Received: 11 April 2018 | Revised: 16 July 2018 | Accepted: 3 August 2018

DOI: 10.1002/jemt.23120

MICROSCOPY RESEARCH & TECHNIQUE WILEY

RESEARCH ARTICLE

The effects of human menstrual blood stem cells-derived granulosa cells on ovarian follicle formation in a rat model of premature ovarian failure

Marjan D. Manshadi[1] | Shadan Navid[1,2] | Yumi Hoshino[3] | Erfan Daneshi[4] | Parastoo Noory[1] | Mehdi Abbasi[1]

[1]Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2]Department of Anatomy, School of Medicine, Qom University of Medical Sciences, Qom, Iran

[3]Laboratory of Reproductive Endocrinology, Graduate School of Biosphere Science, Hiroshima University, Hiroshima, Japan

[4]Department of Anatomy, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

Correspondence
Mehdi Abbasi, Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Email: abbasima@tums.ac.ir

Funding information
University of Medical Sciences, Tehran University of Medical Sciences and Health Services, Grant/Award Number: 3033094

Abstract

Many studies have reported that human endometrial mesenchymal stem cells (HuMenSCs) are capable of repairing damaged tissues. The aim of the present study was to investigate the effects of HuMenSCs transplantation as a treatment modality in premature ovarian failure (POF) associated with chemotherapy-induced ovarian damage. HuMenSCs were isolated from menstrual blood samples of five women. After the in vitro culture of HuMenSCs, purity of the cells was assessed by cytometry using CD44, CD90, CD34, and CD45 FITC conjugate antibody. Twenty-four female Wistar rats were randomly divided into four groups: negative control, positive control, sham, and treatment groups. The rat models of POF used in our study were established by injecting busulfan intraperitoneally into the rats during the first estrus cycle. HuMenSCs were transplanted by injection via the tail vein into the POF-induced rats. Four weeks after POF induction, ovaries were collected and the levels of *Amh*, *Fst*, and *Fshr* expression in the granulosa cell (GC) layer, as well as plasma estradiol (E2) and progesterone (P4) levels were evaluated. Moreover, migration and localization of DiI-labeled HuMenSCs were detected, and the labeled cells were found to be localized in GCs layer of immature follicles. In addition to DiI-labelled HuMenSCs tracking, increased levels of expression of *Amh* and *Fshr* and *Fst*, and the high plasma levels of E2 and P4 confirmed that HuMenSC transplantation had a significant effect on follicle formation and ovulation in the treatment group compared with the negative control (POF) group.

KEYWORDS

Busulfan, HuMenSCs, HuMenSCs transplantation, ovary, POF

1 | INTRODUCTION

Premature ovarian failure (POF) is an infertility disorder characterized by amenorrhea and hypergonadotropic hypoestrogenism in women before the age of 40. The estimated prevalence of POF is almost 1% among women in the general population (Bandyopadhyay et al., 2003; Beck-Peccoz & Persani, 2006; Liu et al., 2014; McGuire et al., 2011; Persani, Rossetti, & Cacciatore, 2010), however, little is known about this devastating disorder. Treatment of cancer with chemotherapy and radiation is one of the major causes of POF due to toxicity-induced DNA damage in the ovary. Alkylating agents such as cyclophosphamide (CTX) and busulfan are known to affect female reproduction through the induction of ovarian cytotoxicity (Generoso, Stout, & Huff, 1971; Kalich-Philosoph et al., 2013; Sakurada et al., 2009). POF is a secondary infertility disorder characterized by high levels of gonadotropins (luteinizing hormone and follicle stimulating hormone) and low levels of gonadal hormones (Anasti, 1998). Previous studies have indicated that lack of estrogen in the inactive ovaries of these patients leads to impeded follicular growth and vaginal and uterine mucosa atrophy (Duncan, Cummings, & Chada, 1993; McGuire et al., 2011). In recent years, much attention has been paid to stem cell therapy as a treatment

modality in POF. Many researchers have investigated the use of stem cell transplant, including human menstrual blood stem cells, Adipose-derived stem cells, Human endometrial mesenchymal stem cells (HuMenSCs), and human amniotic fluid cells (HuAFCs), as a cell therapy for chemotherapy-induced ovarian damaged (Lai et al., 2015; Liu et al., 2010; Liu et al., 2014; Sun et al., 2013).

HuMenSCs are stem cell-like population that exist in menstrual blood, which express the surface markers CD9, CD29, CD41a, CD44, CD59, CD73, CD90, and CD105. These mononuclear cells can be isolated from human menstrual blood via culture expansion (Meng et al., 2007). These cells were first identified by Gargett (2004). HuMenSCs have the ability to differentiate into all the three germ layers, a strong pluripotent characteristic that has been highlighted by a number of studies (Hida et al., 2008; Meng et al., 2007; Patel et al., 2005).

Moreover, these cells are more easily extracted than other adult stem cells. Therefore, they can be an appropriate and safe source for cell therapy, with a higher ability to survive and proliferate in vitro (Borlongan et al., 2010; Mou et al., 2013).

HuMenSCs possess characteristics of both mesenchymal stem cells (high expression of the surface markers CD29, CD44, CD49f, CD90, CD105, and CD117 (and embryonic stem cells (high expression levels of Oct4 and SSEA3/4). In many studies, the ability of these cells to differentiate into other cell types such as adipocytes, osteoblasts, chondrocytes, neurons, endotheliocytes, pulmonary epithelial cells, hepatocytes, cardiac myocytes, and insulin-producing cells has been proven (Hida et al., 2008; Li et al., 2010; Lin, Xiang, Zhang, Allickson, & Xiang, 2011; Meng et al., 2007; Patel et al., 2005; Patel, Park, Kuzman, & Benetti, 2008).

