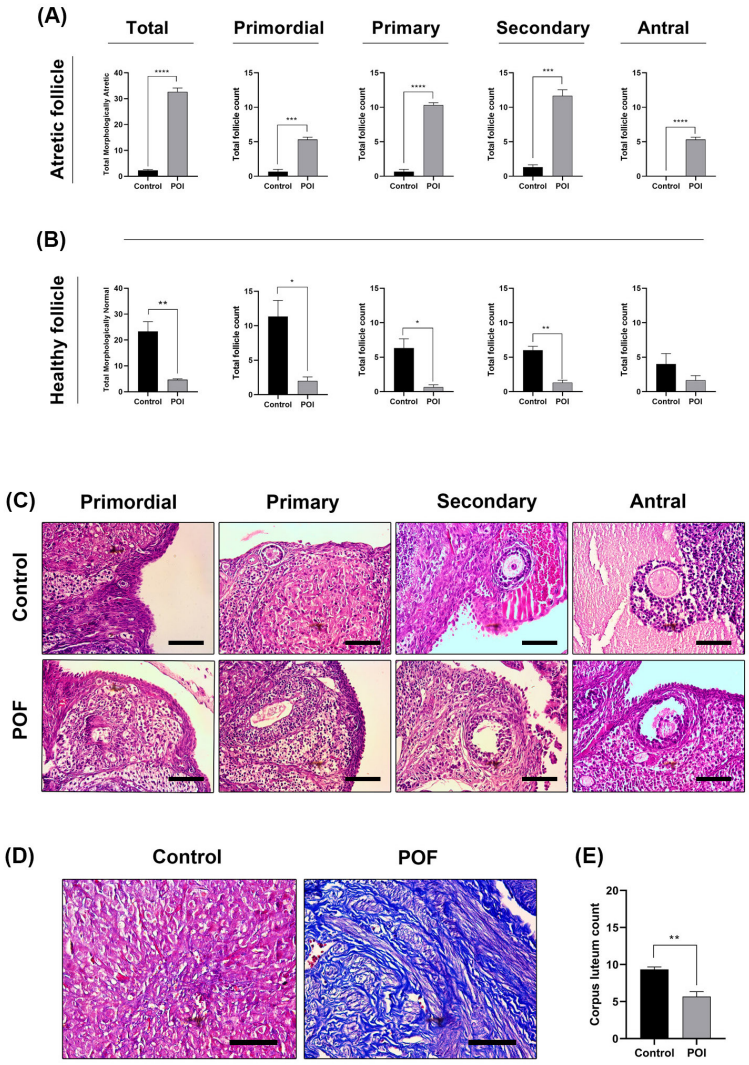


Fig. 1. Morphological assessment of ovarian tissue before the intervention, total atretic and different types of atretic follicles count **(A)**, total healthy and different types of healthy follicles count **(B)**, H&E staining **(C)**, Masson trichrome staining **(D)**, total corpus luteum count **(E)** after POI induction. (Scale bar= 200 μm), **P* < 0.05; ***P* < 0.01; ****P* < 0.001 and *****P* < 0.0001 (n = 3).

CD146⁺ and CD144⁺ cells increased normal follicle number

Four weeks after cell transplantation, healthy and atretic follicles were monitored in terms of density and morphological features. Data revealed that transplantation of CD146⁺ MSCs and CD144⁺ ECs led to the increase ($P<0.01$ and $P<0.05$, respectively) of normal follicles at different development steps compared to the POI control group (Fig. 2A). Interestingly, atretic follicles were declined in both transplanted groups four weeks after the intervention ($P<0.0001$) (Fig. 2B). A general improvement was noticed concerning in the increase of morphologically normal follicles and a decline in the number of atretic follicles compared to the control group (Fig. 2A-B). The histopathological evaluation demonstrated that CD146⁺ MSCs and CD144⁺ ECs can improve the folliculogenesis process in POI rats (Fig. 2C). Data showed the CL number with more promising results in rats that received CD146⁺ cells ($P<0.05$) (Fig. 2D). We noted that CD146⁺ MSCs and CD144⁺ ECs injection ameliorated fibrosis conditions compared to the POI group (Fig. 2E).

