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Placental-Derived Mesenchymal Stem Cells Restore Ovarian Function and Metabolic Profile in the Rat Model for 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. Infertility, insulin resistance, dyslipidemia, and liver dysfunction are perturbations induced by PCOS. Mesenchymal Stem Cells (MSCs) have recently emerged as a potential therapy for metabolic disorders such as PCOS due to their anti-inflammatory, antiapoptotic, proangiogenic, and proliferative properties. 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, we assigned twenty female Wistar rats into four groups (n=5): control, sham, PCOS, and PCOS-PDMSCs. We induced PCOS in the rats by administering letrozole for 21 days. PDMSCs (1×10^6 cells) were injected through the tail vein. Fourteen days after cell infusion, we evaluated 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, we measured the serum levels of cholesterol, Triglyceride (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL). Liver function was determined by evaluating Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels.

Results: The number of corpora luteum, primordial, primary, secondary, and antral follicles significantly elevated in the PCOS-PDMSCs group versus the PCOS group. The number of cystic follicles significantly decreased in the PCOS-PDMSCs group. LH and testosterone levels decreased significantly, while FSH levels increased significantly in the PCOS-PDMSCs group. Fasting blood glucose levels, fasting insulin levels, and insulin resistance notably decreased in the PCOS-PDMSCs group. The lipid profile improved in the PCOS-PDMSCs group with significant cholesterol, LDL, and TG decreases and an increase in HDL. The PCOS-PDMSCs group exhibited marked decreases in the AST and ALT levels.

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Conclusion: Our results suggest that PDMSCs are a potential treatment option for PCOS because they can effectively restore folliculogenesis, correct hormonal imbalances, modify metabolic/lipid profiles, and alleviate 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

Abbreviations: PD-MSCs: 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

Introduction

Polycystic Ovary Syndrome (PCOS) is a clinical syndrome encompassing infertility disorders associated with conditions ranging from chronic anovulation to metabolic dysfunction [1]. A triad of features can diagnose PCOS according to Rotterdam's criteria in premenopausal women: Hyperandrogenism, anovulation or amenorrhea, and polycystic ovary morphology [2]. Additionally, the other features of this syndrome include infertility, irregular menstrual cycles, hirsutism, alopecia, and acne. In addition, long-term PCOS is accompanied by an increased risk of uterine cancer and endometrial hyperplasia. These patients present elevated testosterone and LH serum levels and diminished FSH levels [3]. In 2003, the Rotterdam Consensus established diagnostic criteria for PCOS, 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. Hyperandrogenism is the most prevalent biochemical perturbation in PCOS patients [5]. An increase in the pulse amplitude and frequency of Luteinizing Hormone (LH) and the LH/FSH ratio increase androgen secretion from theca cells in polycystic ovaries [6].

On the other hand, 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 increased secretion of Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus, which results in hypersecretion of LH, increased testosterone, and decreased follicular maturation [8]. In addition, 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, were found in PCOS patients [9]. Prevalent autoimmune diseases such as thyroiditis in patients with PCOS have been reported [10]. Furthermore, there is a transition in macrophage polarization from M2 (anti-inflammatory) to M1 (proinflammatory) in PCOS. This macrophage polarization produces proinflammatory cytokines, including TNF-α and IL-6 [11,12].

Apart from disease management by lifestyle modifications (exercise, weight control, dietary changes, etc.), treatment options for PCOS patients include a combination of supplements and pharmacological interventions [13]. Conventional treatments for PCOS include oral contraceptives for menstrual derangements and hirsutism, antiandrogens for hyperandrogenism, insulin sensitizers for insulin resistance and anovulation, and 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 [14]. Moreover, conventional treatments for PCOS do not produce satisfactory results [15].

Recently, Mesenchymal Stem Cells (MSCs), due to their immunomodulatory, anti-inflammatory, and antiapoptotic properties, have been considered promising for restoring the function of damaged tissues and improving some illnesses [16]. MSCs have multiple potential functions, including self-renewal, secretion of various bioactive mediators, proliferation and differentiation into specialized cells, and migration toward damaged tissues [17]. 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 [18,19]. Moreover, several studies have indicated that MSCs have a strong potential for preventing ovarian dysfunction and uterus inflammation during infertility perturbations such as Premature Ovarian Failure (POF) [20]. MSCs effectively treat other endocrine/metabolic diseases, such as diabetes, by restoring oxidative balance, relieving inflammation, and improving insulin resistance. Since PCOS is a metabolic-reproductive-endocrine and even immunologic disorder, we assumed that MSCs could be a therapeutic option for decreasing PCOS symptoms. Therefore, the present study was carried out to assess the ability of 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 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 10 g of tissue was dissected and treated with collagenase 1 (Sigma–Aldrich) at 37°C in a 5% CO_2 incubator for two h 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. PDMSCs were 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).