In addition to the above-mentioned characteristics of HuMenSCs, we hypothesize that these cells are able to differentiate into ovarian-like cells (particularly, ovarian granulosa cells [GCs]), making them ideal for the treatment of POF. In this study, POF was induced in Rat by busulfan treatment, and HuMenSCs were injected intravenously into the rats via the tail vein. Our findings suggest that HuMenSCs have improved restorative effects on ovarian function. ELISA and real time PCR techniques were used to evaluate the various parameters in our study.

2 | MATERIALS AND METHODS

2.1 | Animal model and experimental grouping

Eight-week old female Wistar rats, weighing 200–250 g, were used for all experiments performed in this study. All procedures in this study were performed according to the guidelines of the Ethical Committee of Tehran University of Medical Sciences.

The rat models of chemotherapy-induced POF were established by intraperitoneal injection of the cytotoxic agent busulfan (Sigma, St. Louis, MO) at a dose of 36 mg/kg (Tan et al., 2010). Busulfan was injected during the first estrus cycle. The predicted period of estrus cycle of the rats was 5 days (Hubscher, Brooks, & Johnson, 2005). Rats were sacrificed 4 weeks after chemotherapy-induced ovarian damage for histologic examination and POF modeling confirmation. The rats were randomly divided into four equal groups: The positive control group (normal control rats) received no treatment. In the negative control group, rats were administered busulfan only. In the treatment group, POF rats were injected intravenously with HuMenSCs (10^6 cells per 200 μL), which had been labeled with DiI (Invitrogen, Carlsbad, CA) 1 ml of phosphate-buffered saline (PBS; Sigma, Steinheim, Germany). In the sham group, 1 ml PBS was injected intravenously to the rat models of POF using a micro injector (Sun et al., 2013).

2.2 | Isolation and culture of HuMenSCs

Mesenchymal cells were collected from ~10 ml of menstrual blood of five women (20–30 years old) on the second day of menstruation after obtaining an informed consent. This study was approved by the Ethical Committee of Tehran University of Medical Sciences (ref. no. 25110, approved April 28, 2014), and the experiments were performed according to national and international guidelines. Informed consent was obtained from all participants.

Menstrual blood samples containing the cells were carefully layered over Ficoll-Paque Plus (GE Healthcare, Amersham, UK) and centrifuged at 400*g* for 15 min to pellet erythrocytes. The supernatant was removed and the plate was added to the medium and was subsequently centrifuged at 1500*g* for 5 min. Finally, the supernatant was discarded and the harvested cell plate was resuspended in a culture medium. The cell suspension (1×10^6 cells/ml) was cultured in DMEM: F12 (1,1) medium containing 10% fetal bovine serum (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin (both from Sigma). The culture medium was replaced every 3 days. The cells were cultured for 2 weeks (Liu et al., 2014). Culture dishes were subsequently incubated in a humidified tissue culture incubator containing 5% CO_2 at 37°C. At the end of the third passage, cultured cells were injected intravenously into the rat models of POF. To determine the identity of HuMenSCs, flow cytometry was performed using a primary antibody conjugated with CD44, CD90 CD34, and CD45 (Wang, Wang, Yang, Li, & Yang, 2017).

2.3 | Flow cytometry for the identification of HuMenSCs

To characterize HuMenSCs, flow cytometry was performed using a conjugated primary antibody. The cell suspension (1×10^6 cells/ml) was stained with the primary antibody labeled with CD90, CD44, CD34, and CD45 FITC conjugate (all from BD Biosciences, San Jose, CA) for 1 hr at room temperature. An isotype antibody (mouse IgG1-FITC; BD Biosciences) was used as a negative control to correct for nonspecific binding. Flow cytometry analysis was carried out using a FC500 flow cytometer. All data were analyzed and presented using Flow Jo software (Liu et al., 2014).

2.4 | Histological assessment

Briefly, ovaries of all rats in the experimental groups were excised and embedded in paraffin (Merck, Darmstadt, Germany). Serial

TABLE 1 The primers sequence *Amh*, *Fst*, *Fshr*, and *GAPDH* of genes

Gene name	Sequence	Product size (bp)	Annealing temp
Amh	For: 5′-TAACCCATCAACCAAGCA-3′	**233**	**629**
	Rev: 5′-TAGGAAGGAGTCAGCACT-3′		
Fst	For: 5'-GCTCTCTCCCAGGTCACATT-3'	**72**	**589**
	Rev: 5'-GACTCATCCAGTAGACCACA-3′		
Fshr	For: 5′-TGGCTGAGTAAGAATGGGA-3′	**182**	**596**
	Rev: 5′-TGGTTTGGTAAGGAATGGA-3′		
GAPDH	For: 5′-AAGTTCAACGGCACAGTCAAGG-3′	**121**	**220**
	Rev: 5′-CATACTCAGCACCAGCATCACC-3′		

sections (5 μm) were cut and stained with hematoxylin–eosin (H&E) for microscopic assessment of the ovarian tissues. Hoechst nuclear staining was also performed for highlighting the nuclei (Sun et al., 2013).

2.5 | ELISA assay

The plasma levels of estradiol (E2) and progesterone (P4) in the rats were evaluated by ELISA kit (Invitrogen Life Technologies, Carlsbad, CA). According to the manufacturer's protocol, 100 μl of diluted rat plasma was added to anti-E2 or anti-P4 antibody precoated Well microtest plate and incubated for 60 min. After washing twice with PBS, the HRP-conjugated detection antibodies were added followed by a substrate solution. The absorbance of the plate was read at 450 nm with the ELISA reader (BIO-RAD Model 550 Microplate reader; Liu et al., 2014).

2.6 | Real-time PCR

The expression of specific ovarian granulosa cell genes such as *Amh* (Anti-Mullerian hormone), *Fst* (Follistatin), and *Fshr* (follicle-stimulating hormone receptor) were examined by real-time PCR. Total RNA was extracted by Trizol reagent (Ready Mini Kit, Qiagen, Valencia, CA), according to the manufacturer's protocol.

