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Use of placental-derived mesenchymal stem cells to restore ovarian function and metabolic profile in a rat model of the polycystic ovarian syndrome

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Abstract

Introduction Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disturbance that affects many women worldwide and is characterized by chronic anovulation, hyperandrogenism, and ovarian dysfunction. Placenta-derived mesenchymal stem cells (PDMSCs) are derived from the placenta and have advantages over other sources of MSCs in terms of availability, safety, and immunomodulation.

Materials and methods In this experimental study, twenty female Wistar rats were assigned to four groups ($n=5$) including control, sham, PCOS, and PCOS+PDMSCs groups. Then, PCOS was induced in the rats through administering letrozole for 21 days. PDMSCs (1×10^6 cells) were injected through the tail vein. Fourteen days after the cell infusion, evaluation was performed on the number of healthy follicles, corpus luteum, and cystic follicles as well as the levels of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), fasting blood glucose, fasting insulin, and insulin resistance. Moreover, the serum levels of cholesterol, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured. Liver function was also determined by the evaluation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

Results The number of corpus luteum and primordial, primary, secondary, and antral follicles was significantly elevated in the PCOS+PDMSCs group compared to the PCOS group. However, the number of cystic follicles significantly decreased in the PCOS+PDMSCs group. The LH and testosterone levels also decreased significantly, while FSH levels increased significantly in the PCOS+PDMSCs group. The levels of fasting blood glucose, fasting insulin, and insulin resistance notably decreased in the PCOS+PDMSCs group. Moreover, the lipid profile improved in the PCOS+PDMSCs group along with a significant decrease of cholesterol, LDL, and TG and an increase in HDL. The PCOS+PDMSCs group exhibited marked decreases in the AST and ALT levels as well.

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Conclusion The results of this study suggest that PDMSCs are a potential treatment option for PCOS because they can effectively restore folliculogenesis and correct hormonal imbalances, lipid profiles and liver dysfunction in a rat model of PCOS. However, further research is needed to establish the safety and effectiveness of PDMSCs for treating PCOS.

Keywords Polycystic ovary syndrome, Mesenchymal stem cell, Hyperandrogenism, Rat

Introduction

The polycystic ovary syndrome (PCOS) is a clinical syndrome involved in infertility disorders and associated with conditions ranging from chronic anovulation to metabolic dysfunction [1]. It also has to do with irregular menstrual cycles, hirsutism, alopecia, and acne. Long-term PCOS is accompanied by an increased risk of uterine cancer and endometrial hyperplasia [2]. Patients with these diseases present elevated testosterone and serum LH levels and diminished FSH levels [3]. In 2003, the Rotterdam Consensus established diagnostic criteria for PCOS, considered as the most commonly used clinical diagnosis and research criteria worldwide. Accordingly, the presence of at least two of the criteria for hyperandrogenism, anovulation or amenorrhea, and polycystic ovary morphology indicate PCOS. Patients with PCOS also suffer from hyperinsulinemia, insulin resistance, hypertension, dyslipidemia, and obesity [4].

PCOS is a multifactorial syndrome, and its causes are not yet well understood. Several etiological factors, including metabolic imbalances and immune system perturbations, are involved in developing heterogeneous clinical signs of PCOS. Several factors imply endocrine axis disturbance as well. Hyperandrogenism is the most prevalent biochemical perturbation in PCOS patients [5]. An increase in the pulse amplitude, the frequency of luteinizing hormone (LH) and the LH/FSH ratio increases androgen secretion from theca cells in polycystic ovaries [6].

In addition, the reduced release of sex hormone-binding globulin (SHGB) from the liver by hyperinsulinemia causes an increase in the bioavailability of free androgen [7]. Hyperinsulinemia also leads to the increased secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which results in hypersecretion of LH, increased testosterone, and decreased follicular maturation [8]. Several studies have shown that chronic inflammation affects the incidence of PCOS symptoms. Recently, specific inflammatory cytokines, namely, IL-6, TNF-α, IL-1, IL-18, and IL-17, have been found in PCOS patients [9]. Prevalent autoimmune diseases such as thyroiditis have been reported in these patients too [10]. Furthermore, there is a transition in macrophage polarization from M2 (anti-inflammatory) to M1 (pro-inflammatory) in PCOS. This macrophage polarization produces proinflammatory cytokines, including TNF-α and IL-6 [11].

Apart from disease management by lifestyle modifications (e.g., exercise, weight control, dietary changes), treatment options for PCOS patients include a combination of supplements and pharmacological interventions [12]. Conventional treatments for PCOS include oral contraceptives for menstrual derangements and hirsutism, antiandrogens for hyperandrogenism, and insulin sensitizers for insulin resistance and anovulation. There are newer treatment options such as statins, aromatase inhibitors, bromocriptine, and vitamin D/calcium. These therapies, however, cause a wide range of adverse metabolic and physiological effects that affect patients' quality of life [13]. Moreover, conventional treatments for PCOS do not yield satisfactory results [14].

Recently, mesenchymal stem cells (MSCs), owing to their immunomodulatory, anti-inflammatory, and anti-apoptotic properties, have been considered promising for restoring the function of damaged tissues and improving some illnesses [15]. MSCs have multiple potential functions, including self-renewal, secretion of various bioactive mediators, proliferation and differentiation into specialized cells, and migration toward damaged tissues [16]. Many previous investigations have demonstrated the therapeutic effect of MSCs or their extracellular secretome (i.e., exosomes) in various diseases, including myocardial infarction, cardiac ischemia, liver injury, and neurological, immunological and metabolic disorders [17]. Several studies have also indicated that MSCs have a strong potential to prevent ovarian dysfunction and uterus inflammation during infertility perturbations such as premature ovarian failure (POF) [18]. MSCs effectively treat other endocrine/metabolic diseases, such as diabetes, by restoring oxidative balance, relieving inflammation, and improving insulin resistance [19]. Since PCOS is a metabolic-reproductive-endocrine and even immunologic disorder, we assumed that MSCs could be a therapeutic option to decrease PCOS symptoms. Therefore, the present study was carried out to assess the ability of the mesenchymal stem cells derived from the placenta (PDMSCs) to restore ovarian function, promote follicular growth, and regulate hormone levels in a mouse model of PCOS.