Animal

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

Estrous cycle monitoring

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

Induction of PCOS and study design

Twenty female rats were assigned into four groups(n=5):

- Control, not receiving any interventions.
- Sham group, received oral gavage 1 ml of 0.5% Carboxymethylcellulose (CMC) and received tail vein injection of 1 ml saline.
- PCOS group, were administered letrozole (1 mg/kg) (Aburaihan Pharma.co., Tehran, Iran), dissolved in 0.5% CMC, orally for 28 days.
- **PCOS-PDMSCs:** The rat in the PCOS-PDMSCs group received a tail vein injection of PDMSCs (1×10^6) dissolved in 1 ml of saline.

Blood sampling

After two weeks of PDMSCs injection, the rats were fasted overnight (12 h) and then anesthetized *via* an IP infusion of ketamine (90 mg/kg) and xylazine (10 mg/kg). 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 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. Ten sections from each rat were selected and stained with hematoxylin-eosin. The numbers of corpus luteum, primordial, primary, secondary, antral, and cystic follicles were analyzed.

Follicle classification

The primordial follicle was observed to display 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, the presence of fluid accumulation between the granulosa cells was documented as a secondary follicle. Any follicles, regardless of their size, that possessed an antral cavity were classified as antral follicles. The corpus lutea showcased distinct luteal cells with a voluminous nucleus and vessels. Furthermore, a cystic 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) and was evaluated according to the following equation:

HOMA-IR=fasting insulin (mIU/l) × fasting glucose (mg/dL)/405

Statistical analysis

The data are presented as mean ± Standard Deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests with Prism 9 (GraphPad Software, Inc.). Statistical significance was set at $P<0.05$.

Results

Isolation and identification of PDMSCs

The flow cytometry results showed that the 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 (Figure 1).

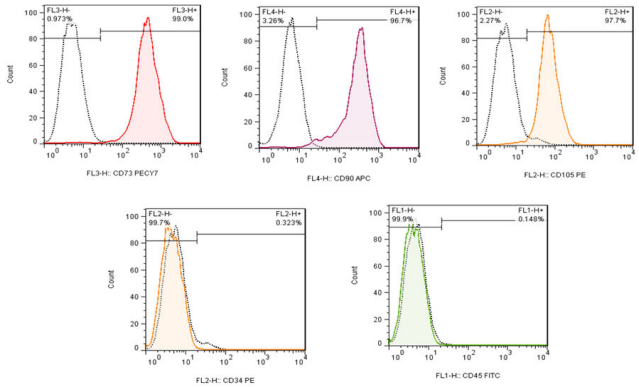

Figure 1. The characterization of human PDMSCs using flow cytometry. Isolated cells were found positive for CD73, CD90 and CD105 and negative for CD34 and CD45.

Estrus cycle analysis

As depicted in Figure 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.

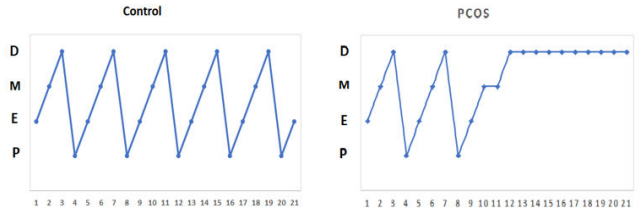

Figure 2. Representative vaginal smears from control and PCOS rats at different stages: Proestrus (P), estrous (E), metestrus (M), and diestrus (D).

PDMSCs treatment improves ovarian morphological dysfunction

Harvested ovaries were assessed using H and E staining to explore the effect of PDMSC treatment on ovarian morphology. The control group displayed normal ovarian morphology with corpora lutea 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 exhibited normal morphology (Figure 3A), a large number of corpus lutea (5.25 ± 1.18 *vs.* 1.66 ± 0.53), primordia (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 (Figure 3B). On the other hand, compared to those in the control rat, the ovaries of PCOS rats exhibited a significant increase in the number of cystic follicles. Furthermore, in the PDMSCs-PCOS group, the number of cystic follicles (9.52 ± 1.97 *vs.* 19.01 ± 0.99) significantly decreased after 14 days (Figure 3B)