Real-time PCR was performed by cDNA synthesis kit (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit, Pittsburgh, PA) using 1 μg of total RNA, according to the manufacturer's protocol. The step one real time PCR system (Applied Biosystems, Waltham, MA) was employed in our study. All samples were normalized against the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using the comparative CT method (ΔΔCT). Primers were designed by the allele ID software. The sequences of primers are shown in Table 1 (Navid, Abbasi, & Hoshino, 2017).

2.7 | Statistical analysis

All data were expressed as mean ± SD. Statistical analysis of the results of all data were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. $p \leq .05$ was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of a rat model of chemotherapy-induced POF

In the present study, we examined the impact of busulfan on the ovaries by H&E staining, in order to *confirm* and validate the rat model of POF. In the normal ovaries, follicles of numerous stages were present and the layers of the surrounding GCs were intact (Figure 1a); however, 1 week after busulfan injection, the ovaries of the rat model of POF were predominantly composed of a high number of collapsed

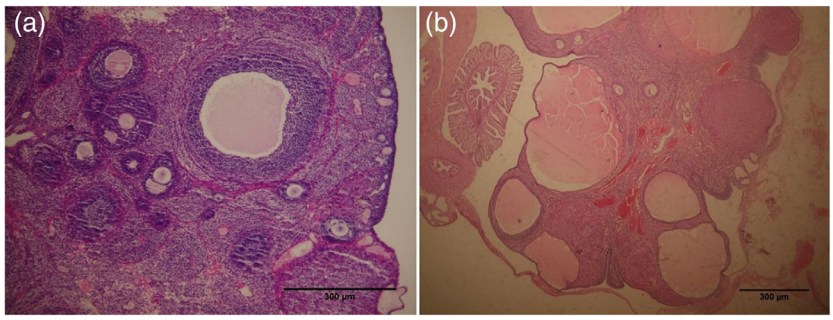

FIGURE 1 Hematoxylin–eosin (H&E) staining for the evaluation of the effect of busulfan on rat ovarian morphology. (a) Various follicles at different stages in the ovaries of a normal rat. (b) Destruction of the ovarian structure and a significant decrease in the premature ovarian failure model rat [Color figure can be viewed at wileyonlinelibrary.com]

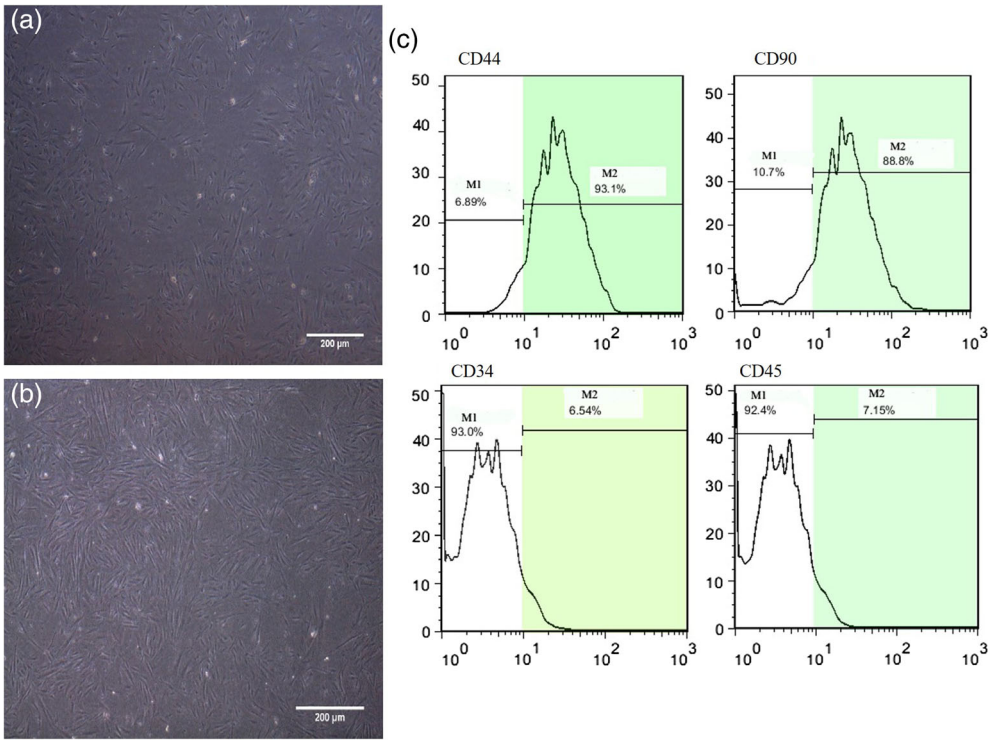

FIGURE 2 Morphology and identification of human endometrial mesenchymal stem cells (HuMenSCs). (a) HuMenSCs exhibited a spindle shape similar to fibroblast-like cells at passage 0. (b) Morphology of passage cells: Passage 3. (c) Flow cytometry analysis of HuMenSCs. The cells were positive for surface markers of CD44, CD90 and negative for surface markers of CD34, CD45 [Color figure can be viewed at wileyonlinelibrary.com]

oocytes with interstitial cells in fibrous matrix. Furthermore, a significant decrease in follicle numbers at all stages were clearly seen (Figure 1b). Thus, we confirmed that busulfan induced ovarian failure in the rat models of POF model.