FSH, LH, and E_2 levels were altered after cell transplantation

Serum hormonal levels were monitored four weeks post-cell transplantation in POI rats. Our findings showed the elevation of FSH in the POI rats ($P<0.01$; Fig. 3A-B). As expected, E2 levels were non-significantly decreased under POI condition ($P>0.05$) (Fig. 3C). Four weeks post-transplantation, the decline pattern was notified for FSH and LH hormones in groups that received CD144⁺ ECs and CD146⁺ MSCs (Fig. 3D-E). The maximum effects were observed in the group that received CD144⁺ cells (Fig. 3E). Regarding the E2 levels, transplantation of CD146⁺ and CD144⁺ cells can significantly elevate E2 more than normal conditions ($P<0.01$; Fig. 3F).

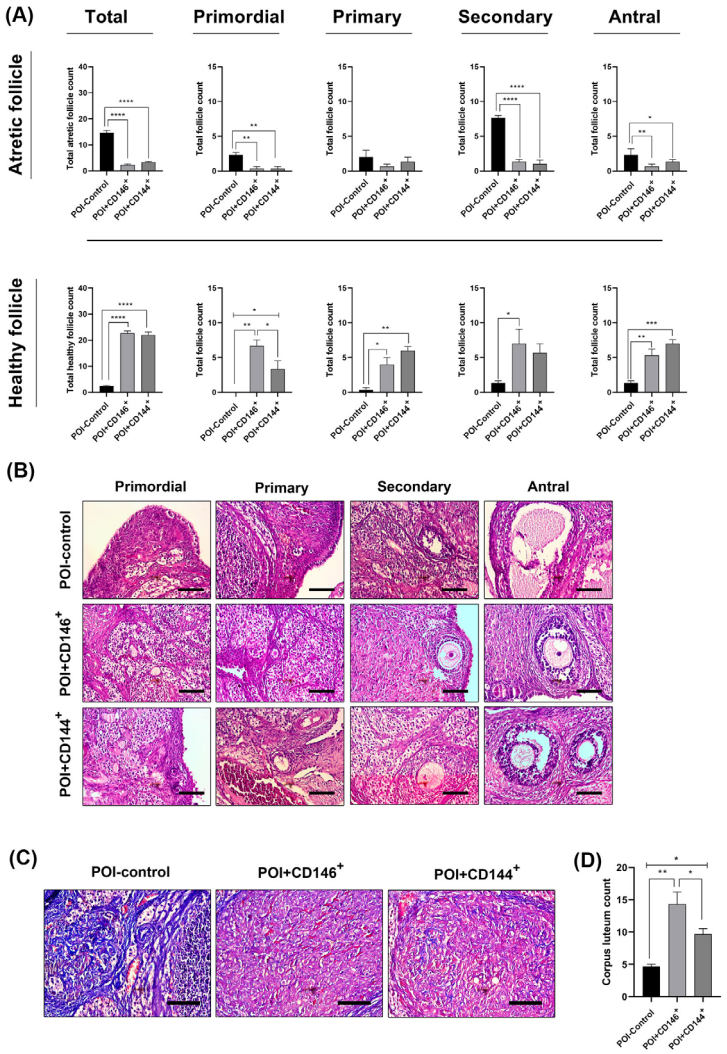


Fig. 2. Morphological assessment of ovarian tissue after CD144⁺ and CD146⁺ cell transplantation, total atretic and different types of atretic follicles count **(A)**, total healthy and different types of healthy follicles count **(B)**, H&E staining **(C)**, Masson trichrome staining **(D)**, total corpus luteum count **(E)** after the intervention. (Scale bar = 200 μm), *P < 0.05; **P < 0.01; ***P < 0.001 and ****P < 0.0001 (n = 3).