Materials and methods

Placenta-derived mesenchymal stem cells

Placenta tissue was obtained from women aged 25–30 years after an uncomplicated elective cesarean section

at Shahid Beheshti Hospital (Kashan, Iran). All the participants had been informed a priori and had consented to donate. Briefly, under sterile conditions, the chorioamniotic membrane layer was removed, and 6–10 g of tissue was dissected and treated with collagenase 1 (Sigma–Aldrich) at 37 °C in a 5% CO2 incubator for two hours until the PDMSCs crawled out of the tissue. The harvested PDMSCs were cultured at 2×10^5 cells/mm^3 in T25 flasks with DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The PDMSCs were passaged for five generations when they reached approximately 80–90% confidence.

The phenotypic markers of the PDMSCs were evaluated using flow cytometry. The PDMSCs were also stained with R-phycoerythrin-conjugated monoclonal antibodies, which included antibodies against CD90 (Bio Legend, USA), CD105, CD73, CD45 (bioscience, USA), and CD34 (Santa Cruz. USA).

Animals

This experimental study was carried out on female Wistar rats (10-week old, weighing 200±30 g). Ethical approval was received from the Ethics Committee of Kashan Medical University (IR.KAUMS.AEC.1400.001). The animals were kept at the animal breeding center of Kashan Medical University at a temperature of 24 °C, humidity of 50±5%, and a 12-hour light/dark cycle. Food and water were also provided ad libitum.

Estrous cycle monitoring

Before the animals were subjected to treatment, they were checked for two sequential cycles and regular estrous cycles. Also, the PCOS induction in animals was confirmed by performing a vaginal smear test. Briefly, saline (50 µL) was injected into the vagina by means of a sampler, smeared on a slide stained with methylene blue, and examined via bright field microscopy.

Induction of PCOS and study design

Twenty female rats were divided into four groups ($n=5$) including (a) a control group not receiving any interventions, (b) a sham group receiving oral gavage 1 ml of 0.5% carboxymethylcellulose (CMC) and a tail vein injection of 1 ml saline, (c) a PCOS group taking letrozole (1 mg/kg) (Aburaihan Pharma.co., Tehran, Iran) dissolved in 0.5% CMC, orally administered for 28 days, and (d) a PCOS+PDMSCs group in which the rats received a tail vein injection of PDMSCs (1×10^6) dissolved in 1 ml of saline.

Dosing volumes for both oral (P.O.) and intravenous (i.v.) administrations were standardized at 1 ml per 250 g body weight. This volume was selected based on universally accepted guidelines for rat studies, ensuring that dosing remained well within safe limits for both routes of administration.

Blood sampling

After two weeks of PDMSCs injection, the rats were put on a fast overnight (12 h) and then anesthetized via an IP infusion of ketamine (90 mg/kg) and xylazine (10 mg/kg). Their blood samples were directly collected from the heart, and serum was separated by centrifugation for 20 min at 3500 RPM.

Histological examination and follicle count

After the last blood collection (14 days after infusion), the rats were euthanized (IP injection of 250 mg/kg of pentobarbital sodium), and the ovaries were immediately separated, freed from the extra fat, and fixed in 10% formaldehyde. The samples were dehydrated in ascending alcohol and xylene and embedded in a paraffin block. Five-µm-thick serial sections were prepared with a microtome. One section per five serial sections was selected and ten sections from each rat were stained with hematoxylin-eosin. Only follicles with a nucleus were counted and The mean numbers of corpus luteum, primordial, primary, secondary, antral, and cystic follicles were analyzed according to the method used for counting the ovarian follicles in previse studies [20, 21].

Follicle classification

The primordial follicle was found to have a flattened layer of granulosa cells that encompassed the oocyte. In instances where the oocyte was encircled by more than two layers of granulosa cells, it was identified as a primary follicle. Alternatively, if there was fluid accumulation between the granulosa cells, it was documented as a secondary follicle. Regardless of size, any follicle that possessed an antral cavity was classified as an antral follicle. The corpus luteum showcased distinct luteal cells with voluminous nuclei and vessels. Furthermore, a cystic entity was recognized as a large fluid-filled structure with an attenuated granulosa cell layer and a thickened theca internal cell layer.

Biochemical profile and hormonal assay

Serum fasting insulin, testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations were assessed via ELISA kits (MyBioSource, USA) following the manufacturer's instructions. The serum levels of aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), and fasting glucose were evaluated via quantitative photometric kits (Pars Azmoon, Iran). Insulin resistance was calculated by the homeostasis model assessment

of insulin resistance (HOMA-IR). The evaluation was according to the following equation [22, 23].

$$\text{HOMA-IR} = fasting\,insulin(mIU/l) \times fasting\,glucose(mg/dL)/405$$

Statistical analysis

The data are presented as mean±standard deviation (SD). Kolmogorov–Smirnov test was used for evaluation of data distribution. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests with SPSS vs. 22 (SPSS Inc., USA). GraphPad Prism 9(GraphPad Software, La Jolla, CA) was used to plot graphs. Statistical significance was set at $P<0.05$.