3.2 | Cultivation and characterization of HuMenSCs

HuMenSCs were harvested from menstrual blood of five women (20–30 years old). The cells grew rapidly in vitro such that cells

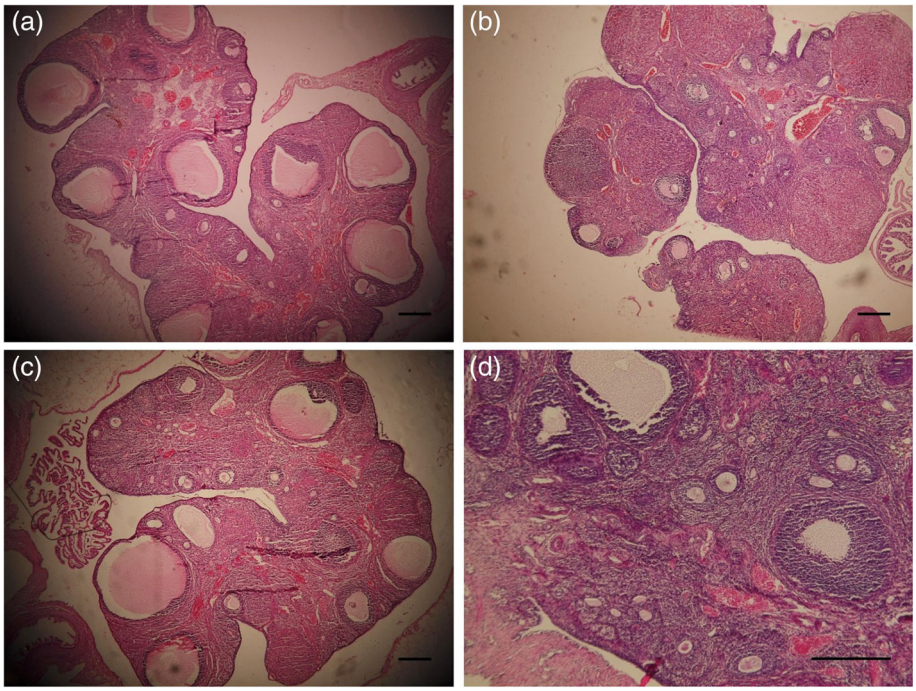

FIGURE 3 Microscopic morphology of ovarian tissue in the different groups. (a) The negative control group. (b) Positive control group. (c) Sham group. (d) Treatment group. (a and c) The number of follicles decreased significantly after injection with busulfan, with most of the follicles in primary and secondary stages. (b) This group had no treatment. (d) The population of follicles recovered after the human endometrial mesenchymal stem cells. (light microscope, scale bar = 200 μm) [Color figure can be viewed at wileyonlinelibrary.com]

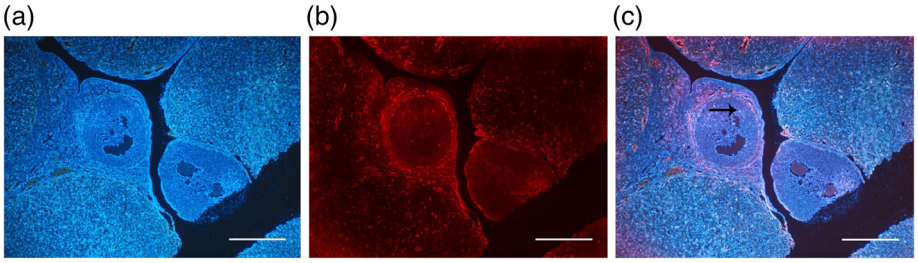

FIGURE 4 Cell labeling of human endometrial mesenchymal stem cells (HuMenSCs) and transplantation. (a) Ovarian sections stained with Hoechst for highlighting nuclei (blue). (b) Cell of HuMenSCs labeled with DiI (red). (c) Merged. A single HuMenSC cell (arrow) distinguished among granulosa cells. (fluorescence microscope, scale bar = 200 μm) [Color figure can be viewed at wileyonlinelibrary.com]

exhibiting spindle shape similar to fibroblast-like cells (Figure 2a) were observed 4 days after culture. After approximately three passages (Figure 2b), flow cytometry was used to assess the expression of surface markers such as CD44 and CD90 (specific mesenchymal stem cell markers), and CD34 and CD45 (specific hematopoietic stem cell markers). The levels of expression of these genes were as follows: CD44 (91.3% ± 3%), CD90 (88.8% ± 2%), CD34 (6.54% ± 4%), and CD45 (7.15% ± 6%; Figure 2c).

The specific mesenchymal stem cell markers were expressed by more than 85% of the population.

3.3 | Histopathological evaluation of ovarian damage

Morphological alterations in the ovaries were analyzed by histological examination using H&E staining. High number of follicles with collapsed oocytes, and many follicles predominantly in primary and secondary stages were observed in the negative control and sham groups. Moreover, the ovaries were atrophic and composed of interstitial cells in fibrous matrix in these groups. However, in the treatment group, we observed high number of healthy follicles especially secondary and antral follicles, and microscopic structure of the ovary was somewhat improved. Unlike the negative control and sham groups, in the positive control group, follicles at all stages of development were observed (Figure 3a,c).

Thus, HuMenSCs transplantation improved the structure and function of the ovaries in the rat models of POF.

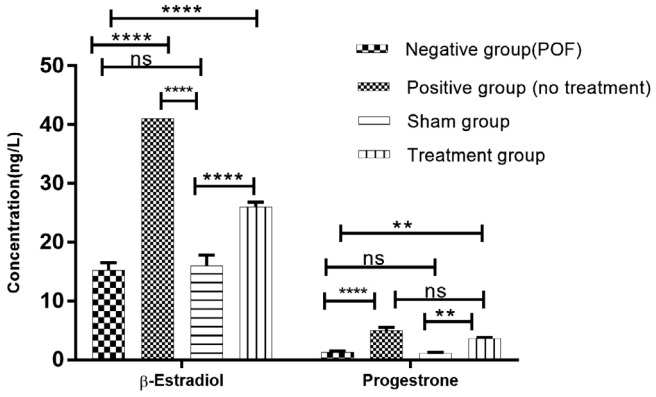

FIGURE 5 Comparison of hormone levels between the different groups. Data are expressed as means ± SD. $^{**}p \leq .01$, $^{****}p \leq .0001$. Ns, not significant

3.4 | Tracking DiI-labeled transplanted HuMenSCs

We examined the ability of these stem cells to differentiate into GCs in the busulfan-treated ovaries. DiI-labeled HuMenSCs were transplanted into the rat models of POF. The labeled cells were localized particularly to the GCs of follicles of the recipient ovaries. One month after HuMenSCs transplantation, stem cell homing and engraftment of the transplanted cells were observed (Figure 4). We observed that the labeled HuMenSCs were able to differentiate into GCs with hormone secretion function after the transplantation. We also examined the oocytes of recipient ovaries for fluorescence expression, but we did not observe any red fluorescence. These results show that HuMenSCs transplantation played a positive role in improving the structure and function of ovaries in the rat.