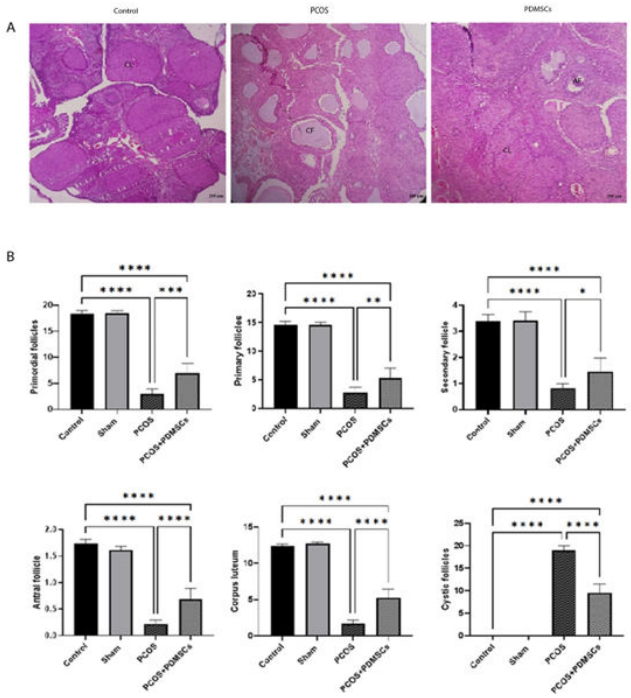

Figure 3. A) Photomicrograph of a section in ovarian tissues in all groups using hematoxylin and eosin staining ovarian tissue section of PCOS+PDMSs group showed a lot of healthy follicles. B) Quantitative analysis of the number of follicles. The PCOS+PDMSCs group shows a reduced number of cystic follicles and an increase in healthy follicles and corpus luteum (mean ± SD). *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001. CL: Corpus Luteum, AF: Antral Follicle, CF: Cystic Follicle.

Hormones assay hormone assay

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

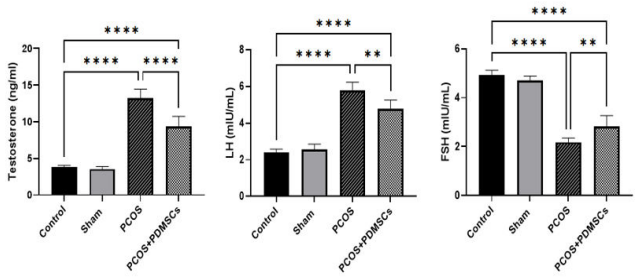

Figure 4. Evaluation of serum hormonal levels in control, sham, PCOS, and PCOS + PDMSCs group: Compared to the PCOS group, PDMSCs treatment significantly decreased serum level of testosterone and LH and increased FSH level: **p<0.005, ****p< 0.0001: Data are shown as mean ± SD. LH: Luteinizing hormone; FSH: Follicle-Stimulating Hormone.

Fasting serum glucose and insulin levels

The serum fasting blood glucose and fasting insulin levels were assessed to evaluate insulin resistance 14 days after 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) (Figure 5).

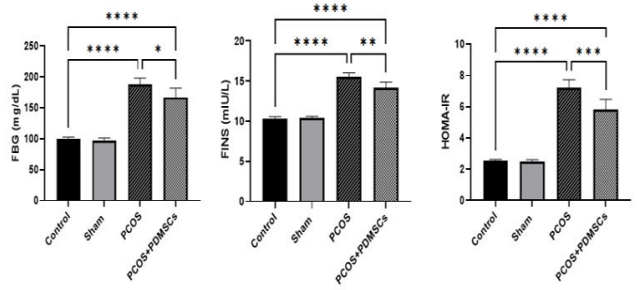

Figure 5. Evaluation of insulin resistance in Control, Sham‹PCOS and PCOS+PDMSCs group: Fasting blood glucose and insulin level as well as HOMA-IR index significantly decreased after PDMSCs intervention: *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001. Data are shown as mean ± SD. FBG: Fasting Blood Glucose, FINS: Fasting Insulin; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.

Lipid profile

The sera levels of triglycerides, cholesterol, LDL, and HDL were also evaluated to assess the effect of PDMSCs on the lipid profile. As Figure 6 demonstrates, the 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) levels 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) levels were significantly lower in the PDMSCs-PCOS 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). However, the HDL level in the PDMSCs-PCOS group was significantly greater than that in the PCOS group (27.35 ± 1.71 *vs.* 31.48 ± 2.80).