Results

Isolation and identification of PDMSCs

The flow cytometry results showed that PDMSCs were positive for CD90 (96.7%), CD73 (99%), and CD105 (97.7%) surface markers and negative for CD45 (0.14%) and CD34 (0.32%) surface markers (Fig. 1).

Estrus cycle analysis

As depicted in Fig. 2, the rats in the control group exhibited a normal estrous cycle, whereas the rats with PCOS demonstrated a disrupted estrous cycle, primarily characterized by prolonged diestrous phase.

Improving effect of PDMSCs treatment on ovarian morphological dysfunction

Harvested ovaries were assessed using H&E staining to explore the effect of PDMSC treatment on ovarian morphology. The control group displayed normal ovarian morphology with corpora luteum and healthy follicles. In the PCOS group, the number of healthy follicles significantly decreased compared to that in the control group. In the PDMSCs-PCOS group, the ovaries had normal morphology (Fig. 3A), a large number of corpus luteum (5.25±1.18 vs. 1.66±0.53), primordial (6.93±1.91 vs. 2.97±0.95), primary (5.31±1.71 vs. 2.74±0.95), secondary (1.45±0.51 vs. 0.82±0.16) and antral follicles (0.68±0.20 vs. 0.22±0.07) versus those in the PCOS group (Fig. 3B). Also, compared to the ovaries in the control rats, those in the PCOS rats had a significantly increased number of cystic follicles. As for the PCOS+ PDMSCs group, the number of cystic follicles (9.52±1.97 vs. 19.01±0.99) significantly decreased after 14 days (Fig. 3B).

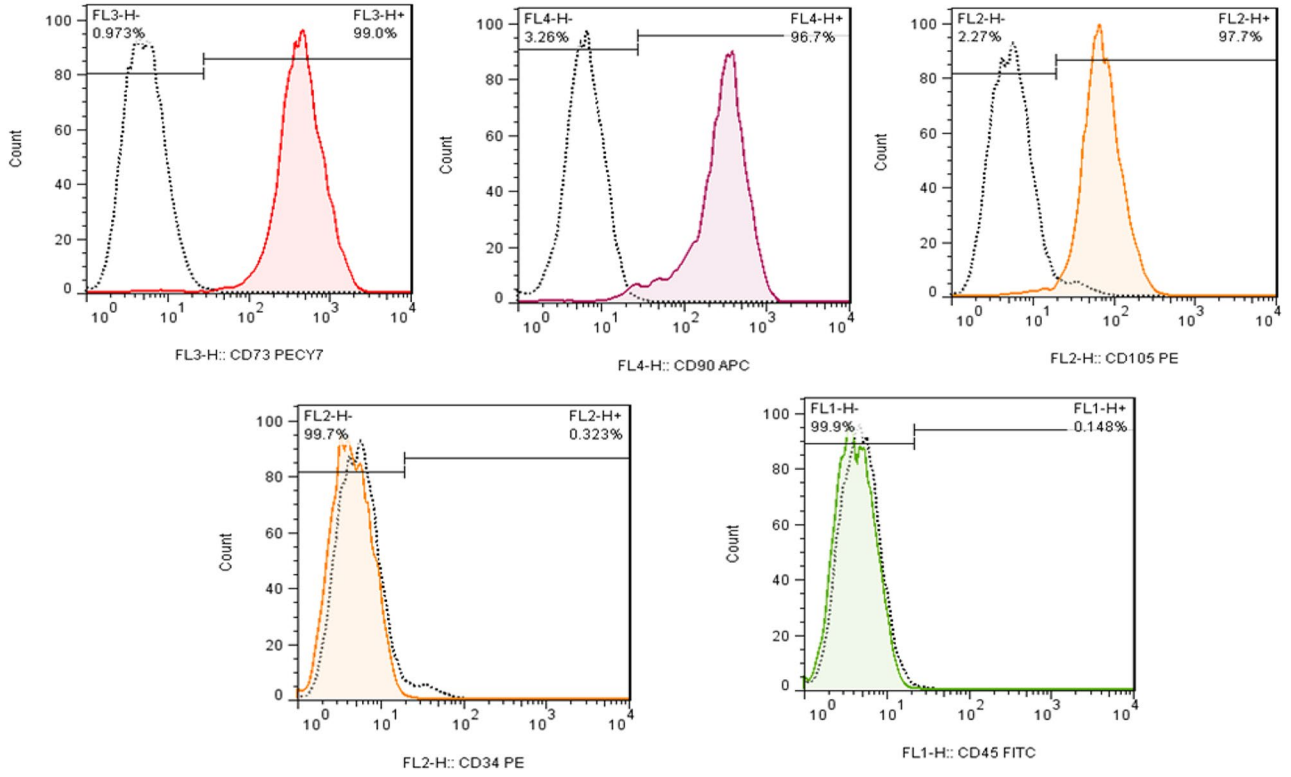

Fig. 1 The characterization of human PDMSCs using flow cytometry: Isolated cells were found positive for CD73, CD90 and CD105 and negative for CD34 and CD45