3.5 | Transplanted HuMenSCs improve hormone secretion in POF rats

Measurement of hormone (estradiol and progesterone) secretion was carried out using ELISA kit, and the following results were obtained: negative control group (E2:1.25 ± 15.25 ng/L; P4: 1.35 ± 0.12 ng/L), positive control group (E2:41 ± 0.005 ng/L; P4: 5.02 ± 0.47 ng/L), sham group (E2: 16 ± 1.82 ng/L; P4: 1.39 ± 0.38 ng/L), and treatment group (E2: 26 ± 0.816 ng/L; P4: 3.4 ± 0.181 ng/L). The results indicate that plasma levels of E2 and P4 in the negative control group were significantly lower than in the other three groups; E2 ($p \leq .0001$ vs. positive control and treatment group), P4 ($p \leq .0001$ vs. positive control), and P4 ($p \leq .01$ vs. treatment). However, there was no significant difference in hormone secretion when the sham group was compared with the negative control group. These results suggest that transplanted HuMenSCs improved hormone secretion in POF rats (Figure 5).

3.6 | Real-time PCR findings

Real-time PCR was used to evaluate the levels of expression of the ovarian granulosa cell-specific genes *Amh*, *Fst*, and *Fshr*. The levels of *Amh*, *Fst*, and *Fshr* expression in the treatment group were higher than in the negative control group. Moreover, levels of *Amh* and *Fshr* gene expression in the treatment group were significantly higher than compared with the negative control group. Based on these results, it appears that transplantation of HuMenSCs had positive effect on the expression levels of ovarian granulosa cell-specific genes (Figure 6).

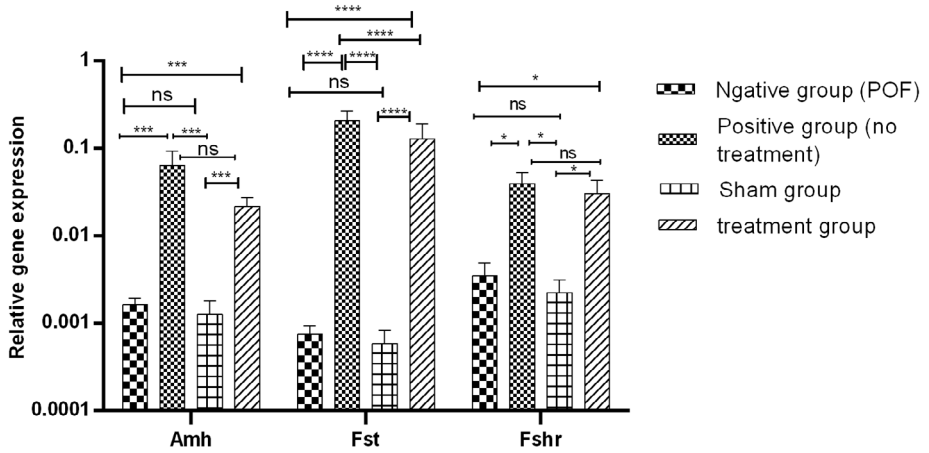

FIGURE 6 Expression patterns of *Amh*, *Fst*, and *Fshr* genes between the different groups analyzed by real-time PCR. Levels of *Amh*, *Fst*, and *Fshr* in the treatment group increased compared with the negative control group. Data are expressed as means ± SD. *$p \leq .1$, ***$p \leq .001$, ****$p \leq .0001$. Ns, not significant

4 | DISCUSSION

In the present study, we transplanted HuMenSCs into POF rats via tail vein injection. Using the current protocol, HuMenSCs were isolated from menstrual blood samples of five women. In recent years, much attention has been paid to the use of these stem cells as possible cell therapy for POF treatment. Given that these cells are derived from endometrial cells, they can play an effective role in the restoration of ovaries than other stem cells. Our results show that HuMenSCs induced the expression of granulosa cell-specific genes in the ovaries of POF rats.

Although the use of these stem cells for cell therapy has been criticized and vigorously challenged by some researchers, HuMenSCs transplantation has been used to treat irreversible genetic disorders such as muscular dystrophies, diabetes, heart failure, and spinal cord injury. As a source of mesenchymal stem cells (MSCs), HuMenSCs have several interesting features, including their ability to grow rapidly, and are minimally invasive and safe for autologous transplantation (Gargett, 2006; Hida et al., 2008; Lin et al., 2011; Meng et al., 2007; Patel et al., 2008; Rajabi et al., 2018; Santamaria, Massasa, Feng, Wolff, & Taylor, 2011). HuMenSCs can also be induced to differentiate into specific tissue or organ-like cells. These cells, however, do not develop into oocytes. As shown in Figure 4, HuMenSCs participated directly in the ovarianfollicle formation. These characteristics make HuMenSCs viable for cell therapy. Figure 2 shows that these cells express more than 85% of the specific mesenchymal markers. This finding, similar to that of Dongmei et al (2015), suggests that HuMenSCs expressed the markers of MSCs obtained from endometrial tissue (Lai et al., 2015).

In recent years, the use of MSCs as cell therapy for the treatment of POF has been investigated in many studies. MSCs from various sources have been used for this purpose, and it has been found that these cells have different abilities in improving ovarian function (Fu, He, Xie, & Liu, 2008; Song et al., 2016; Sun et al., 2013; Xiao et al., 2016). In a previous study, a successful treatment of POF mice using MSC transplantation was reported (Lai et al., 2015; Liu et al., 2014; Wang et al., 2017).