CD146⁺ and CD144⁺ cells can alter angiogenesis and fibrosis in POI rats

Our findings showed the up-regulation of *Angpt2* and *vegfr-2* transcripts following CTX administration with significant differences for *Angpt2* (P<0.05). Transcription of *Angpt2* and *vegfr-2* was reduced in the groups that were subjected to cell transplantation. However, these features were statistically significant in rats subjected to CD144⁺ ECs transplantation (Fig. 4). The induction of POI led to the significant expression of *Smad-4* and a non-significant increase of TGF-β1 while the transcription levels of *Smad-2* and *-6* remained unchanged after CTX administration. We found that the expression of *Smad-2* was significantly reduced when rats received CD144⁺ ECs compared to the POI group (P<0.01). Despite the down-regulation of this gene after CD146⁺ MSCs transplantation, non-significant differences were obtained (P>0.05). Transplantation of CD144⁺ ECs non-significantly reduced the expression of *Smad-4*. Unexpectedly, the injection of CD146⁺ MSCs into the POI rats caused an increase in the expression of *Smad-6* (P<0.05) while these values were not statically significant (P>0.05) (Fig. 4). Similar to SMAD-6, the injection of CD146⁺ MSCs promoted the transcription of TGF-β1 compared to the POI group (P<0.05). In contrast, CD144⁺ ECs reduced the expression of *Tgf-β1* compared to the POI + CD146 and POI groups (P<0.05). These features indicated that CD144⁺ ECs can alter the expression of fibrosis-related genes in comparison with CD146⁺ MSCs in terms of POI ovarian tissue.

Discussion

Here, the restorative effects of bone marrow CD146⁺ MSCs and CD144⁺ ECs were examined in the POI rat model. Induction of the POI model was successfully induced using CTX. Depending on age and dose, CTX administration can cause adverse effects on the female reproductive organs, especially the ovarian tissue.[9,14,36] Along with our previous data, we confirmed that CTX administration for 14 consecutive days can significantly

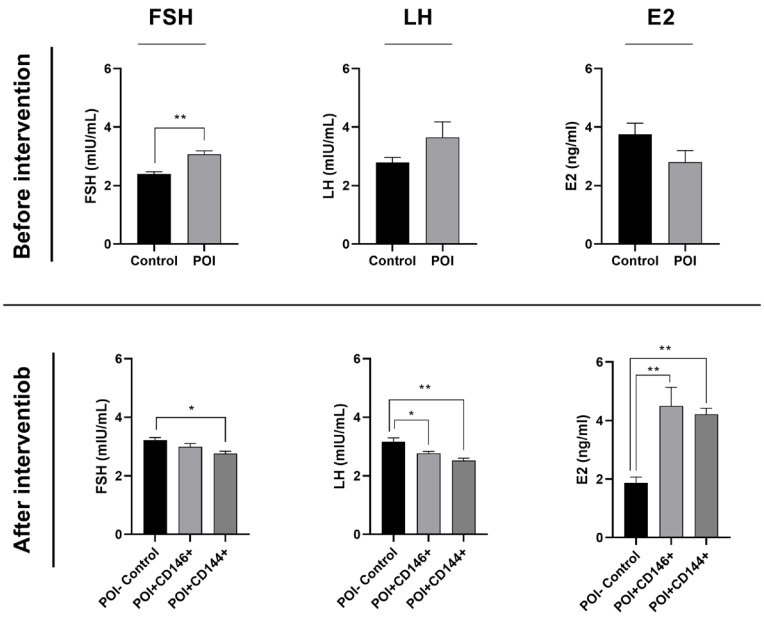


Fig. 3. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2), before also after CD144⁺ and CD146⁺ cell transplantation. One-way analysis of variance (ANOVA) and least significant difference (LSD) as the post hoc analysis was considered. **P* < 0.05 and ***P* < 0.01 (n = 3).

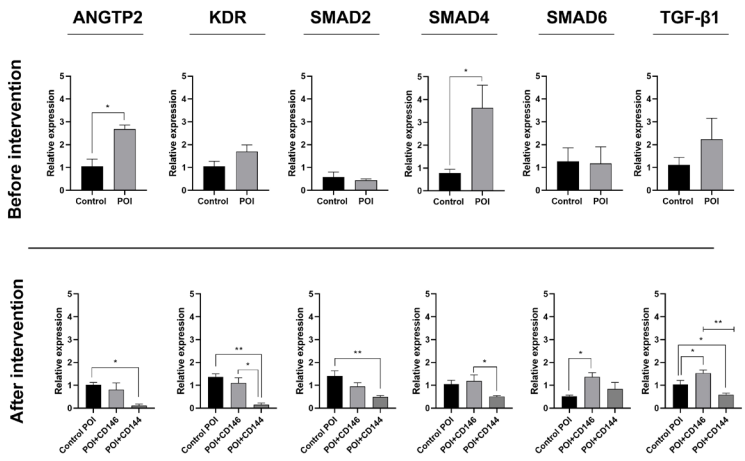


Fig. 4. Relative expression of *ANGPT2*, *KDR, SMAD2, SMAD4, SMAD6,* and *TGF-β1* genes in ovarian tissue before also after CD144⁺ and CD146⁺ cell transplantation. One-way analysis of variance (ANOVA) and least significant difference (LSD) as the post hoc analysis was considered. **P* < 0.05; and ***P* < 0.01 (n = 3).