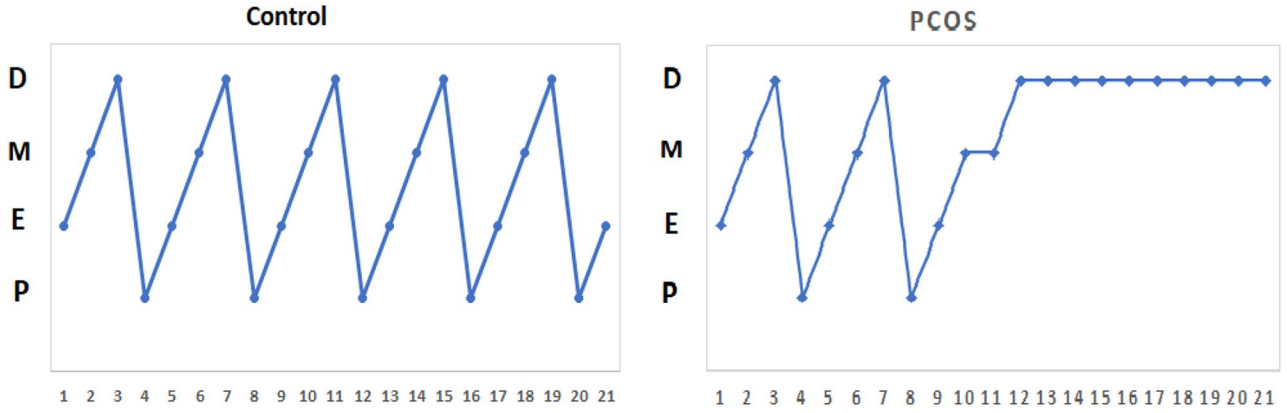

Fig. 2 Representative vaginal smears from the control and PCOS rats at different stages: proestrus (P), estrous (E), metestrus (M), and diestrus (D)

Hormone assays

The serum levels of testosterone, LH, and FSH were quantified to assess the hormonal alterations after the administration of PDMSCs (Fig. 4). As the results indicated, the injection of letrozole in the PCOS and PCOS+PDMSCs groups caused a substantial increase in the serum concentrations of testosterone and LH, compared to those in the control and sham groups. Furthermore, the serum levels of testosterone (9.41±1.32 vs. 13.20±1.27) and LH (4.74±0.48 vs. 5.78±0.43) were significantly lower in the PCOS+PDMSCs group than in the PCOS group following two weeks of treatment with PDMSCs. The serum concentrations of FSH were also significantly lower in the PCOS group than in the healthy control and sham groups. They were elevated in the PCOS+PDMSCs (2.83±0.43 vs. 2.18±0.17) group versus the PCOS group.

Fasting serum glucose and insulin levels

The serum levels of fasting blood glucose and fasting insulin were assessed to evaluate insulin resistance 14 days after the treatment. The findings indicated a significant increase in the serum levels of fasting insulin, fasting glucose, and HOMA-IR in the PCOS group compared to those in the control and sham groups. However, the FBG, FINS, and HOMA-IR were significantly lower in the PDMSCs+PCOS group (166.9±14.96, 14.17±0.73, 5.84±0.62, respectively) than in the PCOS group (188.2±9.84, 15.58±0.46, 7.24±0.48, respectively) (Fig. 5).

Lipid profile

The serum levels of triglycerides, cholesterol, LDL, and HDL were evaluated to assess the effect of PDMSCs on the lipid profile. As Fig. 6 demonstrates, the levels of triglyceride (120.6±5.66 vs. 56.11±1.21), cholesterol (95.04±4.48 vs. 62.49±0.70), and LDL (27.40±0.78 vs. 17.02±0.20) in the PCOS groups were significantly elevated compared to those in the control group. However, triglyceride (120.6±5.66 vs. 103.6±9.13), cholesterol (95.04±4.48 vs. 85.57±4.39), and LDL (27.40±0.78 vs. 25.64±1.51) were significantly lower in the PCOS+PDMSCs group than in the PCOS group ($p<0.05$). Additionally, the HDL levels of PCOS groups were lower than those of the control group (27.35±1.71 vs. 42.28±0.56). As for the HDL level in the PCOS+PDMSCsgroup, it was significantly greater than that in the PCOS group (31.48±2.80 vs. 27.35±1.71).

ALT and AST levels

The effect of PDMSCs on liver function was assessed by measuring the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). As Fig. 7 demonstrates, compared to those in the control group, the serum ALT (127.2±7.67 vs. 64.14±2.01) and AST (133.0±10.23 vs. 57.01±1.33) levels were significantly elevated in the PCOS group ($P<0.01$). Moreover, two weeks after the cell infusion, the serum levels of AST (117.2±7.45 vs. 133.0±10.23) and ALT (108.3±9.56 vs.127.2±7.67) in the PCOS+PDMSCs group were notably lower than those in the PCOS group.

Discussion and conclusion

The polycystic ovary syndrome (PCOS) is a medical condition that impacts both the reproductive and endocrine systems and is characterized by three critical diagnostic features, including chronic anovulation, hyperandrogenism, and the presence of polycystic ovaries. In the present study, PDMSCs were isolated from the human placenta and then infused into PCOS animals to evaluate their treatment effects. In recent years, various types of stem cells, such as human umbilical cord mesenchymal stem cells (HUMSCs), bone marrow mesenchymal stromal cells (BMSCs), and adipose mesenchymal stem cells (ADSCs), have been investigated for their ability to treat infertility diseases [24].Placenta mesenchymal stem cells have attracted widespread attention because of their low