In the present study, we found that HuMenSCs improved ovarian follicular structure, however, in some studies, Adipose-derived stem cells did not have any positive effects on follicular structure or oocytes development (Sun et al., 2013).

We established a POF rat model using busulfan as a chemotherapeutic agent (Tan et al., 2010). It is well established that busulfan induces apoptosis in GCs of follicles (Fox, 1992). GCs of follicles which surround oocytes play a vital role in the development of follicles through secretion of growth factors and hormones (Chavakis, Urbich, & Dimmeler, 2008; Iorio et al., 2014). Damage to GCs can thus impair growth, development and survival of oocytes (Wang et al., 2017).

A week after the injection of busulfan, histological analyses clearly showed that the number of follicles at different stages of development, especially early antral and preovulatory, were reduced, and follicles exhibited poor development and maturity. This result is consistent with that of Wang et al (Wang et al., 2017). Chemotherapy induced fibrosis formation in ovarian stroma has also been reported, which is similar to the findings of our study (Sun et al., 2013; Wang et al., 2017).

Previous mice model studies have shown that busulfan targets GCs in follicles and spermatogonial stem cells in the testes (Brinster et al., 2003; Bucci & Meistrich, 1987).

After transplantation, HuMenSCs were tracked and found to be localized to the granulosa cell layer of follicles. In the HuMenSCs transplant group, ovarian function significantly improved, and expression levels of ovarian granulosa cell-specific genes (*Amh*, *Fst*, and *Fshr*) were increased. Consequently, hormone levels were normalized, as well, the number of follicles at all stages of development was increased. FSH is secreted by adenohypophysis of the pituitary gland. FSH receptor is expressed by GCs (Lazaros et al., 2012), which when bound by TSH, stimulates the secretion of E2. E2, on the other, has a negative feedback effect on the secretion of FSH in the hypothalamus-pituitary-gonadal axis. Under the influence of chemotherapeutic agent in the POF models, E2 levels decreased, leading to a sharp risein the level of FSH. This result is consistent with that of a previous study (Yacobi, Wojtowicz, Tsafriri, & Gross, 2004). *Amh* is

also secreted by granulosa cells in primary and early antral follicles, and its level of secretion is usually constant throughout the menstrual cycle. This hormone acts as an inhibitory growth factor in the early stages of folliculogenesis and its secretion is directly related to the size of the follicular pool (Visser, Schipper, Laven, & Themmen, 2012; Weenen et al., 2004). Therefore, evaluating the levels of ovarian granulosa cell-specific genes (*Amh*, *Fst*, and *Fshr*) and The plasma levels of (E2 and P4) can be useful in assessing the function of GCs.

These results are in line with the results of other previous studies (Liu et al., 2014; Sheikhansari, Aghebati-Maleki, Nouri, Jadidi-Niaragh, & Yousefi, 2018; Song et al., 2016; Sun et al., 2013; Woods et al., 2013; Xiao et al., 2016).

DiI-labeled HuMenSCs were localized to the ovarian stroma and GCs of follicles in the damaged tissues. Previous studies have shown that MSCs secrete cytokinins, hence, they have a positive effect on proliferation, survival and repair of damaged tissues (Cheng et al., 2013; Ding et al., 2014; Fujita et al., 2004; Wang et al., 2017). Many other previous studies have reported that MSCs implanted into damaged tissues can induce the secretion of bioactive molecules which promote repair of damaged tissues (Lai et al., 2015; Lai, Wang, Dong, & Zhang, 2014; Ranganath, Levy, Inamdar, & Karp, 2012; Schächinger et al., 2006). Likewise, Wang et al. (2017) showed that MenSC-derived conditioned media has a significant cytoprotective and anti-apoptotic properties. MenSC-derived conditioned media contain fibroblast growth factor-2 which is an essential factor for the proliferation of endometrial cells and regeneration of damaged tissues (Wang et al., 2017).

Our findings indicate that HuMenSCs can differentiate into ganulosa cells in POF. These differentiated cells can improve folliculogenesis and hormones secretion in the ovary. In conclusion, HuMenSCs transplantation can be considered as an appropriate therapy for the treatment of POF.

ACKNOWLEDGMENTS

The results described in this article were part of a student thesis. All authors want to say that this is an original article which was supported by Tehran University of Medical Sciences and they are grateful for the funding support by the University.

DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest that would prejudice the impartiality of this scientific work. There were also no conflicts of interest in designing and carrying out this current work. The authors alone are responsible for the content and writing of this article.

ORCID

Mehdi Abbasi https://orcid.org/0000-0002-4310-0381

REFERENCES

Anasti, J. N. (1998). Premature ovarian failure: An update. *Fertility and Sterility*, *70*, 1–15.

Bandyopadhyay, S., Chakrabarti, J., Banerjee, S., Pal, A. K., Goswami, S. K., Chakravarty, B. N., & Kabir, S. N. (2003). Galactose toxicity in the rat as a model for premature ovarian failure: An experimental approach readdressed. *Human Reproduction*, *18*, 2031–2038.

Beck-Peccoz, P., & Persani, L. (2006). Premature ovarian failure. *Orphanet Journal of Rare Diseases*, *1*, 9.

Borlongan, C. V., Kaneko, Y., Maki, M., Yu, S.-J., Ali, M., Allickson, J. G., … Sanberg, P. R. (2010). Menstrual blood cells display stem cell-like phenotypic markers and exert neuroprotection following transplantation in experimental stroke. *Stem Cells and Development*, *19*, 439–452.

Brinster, C. J., Ryu, B.-Y., Avarbock, M. R., Karagenc, L., Brinster, R. L., & Orwig, K. E. (2003). Restoration of fertility by germ cell transplantation requires effective recipient preparation. *Biology of Reproduction*, *69*, 412–420.