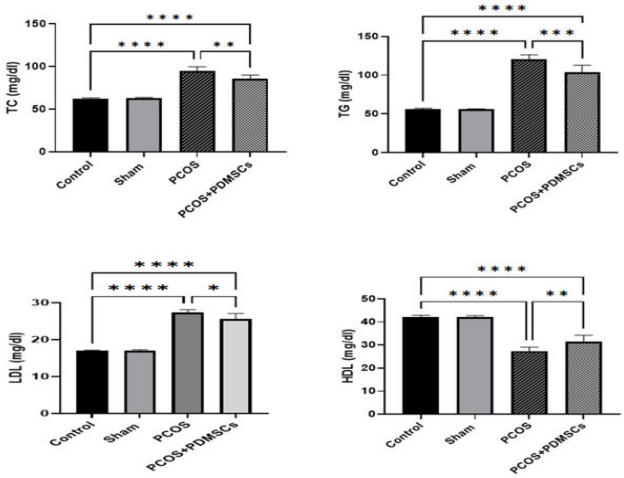

Figure 6. Evaluation of lipid profile in Control, Sham, PCOS, and PCOS+PDMSCs group: After treatment with PDMSCs, the serum level of triglyceride, cholesterol, and LDL significantly decreased, and the serum level of HDL significantly increased compared to the PCOS group: *p<0.05, **p<0.005, ****p<0.0001: Data are shown as mean ± SD. TG: Triglyceride; TC: Total Cholesterol; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein.

ALT and AST levels

The effect of the PDMSCs on liver function was assessed by measuring the serum levels of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). As Figure 7 demonstrates, compared with 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 cell infusion, the serum levels of AST (133.0 ± 10.23 *vs.* 117.2 ± 7.45) and ALT (127.2 ± 7.67 *vs.* 108.3 ± 9.56) in the PDMSCs-PSCO group were notably lower than those in the PCOS group.

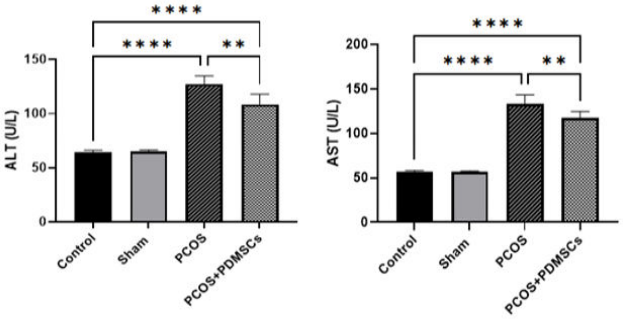

Figure 7. Evaluation of liver marker in Control, Sham PCOS, and PCOS+PDMSCs group. Injection of PDMSCs significantly increased serum level of ALT and AST compared to the PCOS group: **p< 0.005, ****p<0.0001: Data are shown as mean ± SD. AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase.

Discussion

Polycystic Ovary Syndrome (PCOS) is a medical condition impacting both the reproductive and endocrine systems and is characterized by three critical diagnostic features: Chronic anovulation, hyperandrogenism, and the presence of polycystic ovaries. The present study isolated PDMSCs from the human placenta and then infused them 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. Placenta Mesenchymal Stem Cells (PMSCs) have attracted widespread attention because of their low immunogenicity, accessible collection, lack of ethical issues, and high differentiation potential.

Here, similar to the findings of many previous investigations, we observed that treating rats with letrozole led 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 were increased after PDMSCs infusion. Additionally, PDMSCs could reduce cystic follicle numbers, which were significantly elevated in the PCOS group. In line with our findings, Kim et al., demonstrated the substantial potential of PDMSCs in restoring ovarian function in models of Premature Ovarian Failure (POF). Another study reported that injection of PDMSCs in a rat OVX model can promote primordial follicle activation.

Numerous reports have indicated that the effect of MSC transplantation on ovarian function could occur via paracrine effects and regulation of signaling pathways in the ovary. For example, Choi et al., 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., showed that MSCs can 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 developmental stages of folliculogenesis.

Additionally, Kalhori et al., 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 ovarian function. Angiotensin, Fibroblast Growth Factor-2 (FGF-2), and VEGF secreted from MSCs promote neovascularization and facilitate blood perfusion of damaged ovarian tissues. Additionally, studies have shown that androgen promotes granulosa cell apoptosis, which causes a decrease in the number of antral follicles. Wang et al., 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. Published studies have reported remarkable increases in circulating hormone levels, such as testosterone and LH, as well as lower serum FSH levels in patients with PCOS. 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 expression of the 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 persistent features of PCOS.