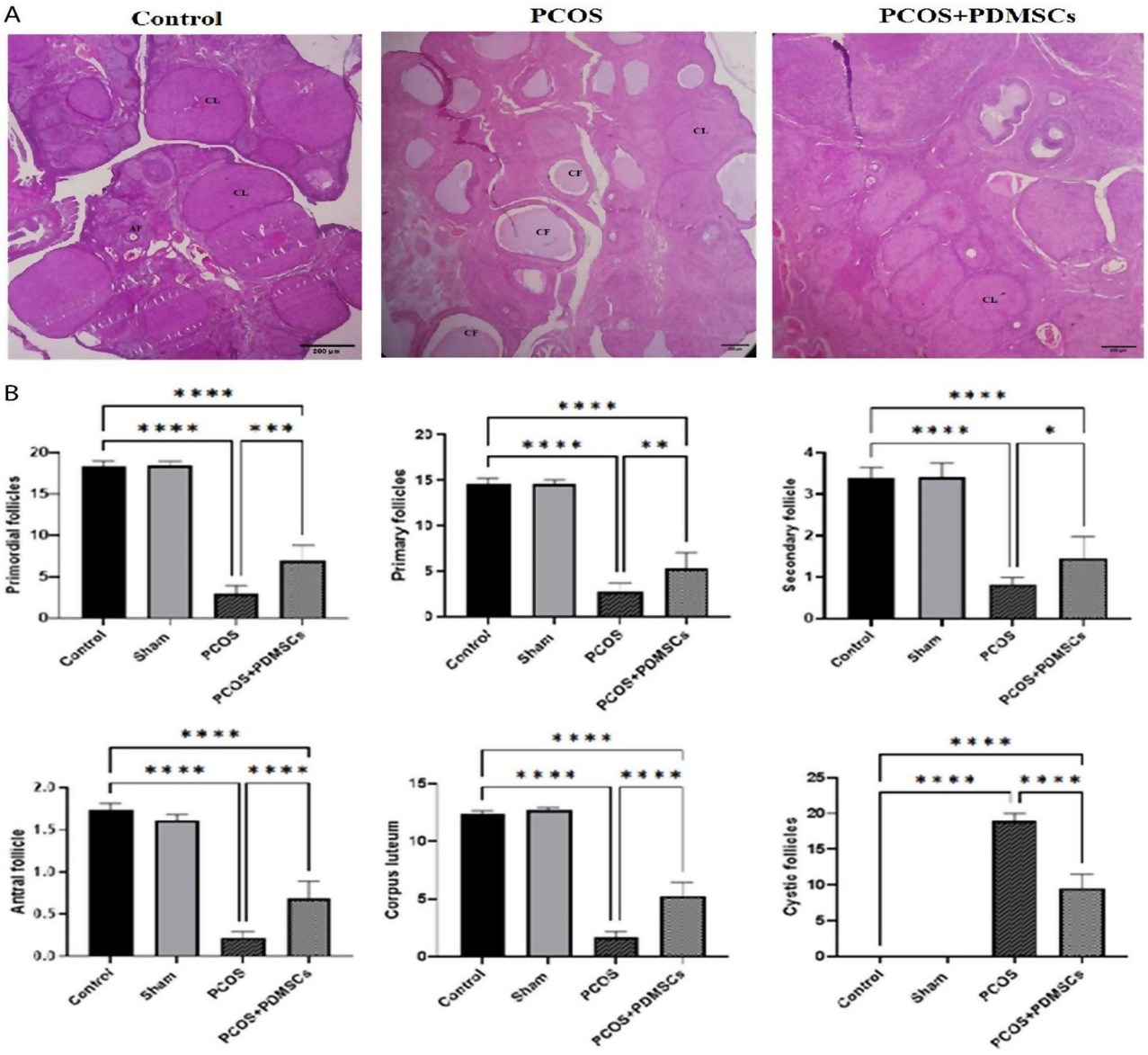

Fig. 3 (**A**) Photomicrograph of a section in the ovarian tissues in all the groups using hematoxylin and eosin staining: The ovarian tissue section of the PCOS + PDMSCs group showed a lot of healthy follicles. (**B**) Quantitative analysis of the number of follicles: The PCOS + PDMSCs group showed a reduced number of cystic follicles but increased numbers of healthy follicles and corpus luteum (mean ± SD) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). CL: corpus luteum, AF: antral follicle, CF: cystic follicle

immunogenicity, accessible collection, lack of ethical issues, and high differentiation potential [25].

Similar to many previous investigations, in this study, it was observed that treating rats with letrozole could lead to significant histological changes, such as the development of cystic follicles and a decrease in healthy follicles. However, the number of healthy follicles (in all developmental stages) and the corpus luteum was increased after PDMSCs infusion. Additionally, PDMSCs could reduce the number of cystic follicles, which were significantly elevated in the PCOS group. In line with our findings, Seok et al. [26]demonstrated the substantial potential of PDMSCs in restoring the ovarian function in a rat ovariectomized (OVX) model. Another study [27]reported that injection of PDMSCs into a rat OVX model can promote primordial follicle activation.

Numerous reports have indicated that the effect of MSC transplantation on the ovarian function can occur via paracrine effects and the regulation of signaling pathways in the ovary. For example, Choi et al. [27]showed that PDMSCs activate the PI3K/AKT and ERK pathways. The PI3K/Akt pathway substantially influences follicular activation, granulosa cell development, and oocyte quality. In addition, Kim et al. [28]showed that MSCs can