Bucci, L. R., & Meistrich, M. L. (1987). Effects of busulfan on murine spermatogenesis: Cytotoxicity, sterility, sperm abnormalities, and dominant lethal mutations. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *176*, 68–259.

Chavakis, E., Urbich, C., & Dimmeler, S. (2008). Homing and engraftment of progenitor cells: A prerequisite for cell therapy. *Journal of Molecular and Cellular Cardiology*, *45*, 514–522.

Cheng, K., Rai, P., Plagov, A., Lan, X., Kumar, D., Salhan, D., … Palestro, C. J. (2013). Transplantation of bone marrow-derived MSCs improves cisplatinum-induced renal injury through paracrine mechanisms. *Experimental and Molecular Pathology*, *94*, 466–473.

Ding, L., Li, X.'a., Sun, H., Jing, S., Lin, N., Péault, B., … Yali, H. (2014). Transplantation of bone marrow mesenchymal stem cells on collagen scaffolds for the functional regeneration of injured rat uterus. *Biomaterials*, *35*, 4888–4900.

Duncan, M., Cummings, L., & Chada, K. (1993). Germ cell deficient (gcd) mouse as a model of premature ovarian failure. *Biology of Reproduction*, *49*, 221–227.

Fox, H. (1992). The pathology of premature ovarian failure. *The Journal of Pathology*, *167*, 357–363.

Fu, X.-f., He, Y.-l., Xie, C.-h., & Liu, W. (2008). Bone marrow mesenchymal stem cell transplantation improves ovarian function and structure in rats with chemotherapy-induced ovarian damage. *Cytotherapy*, *10*, 353–363.

Fujita, M., Ishihara, M., Simizu, M., Obara, K., Ishizuka, T., Saito, Y., … Matsui, T. (2004). Vascularization in vivo caused by the controlled release of fibroblast growth factor-2 from an injectable chitosan/non-anticoagulant heparin hydrogel. *Biomaterials*, *25*, 699–706.

Gargett, C. E. (2004). Stem cells in gynaecology. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *44*, 380–386.

Gargett, C. E. (2006). Identification and characterisation of human endometrial stem/progenitor cells. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *46*, 250–253.

Generoso, W. M., Stout, S. K., & Huff, S. W. (1971). Effects of alkylating chemicals on reproductive capacity of adult female mice. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *13*, 171–184.

Hida, N., Nishiyama, N., Miyoshi, S., Kira, S., Segawa, K., Uyama, T., … Cui, C. H. (2008). Novel cardiac precursor-like cells from human menstrual blood-derived mesenchymal cells. *Stem Cells*, *26*, 1695–1704.

Hubscher, C. H., Brooks, D. L., & Johnson, J. R. (2005). A quantitative method for assessing stages of the rat estrous cycle. *Biotechnic & Histochemistry*, *80*, 79–87.

Iorio, R., Castellucci, A., Ventriglia, G., Teoli, F., Cellini, V., Macchiarelli, G., & Cecconi, S. (2014). Ovarian toxicity: From environmental exposure to chemotherapy. *Current Pharmaceutical Design*, *20*, 5388–5397.

Kalich-Philosoph, L., Roness, H., Carmely, A., Fishel-Bartal, M., Ligumsky, H., Paglin, S., … Meirow, D. (2013). Cyclophosphamide triggers follicle activation and "burnout"; AS101 prevents follicle loss and preserves fertility. *Science Translational Medicine*, *5*, 185ra62.

Lai, D., Wang, F., Dong, Z., & Zhang, Q. (2014). Skin-derived mesenchymal stem cells help restore function to ovaries in a premature ovarian failure mouse model. *PLoS One*, *9*, e98749.

Lai, D., Wang, F., Yao, X., Zhang, Q., Wu, X., & Xiang, C. (2015). Human endometrial mesenchymal stem cells restore ovarian function through improving the renewal of germline stem cells in a mouse model of premature ovarian failure. *Journal of Translational Medicine*, *13*, 155.

Lazaros, L. A., Hatzi, E. G., Pamporaki, C. E., Sakaloglou, P. I., Xita, N. V., Markoula, S. I., … Georgiou, I. A. (2012). The ovarian response to standard gonadotrophin stimulation depends on FSHR, SHBG and CYP19 gene synergism. *Journal of Assisted Reproduction and Genetics*, *29*, 1185–1191.

Li, H.-Y., Chen, Y.-J., Chen, S.-J., Kao, C.-L., Tseng, L.-M., Lo, W.-L., … Twu, N.-F. (2010). Induction of insulin-producing cells derived from endometrial mesenchymal stem-like cells. *Journal of Pharmacology and Experimental Therapeutics*, *335*, 817–829.

Lin, J., Xiang, D., Zhang, J.-l., Allickson, J., & Xiang, C. (2011). Plasticity of human menstrual blood stem cells derived from the endometrium. *Journal of Zhejiang University-Science B*, *12*, 372–380.

Liu, T., Huang, Y., Zhang, J., Qin, W., Chi, H., Chen, J., … Chen, C. (2014). Transplantation of human menstrual blood stem cells to treat premature ovarian failure in mouse model. *Stem Cells and Development*, *23*, 1548–1557.

Liu, T., Xu, F., Xiling, D., Lai, D., Liu, T., Zhao, Y., … Cheng, W. (2010). Establishment and characterization of multi-drug resistant, prostate carcinoma-initiating stem-like cells from human prostate cancer cell lines 22RV1. *Molecular and Cellular Biochemistry*, *340*, 265–273.

McGuire, M. M., Bowden, W., Engel, N. J., Ahn, H. W., Kovanci, E., & Rajkovic, A. (2011). Genomic analysis using high-resolution single-nucleotide polymorphism arrays reveals novel microdeletions associated with premature ovarian failure. *Fertility and Sterility*, *95*, 1595–1600.

Meng, X., Ichim, T. E., Zhong, J., Rogers, A., Yin, Z., Jackson, J., … Chan, K. W. (2007). Endometrial regenerative cells: A novel stem cell population. *Journal of Translational Medicine*, *5*, 57.