reduce healthy follicles at different stages that coincided with atretic changes. Monitoring sex-related hormones revealed an increase in systemic FSH and LH levels while serum contents of E2 increased.[37,38] Consistent with our data, Tang and co-workers claimed that i.p. injection of CTX plus busulfan in rats leads to mild degeneration of granulosa cells plus oocytes within the ovarian tissue with significant reduction of all follicle types (primordial, primary, secondary, and mature follicles).[39]

Bone marrow medullary cavities contain several cell types with varied regenerative potential.[40] According to our findings, transplantation of both bone marrow ECs and MSCs can blunt detrimental effects of CTX within the ovarian tissue via an increase of normal follicles at different stages of development. In line with these changes, we indicated that the atretic changes were diminished after the direct transplantation of CD146⁺ MSCs and CD144⁺ ECs.[6,41] It has been indicated that transplantation of ECs like different stem cell types can improve the healing process in injured tissues via the juxtacrine and paracrine interaction with homotypic and heterotypic cells.[42] One reason for the increase of normal follicles at different stages would be that MSCs are potent cells to produce several types of cytokines involved in the inhibition of apoptosis

in oocytes and granulosa cells via the alteration of specific factors such as Gadd45b, CyclinB1, and pCDC2.[43] Similar to our study, Ling et al found that xenogeneic transplantation of human amnion-derived MSCs in POI rats via systemic route can improve the function of ovaries mainly via the promotion of paracrine manner. In rats that received MSCs, the systemic levels of E_2 and AMH increased while FSH levels were reduced compared to the POI group. TUNEL staining and gene expression analysis revealed the reduction of apoptotic granulosa cells and Bax/Bcl2 in POI rats following MSC transplantation.[44] It has been shown that the modulation of certain intracellular effectors like p53, Cyclin D2, Bax, and p21 in the presence of MSCs can lead to the acceleration of cell proliferation within the injured tissues.[38,45] Relative similar results were obtained in POI + CD144$^+$ group. It was suggested that CD144$^+$ cells are mature ECs with the capacity to release angiocrine. These cells can form a passive vascular conduit to support blood and oxygen supplementation toward the injured tissues.[46] The development of nascent vessels not only increases the recruitment of several progenitor types from the blood into the ovarian niche but also can remove the toxic substance rapidly.[47]

Based on our data, transplantation of bone marrow CD146$^+$ MSCs and CD144$^+$ ECs decreased notably collagen fiber deposition compared to the POI rats. The reduction of excessive collagen fibers is touted as a putative sign for tissue regeneration and prevention of end-stage fibrosis.[48] Likewise, it was previously suggested that menstrual blood-derived stem cells can restore the function of POI ovarian tissue via the control of fibrosis rate.[9] This activity is orchestrated via the paracrine modulation of the TGF-β3 signaling axis, resulting in the reduction of α-smooth muscle actin fibroblast viability and collagen synthesis capacity.[49] In an experiment conducted by Fouad and co-workers, they indicated that adipose tissue and especially amniotic membrane MSCs can reduce the fibrotic changes in POI rats.[50] In a similar work conducted by Cui and co-workers, it was found that MSCs can reduce pathological fibrosis in a rat model of POI via the reduction of TGF-β1 and p-Smad3.[51] As shown in this study, the expression of *smad-6* expression was induced in the POI + CD146 group. It seems that CD146$^+$ MSCs can inhibit the fibrosis phenomenon via activation of inhibitory SMADs like SMAD-6 rather than direct suppression of stimulatory SMADs such as SMAD-3 and -4. Interestingly, the injection of CD144$^+$ ECs exerted more prominent effects in the regulation of the TGF-β1 signaling pathway as compared to CD146$^+$ MSCs. In contrast to CD146$^+$ MSCs, CD144$^+$ ECs blunted fibrotic changes via direct suppression of stimulatory SMADs such as *smad-2*, and *-4*. Of note, abnormal EC function can increase the possibility of fibrosis in the injured tissue. The introduction of naïve and functional ECs can reverse these conditions and faint the progression of fibroblast activity and maladaptive fibrosis.[49] The activation of relevant factors such as Wnt, metalloprotease-14, and the CXCR7 axis increases the activity of resident stem cells and prevents further fibrotic changes.[42] These features indicated that ECs and MSCs exploited different mechanisms to modulate the process of fibrosis in POI ovarian tissue after 4 weeks.