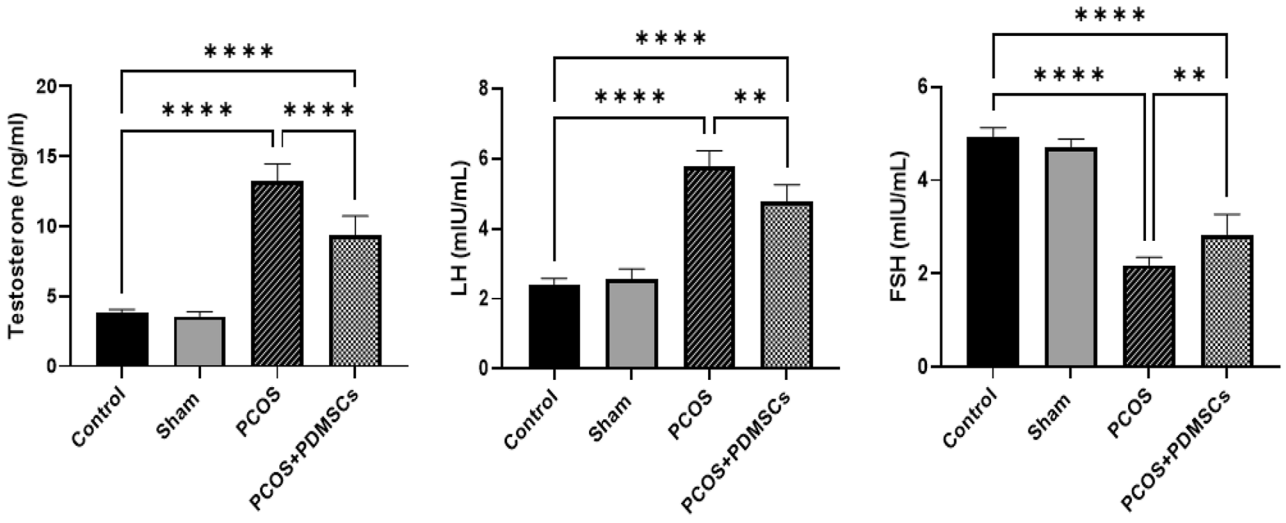

Fig. 4 Evaluation of the serum hormonal levels in the control, sham, PCOS, and PCOS + PDMSCs groups: Compared to the PCOS group, PDMSCs treatment significantly decreased the serum levels of testosterone and LH and increased the FSH level (** $p < 0.01$, **** $p < 0.0001$). The data are shown as mean ± SD. LH: luteinizing hormone, FSH: follicle-stimulating hormone

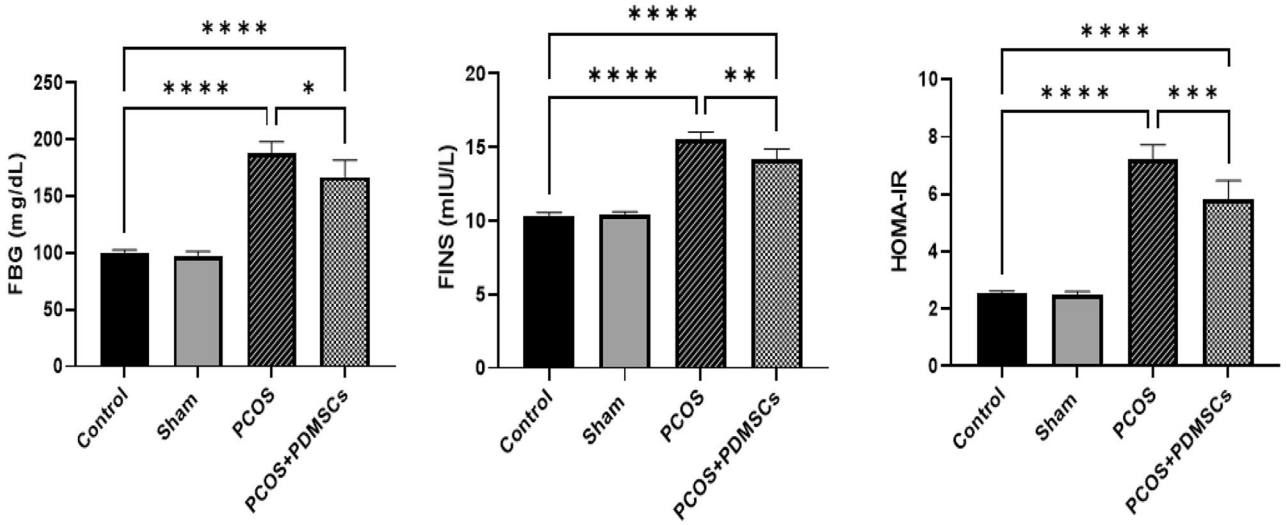

Fig. 5 Evaluation of the insulin resistance in the control, sham. PCOS and PCOS + PDMSCs groups: Fasting blood glucose, insulin level, and HOMA-IR index significantly decreased after PDMSCs intervention (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). The data are shown as mean ± SD. FBG: fasting blood glucose, FINS: fasting insulin, HOMA-IR: homeostasis model assessment of insulin resistance

change the expression of circulating proteins and miRNAs associated with follicle development via bone morphogenetic protein (BMP) signaling and steroidogenesis in ovaries. BMPs are among the many growth factors secreted by MSCs. These proteins play a crucial role in female fertility and are involved in all the developmental stages of folliculogenesis.

Similarly, Kalhori et al. [29] reported that MSCs could repair damaged ovarian function by secreting several growth factors and antiapoptotic cytokines, such as TGF-β and VEGF. Angiogenesis is an essential mechanism in the recovery of the ovarian function [30]. Angiotensin, fibroblast growth factor-2 (FGF-2), and VEGF are secreted from MSCs to promote neovascularization and facilitate the blood perfusion of damaged ovarian tissues [31]. Studies have also shown that androgen promotes granulosa cell apoptosis, which causes a decrease in the number of antral follicles [1]. Wang et al. [32] reported that MSCs can inhibit GC apoptosis and enhance GC proliferation by upregulating Bcl-2 expression and releasing growth factors, including HGF, IGF-1, and VEGF.