Mou, X.-z., Lin, J., Chen, J.-y., Li, Y.-f., Wu, X.-x., Xiang, B.-y., … Xiang, C. (2013). Menstrual blood-derived mesenchymal stem cells differentiate into functional hepatocyte-like cells. *Journal of Zhejiang University. Science B*, *14*, 961–972.

Navid, S., Abbasi, M., & Hoshino, Y. (2017). The effects of melatonin on colonization of neonate spermatogonial mouse stem cells in a three-dimensional soft agar culture system. *Stem Cell Research & Therapy*, *8*, 233.

Patel, A. N., Geffner, L., Vina, R. F., Saslavsky, J., Urschel, H. C., Kormos, R., & Benetti, F. (2005). Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: A prospective randomized study. *The Journal of Thoracic and Cardiovascular Surgery*, *130*, 1631–1638 e2.

Patel, Amit N, Eulsoon Park, Michael Kuzman, Federico Benetti, Francisco J Silva, and Julie G Allickson. 2008. 'Multipotent menstrual blood stromal stem cells: Isolation, characterization, and differentiation', *Cell Transplantation*, *17*: 303–11.

Persani, L., Rossetti, R., & Cacciatore, C. (2010). Genes involved in human premature ovarian failure. *Journal of Molecular Endocrinology*, *45*, 257–279.

Rajabi, Z., Yazdekhasti, H., Mugahi, S. M. H. N., Abbasi, M., Kazemnejad, S., Shirazi, A., … Zarnani, A.-H. (2018). Mouse preantral follicle growth in 3D co-culture system using human menstrual blood mesenchymal stem cell. *Reproductive Biology*, *18*(1), 122–131.

Ranganath, S. H., Levy, O., Inamdar, M. S., & Karp, J. M. (2012). Harnessing the mesenchymal stem cell secretome for the treatment of cardiovascular disease. *Cell Stem Cell*, *10*, 244–258.

Sakurada, Y., Kudo, S., Iwasaki, S., Miyata, Y., Nishi, M., & Masumoto, Y. (2009). Collaborative work on evaluation of ovarian toxicity dose studies and fertility study of busulfan in female rats. *The Journal of Toxicological Sciences*, *34*, SP65–SP72.

Santamaria, X., Massasa, E. E., Feng, Y., Wolff, E., & Taylor, H. S. (2011). Derivation of insulin producing cells from human endometrial stromal stem cells and use in the treatment of murine diabetes. *Molecular Therapy*, *19*, 2065–2071.

Schächinger, V., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., … Hamm, C. W. (2006). Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *New England Journal of Medicine*, *355*, 1210–1221.

Sheikhansari, G., Aghebati-Maleki, L., Nouri, M., Jadidi-Niaragh, F., & Yousefi, M. (2018). Current approaches for the treatment of premature ovarian failure with stem cell therapy. *Biomedicine & Pharmacotherapy*, *102*, 254–262.

Song, D., Zhong, Y., Qian, C., Zou, Q., Jian, O., Shi, Y., … Li, H. (2016). Human umbilical cord mesenchymal stem cells therapy in cyclophosphamide-induced premature ovarian failure rat model. *BioMed Research International*, *2016*, 13.

Sun, M., Wang, S., Li, Y., Yu, L., Fang, G., Wang, C., & Yao, Y. (2013). Adipose-derived stem cells improved mouse ovary function after chemotherapy-induced ovary failure. *Stem Cell Research & Therapy*, *4*, 80.

Tan, S.-J., Yeh, Y.-C., Shang, W.-J., Wu, G.-J., Liu, J.-Y., & Chen, C.-H. (2010). Protective effect of a gonadotropin-releasing hormone analogue on chemotherapeutic agent-induced ovarian gonadotoxicity: A mouse model. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *149*, 182–185.

Visser, J. A., Schipper, I., Laven, J. S. E., & Themmen, A. P. N. (2012). Anti-Müllerian hormone: An ovarian reserve marker in primary ovarian insufficiency. *Nature Reviews Endocrinology*, *8*, 331–341.

Wang, Z., Wang, Y., Yang, T., Li, J., & Yang, X. (2017). Study of the reparative effects of menstrual-derived stem cells on premature ovarian failure in mice. *Stem Cell Research & Therapy*, *8*, 11.

Weenen, C., Laven, J. S. E., von Bergh, A. R. M., Cranfield, M., Groome, N. P., Visser, J. A., … Themmen, A. P. N. (2004). Anti-Müllerian hormone expression pattern in the human ovary: Potential implications for initial and cyclic follicle recruitment. *MHR: Basic Science of Reproductive Medicine*, *10*, 77–83.

Woods, D. C., White, Y. A. R., Niikura, Y., Kiatpongsan, S., Lee, H.-J., & Tilly, J. L. (2013). Embryonic stem cell–derived granulosa cells participate in ovarian follicle formation in vitro and in vivo. *Reproductive Sciences*, *20*, 524–535.

Xiao, G.-Y., Cheng, C.-C., Chiang, Y.-S., Cheng, W. T.-K., Liu, I.-H., & Shinn-Chih, W. (2016). Exosomal miR-10a derived from amniotic fluid stem cells preserves ovarian follicles after chemotherapy. *Scientific Reports*, *6*, 23120.

Yacobi, K., Wojtowicz, A., Tsafriri, A., & Gross, A. (2004). Gonadotropins enhance caspase-3 and-7 activity and apoptosis in the theca-interstitial cells of rat preovulatory follicles in culture. *Endocrinology*, *145*, 1943–1951.

How to cite this article: Manshadi MD, Navid S, Hoshino Y, Daneshi E, Noory P, Abbasi M. The effects of human menstrual blood stem cells-derived granulosa cells on ovarian follicle formation in a rat model of premature ovarian failure. *Microsc Res Tech*. 2019;82:635–642. https://doi.org/10.1002/jemt.23120