In line with previous observations, serum levels of sex-related hormones were relatively restored following the administration of CD146$^+$ MSCs and CD144$^+$ ECs.[9,14] The reduction of follicular atresia after the introduction of CD146$^+$ MSCs and CD144$^+$ ECs seems one possible mechanism involved in the restoration of the hormone profile via the function hypothalamic-pituitary-ovarian axis. These features can lead to increased E2 hormone levels in POI rats that received ECs or MSCs.

The increase of angiogenesis factors *Angpt2* and *vegfr-2* in this study would be due to the activation of inflammatory response and aberrant remodeling following CTX administration.[6,52] The reduction of these factors is more prominent in rats that received CD144$^+$ ECs rather than CD146$^+$ MSCs. It was suggested that injection of MSCs can increase protein levels of VEGF in ovarian tissue with POI changes.[44] According to previous studies, regulation of angiogenesis factors is an efficient strategy to prevent the progression of fibrotic changes.[53] There is a close relationship between serum ANGPT2 levels, CD31 expression, collagen deposition, and VEGFR signaling cascade in patients with fibrotic hepatic diseases.[54] In this study, we indicated that the introduction of freshly isolated bone marrow CD144$^+$ ECs led to the suppression of *Angpt2* and *vegfr-2*. It seems that transplantation of functional ECs into the inflamed niched can normalize inflammatory angiogenesis response in POI ovarian tissue which is indicated by the suppression of *Angpt2* and *vegfr-2*. These findings may support the fact that the reduction of aberrant angiogenesis progression is one possible effect after the transplantation of CD144$^+$ ECs in POI rats.

Conclusion

This study highlighted that the modulation of angiogenesis is a critical step in the restoration of injured ovarian tissue in POI candidates. The current experiment examined the regenerative potential of bone marrow-derived CD144$^+$ ECs and CD146$^+$ MSCs on rat POI ovarian tissue with a focus on angiogenesis properties and fibrotic changes. Based on the present data, ECs possess superior properties compared to CD146$^+$ MSCs to alleviate the POI condition via the reduction of fibrotic changes and alteration of gene expression related to the TGF-β signaling pathway. It was suggested that the suppression of inflammatory angiogenesis is more evident in POI rats that received CD144$^+$ ECs compared to the group transplanted with CD146$^+$ MSCs. These data support the notion that the inhibition of inflammatory angiogenesis and fibrotic changes by EC lineage can be effective to prohibit further

Research Highlights

What is the current knowledge?

√ Varied cell types such as endothelial cells (ECs) and mesenchymal stem cells (MSCs) are valid therapeutic tools.

What is new here?

√ The therapeutic mechanisms of ECs and MSCs are different in the context of POI rats.

follicle atresia. Taken together, cell transplants should be selected under specific pathological conditions that affect the target organ. Besides, the selection of an appropriate cell source alone or in combination with other lineages can yield better regenerative outcomes in POI candidates subjected to cell-based therapies.

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Authors' Contribution

Conceptualization: Reza Rahbarghazi, Mahdi Mahdipour.
Formal analysis: Mahdi Mahdipour.
Funding acquisition: Mahdi Mahdipour.
Investigation: Nahideh Nazdikbin Yamchi, Farhad Amjadi, Rahim Beheshti, Mehdi Hassanpour, Reza Shirazi, Amin Tamadon.
Project administration: Mahdi Mahdipour.
Supervision: Reza Rahbarghazi, Mahdi Mahdipour.
Visualization: Mahdi Mahdipour.
Writing–original draft: Nahideh Nazdikbin Yamchi.
Writing–review editing: Reza Rahbarghazi, Mahdi Mahdipour.

Competing Interests

The authors declare no competing Interests.

Ethical statement

All experimental protocols were approved by the Local Ethics Committee of Tabriz University of Medical Sciences [IR.TBZMED.VCR.REC.1398.374]. All methods were carried out under previously published principles [NIH, 1986]. The study was carried out in compliance with the ARRIVE guidelines.

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