On the other hand, serum Sex Hormone-Binding Globulin (SHBG) levels are significantly lower in PCOS individuals. A reduction in SHBG leads to elevation-free and biologically active androgens. In alignment 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 reducing the serum testosterone and LH concentrations and upregulating the FSH level compared with those in the PCOS group. Consistent with our results, Kalhori et al., reported that MSC transplantation in PCOS models can regulate sex hormone levels, such as LH and testosterone. Additionally, Chugh et al., showed that the MSC secretome could inhibit androgen production by reducing the expression of steroidogenic-related genes such as DENND1A, CYP11A1, and CYP17A1. Additionally, in another *in vitro* study, Chugh et al., showed that BMP-2 secreted by MSCs can inhibit androgen production in the H295R cell line.

Insulin resistance is a prevalent abnormality in PCOS patients. In our study, the fasting glucose level and HOMA-IR index notably increased, indicating insulin resistance in PCOS rats. However, we found that PDMSCs improved HOMA-IR and significantly reduced FBG and FIN levels. In PCOS, serine phosphorylation of Insulin Receptor Substrate (IRS) leads to inhibited translocation of Glucose Transporter 4 (GLUT4) into the plasma membrane and reduced glucose uptake. Additionally, serine phosphorylation of IRS1 can inhibit the response to insulin receptor activation through reduced PI3K/AKT signaling. Chen et al. showed that MSCs can enhance the expression of GLUT4 and translocation activity by regulating the PI3K/AKT and MAPK pathways. In addition, hyperandrogenism activates Endoplasmic Reticulum (ER) stress. ER stress can lead to serine phosphorylation of IRS1 by activating the JNK signaling pathway. As reported by Sanap et al., MSCs can significantly decrease the expression of proteins related to ER stress, such as CHOP1 and IRE1.

Several studies have indicated interactions between hyperandrogenism and insulin resistance and liver dysfunctions such as dyslipidemia and Nonalcoholic Fatty Liver Disease (NAFLD) in patients with PCOS. Approximately 70% of women with PCOS exhibit abnormal lipid profiles. Dyslipidemia, characterized by increased

triglycerides, LDL, and cholesterol, is common in PCOS patients. Cui et al., 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. It has also been documented that testosterone can reduce LDH levels by increasing the expression of genes involved in LDH catabolism, such as SR-B1. 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 indicate the risk of NAFLD.

This study showed that letrozole adversely affected liver function and significantly enhanced LDL, TC, TG, ALT, and AST in the PCOS group. Additionally, significant reductions in HDL levels were 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 our study, Frodermann et al., reported that BM-MSCs significantly decreased serum cholesterol and low-density lipoproteins (VLDLs) in LDLR-/- mice. Additionally, transplantation of Ad-MSCs significantly improved LDL, cholesterol, and HDL levels in patients with arteriosclerosis. In addition, Shi et al., showed that injecting MSCs into ApoE-/- mice decreases total cholesterol and LDL levels. Moreover, a study reported that Hepatic Growth Factor (HGF) secreted from MSCs has antiapoptotic effects on hepatocytes and restores liver injury. Another study demonstrated that injection of UC-MSCs ameliorated NAFLD and improved lipid metabolism through upregulation of the HNF4α-CES2 pathway, which plays a vital role in lipid and glucose metabolism in the liver.

Conclusion

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. On the other hand, PCOS, as an inflammatory, endocrine, and metabolic syndrome, can be an exciting candidate for MSC therapy. Here, we 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 of the molecular mechanisms of regeneration, infertility rectification, and safety concerns associated with using PDMSCs to treat PCOS are needed.

Ethics Approval and Consent to Participate

This study was approved by the ethics committee of Kashan University of Medical Sciences with the code of ethics No (IR.KAUMS.AEC.1400.001).

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests

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Authors' Contributions

Alireza Rajabzadeh and Tahereh Mazoochi designed the project. Mojtaba Sarvestani performed the experiment. Mojtaba Sarvestani and Mansooreh Samimi analyzed the data. Alireza Rajabzade and Mojtaba Sarvestani wrote the initial draft of manuscript. Faezeh Moradi and Mohsen Navari revised the manuscript. Final edit was accomplished by Alireza Rajabzade and Faezeh Moradi.

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