Although the exact cause of PCOS is unclear, factors such as hyperandrogenism are essential for its occurrence. The studies published in the field have reported remarkable increases in circulating hormone levels, such as testosterone and LH, as well as lower serum FSH levels

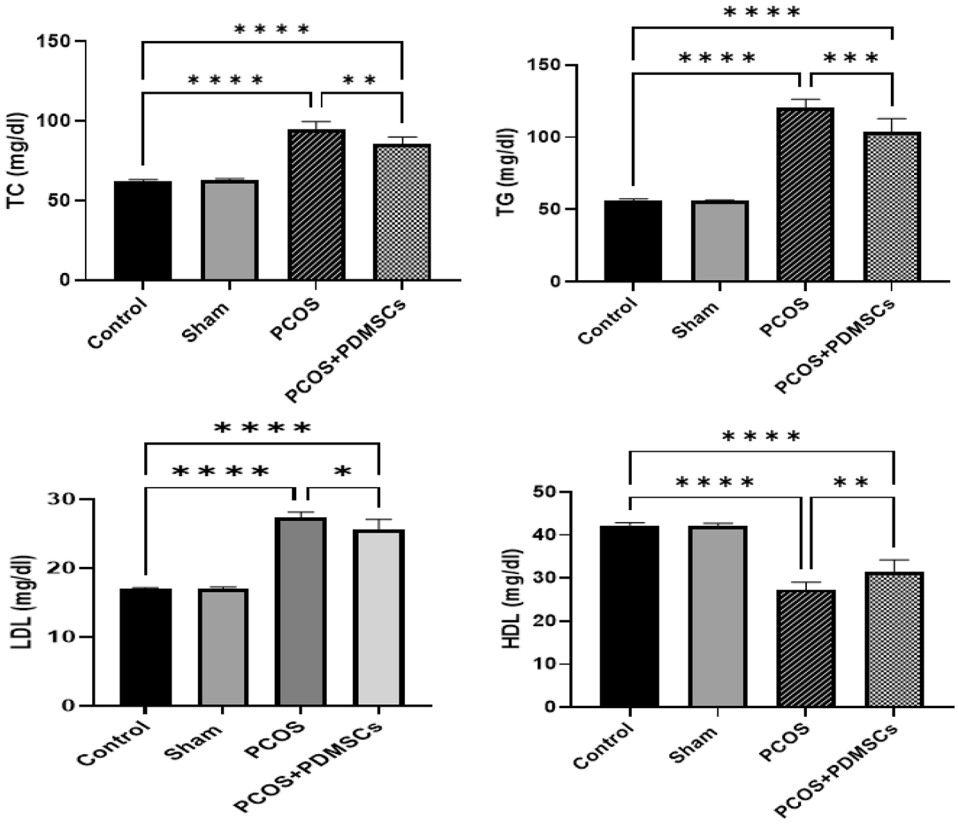

Fig. 6 Evaluation of the lipid profile in the control, sham, PCOS, and PCOS + PDMSCs groups: After treatment with PDMSCs, the serum level of triglyceride, cholesterol, and LDL significantly decreased, but the serum level of HDL significantly increased compared to the PCOS group (* $p < 0.05$, ** $p < 0.01$,*** p<0.001 , **** $p < 0.0001$). The data are shown as mean ± SD. TG: triglyceride, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein

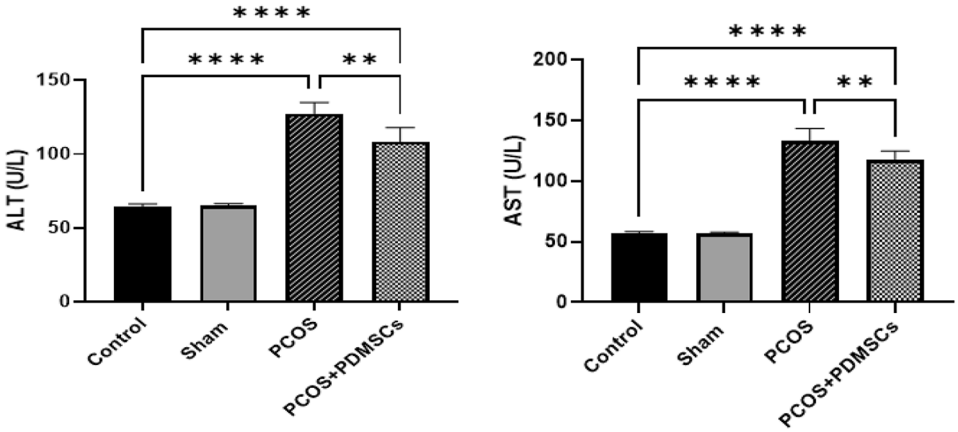

Fig. 7 Evaluation of the liver marker in the control, sham, PCOS, and PCOS + PDMSCs groups: Injection of PDMSCs significantly decreased the serum level of ALT and AST compared to the PCOS group (** $p < 0.01$, **** $p < 0.0001$). The data are shown as mean ± SD. AST: aspartate aminotransferase, ALT: alanine aminotransferase

in patients with PCOS [33]. In these patients, an increase in the pulse frequency of gonadotropin-releasing hormone (GnRH) enhances LH release into the ovary. LH can directly stimulate androgen production by upregulating androgenic enzymes in theca cells. Increased expressions of CYP17A1 and CYP11A mRNAs, as well as increased activity of the 17βHSD, 3βHSD and P450c17 enzymes in theca cells, resulting in increased production of progesterone, 17OHP, and testosterone, are the persistent features of PCOS [34].

In addition, sex hormone-binding globulin (SHBG) levels are significantly lower in in the serum of PCOS

individuals. A reduction in SHBG leads to elevation-free and biologically active androgens [35]. In line with these reports, we observed that the serum levels of several sex hormones, such as LH and testosterone, were significantly elevated and that the level of FSH was significantly reduced in the PCOS group. However, after PD-MSC intervention, these changes were reversed by the reduction of the serum testosterone and LH concentrations and the upregulation of the FSH level, compared to those in the PCOS group. Consistent with our results, Kalhori et al. [29] reported that MSC transplantation in PCOS models could regulate sex hormone levels, such as LH and testosterone. Moreover, Chugh et al. [36]showed that the MSC secretome could inhibit androgen production by reducing the expression of steroidogenic-related genes such as DENND1A, CYP11A1, and CYP17A1. In another in vitro study by Chugh et al. [37], BMP-2 secreted by MSCs could inhibit androgen production in the H295R cell line.

Insulin resistance is a prevalent abnormality in PCOS patients [38]. In our study, the fasting glucose level and HOMA-IR index notably increased, indicating the insulin resistance in PCOS rats. However, we found that PDMSCs improved HOMA-IR and significantly reduced FBG and FIN levels. In PCOS, the serine phosphorylation of the insulin receptor substrate (IRS) leads to the inhibited translocation of glucose transporter 4 (GLUT4) into the plasma membrane and the reduction of glucose uptake [39]. Additionally, the serine phosphorylation of IRS1 can inhibit the response to insulin receptor activation through reduced PI3K/AKT signaling [40]. As Chen et al. [41]. showed, MSCs could enhance the expression of GLUT4 and translocation activity by regulating the PI3K/AKT and MAPK pathways. Hyperandrogenism also activated the endoplasmic reticulum (ER) stress [42]. This stress can lead to the serine phosphorylation of IRS1 by activating the JNK signaling pathway [43]. As reported by Sanap et al. [44], MSCs can significantly decrease the expression of proteins related to the ER stress, such as CHOP1 and IRE1.

Several studies have indicated the interactions between hyperandrogenism and insulin resistance and liver dysfunctions such as dyslipidemia and the nonalcoholic fatty liver disease (NAFLD) in patients with PCOS [45]. Approximately 70% of women with PCOS exhibit abnormal lipid profiles [46]. Dyslipidemia, characterized by increased triglycerides, LDL, and cholesterol, is common in PCOS patients [46]. Cui et al. [47]reported that insulin resistance and elevated androgen levels contribute to hepatic steatosis and change lipid metabolism in the liver. Hyperinsulinemia through the inhibition of lipolysis results in an increase in no-esterified fatty acids. A high level of no-esterified fatty acids causes an increase in the TG level and reduces the level of HDL.

Moreover, hyperinsulinemia can elevate free testosterone (FT) levels through reduced hepatic SHBG synthesis [48]. It has also been documented that testosterone can reduce LDH levels by increasing the expression of the genes involved in LDH catabolism, such as SR-B1 [6]. Furthermore, patients with PCOS have higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the liver. Elevated ALT and AST levels reflect nonspecific hepatocellular damage and a risk of NAFLD [49].

In the present study, letrozole adversely affected liver function and significantly enhanced LDL, TC, TG, ALT, and AST in the PCOS group. Significant reductions in HDL levels were also observed in PCOS rats. However, treatment with PDMSCs decreased the levels of triglycerides, cholesterol, and LDL, which led to significantly increased HDL cholesterol levels. In addition, PCOS rats presented elevated levels of serum AST and ALT, which were reversed by PDMSCs.

Like in this study, Frodermann et al. [50]. reported that BM-MSCs significantly decreased the serum cholesterol and low-density lipoproteins (VLDLs) in LDLR-/- mice. The transplantation of Ad-MSCs also significantly improved the LDL, cholesterol, and HDL levels in patients with arteriosclerosis [51]. In this context, Shi et al. [52] showed that injecting MSCs into ApoE-/- mice could decrease total cholesterol and LDL levels. Another study [53] reported that the hepatic growth factor (HGF) secreted from MSCs has antiapoptotic effects on hepatocytes and restores liver injury. Researchers also demonstrated that the injection of UC-MSCs ameliorated NAFLD and improved lipid metabolism through upregulating the HNF4α-CES2 pathway, which plays a vital role in lipid and glucose metabolism in the liver [54].

As a result, mesenchymal stem cells improve damaged and dysfunctional tissues due to their inherent protective effects, such as anti-inflammatory, antiapoptotic, proangiogenic, and proliferative effects. In addition, PCOS, as an inflammatory, endocrine, and metabolic syndrome, can be an exciting candidate for MSC therapy. In this study, it was found that PDMSCs can significantly modify ovarian morphology, improve the imbalance of sex hormone levels, and enhance insulin sensitivity in rats with PCOS induced with letrozole. Moreover, PDMSCs had beneficial effects on liver function and lipid metabolism. However, additional investigations are needed on the molecular mechanisms of regeneration, infertility rectification, and safety concerns associated with the use of PDMSCs to treat PCOS.

A limitation of this study is the lack of direct evidence for PDMSC homing to the ovaries. While our results strongly suggest PDMSC-mediated effects based on restoring lipid profile, hormonal balance and liver and ovarian functioning markers, future studies employing

molecular tracking techniques such as PCR, immunohistochemistry, or in vivo imaging would provide valuable insights into the precise localization and mechanism of action of PDMSCs in ovarian tissue repair.

Abbreviations

PDMSCs	Placenta-derived mesenchymal stem cells
PCOS	Polycystic ovary syndrome
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
TNF-α	Tumor Necrosis Factor-Alfa
IL-6	Interleukin 6
FBG	Fasting blood glucose
FINS	Fasting insulin
SHGB	Sex hormone binding globulin

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Author contributions
A.R. and T.M. designed the project. M.S. performed the experiment. M.S. and M.S. analyzed the data. A.R. and M.S. wrote the initial draft of the manuscript. F.M. and M.N. revised the manuscript. The final edit was accomplished by A.R and F.M.

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Data availability
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of Kashan University of Medical Sciences with the ethics code number of IR.KAUMS.AEC.1400.001.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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