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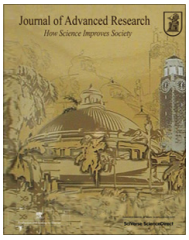

ORIGINAL ARTICLE

Therapeutic efficacy of amniotic membrane stem cells and adipose tissue stem cells in rats with chemically induced ovarian failure

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ABSTRACT

The present study was conducted to compare between the therapeutic efficacies of human amniotic membrane-derived stem cells (hAM-MSCs) vs. adipose tissue derived stem cells (AD-MSCs) in cyclophosphamide (CTX)-induced ovarian failure in rats. Forty-eight adult female rats were included in the study; 10 rats were used as control group. Thirty-eight rats were injected with CTX to induce ovarian failure and divided into four groups: ovarian failure (IOF) (IOF group), IOF + phosphate buffer saline (PBS group), IOF + hAM-MSCs group and IOF + AD-MSCs group. Serum levels of FSH and estradiol (E2) were assessed. Histopathological examination of the ovarian tissues was performed and quantitative gene expressions of *Oct-4*, *Stra8* and *integrin beta-1* genes were conducted by quantitative real time PCR. Results showed that IOF and IOF + PBS rat groups exhibited decreased ovarian follicles, increased interstitial fibrosis with significant decrease of serum E2, significant increase serum FSH level and significant down-regulation of *Stra8* and *integrin beta-1*. In hAM-MSCs and AD-MSCs rat groups, there were increased follicles and corpora with evident the presence of oocytes, significant increase in serum E2, significant decrease in serum FSH levels (in hAM-MSCs treated group only) and significant up-regulation of the three studied genes with higher levels in hAM-MSCs treated rats group when compared to AD-MSCs treated rats group. In Conclusion, administration of either hAM-derived MSCs or AD-MSCs exerts a significant therapeutic efficacy in chemotherapy induced ovarian insult in rats. hAM-MSCs exert higher therapeutic efficacy as compared to AD-MSCs.

Introduction

Premature ovarian failure (POF) or premature ovarian insufficiency (POI) is defined as cessation of menstrual periods (amenorrhea for 4 months or more), increased levels of FSH to menopausal level (usually over 40 IU L^{-1} obtained on 2 occasions at least 1 month apart), and diminished levels of

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estrogens (estradiol levels less than 50 pg mL^{-1}) before the age of 40 [1].

At present about 25% of all forms of POF can be classified as iatrogenic and related to cancer treatment [2] such as chemotherapy or radiotherapy. Also, POI may be caused by autoimmunity, genetic factors as Turner's syndrome, fragile X syndrome and inhibin alpha *(INHA)* gene affection. In addition, metabolic disorders such as classic galactosemia, virus infection such as HIV and mumps, toxins and lifestyle factors such as cigarette smoking are associated with the development of POI [3]. However, the majority of cases remain idiopathic.

Bone marrow derived MSCs (BM-MSCs) are the most common source of clinically used MSCs. However, adult BM has limitations; it needs invasive harvesting which is accompanied by a risk of infection. It yields a small number of cells. In addition, the number, differentiation potential, and life span of BM-MSCs decline with patient age [4].

Amniotic membrane (AM)-derived stem cells are isolated from amnion that is normally discarded. These cells are therefore readily available, and ethical concerns regarding the isolation of stem cells from the amniotic membrane are minimized [5]. In addition to that, amniotic stem cells have not been found to form teratomas when transplanted *in vivo* [6]. Furthermore, hAM-MSCs may be considered superior to adult MSCs in their proliferation and differentiation potential [6].

hAM-MSCs express pluripotency markers including *OCT4*, *SOX2*, and *NANOG* [7], high expansion *in vitro* and multilineage differentiation capacity potential into cells derived from the three germ layers [8]. However, at this time whether hAM-MSCs can restore ovarian function is unclear.

Oct-4 gene is required for primordial germ cell survival and loss of Oct4 function leads to apoptosis of primordial germ cells (PGCs) so it is essential for maintaining viability of mammalian germ line [9]. The discovery of the essential role of *Oct4* in induced pluripotent stem (iPS) cell technology further highlights its irreplaceable function in establishing pluripotency [10].

Integrins are transmembrane receptors that link the extracellular matrix (ECM) environment with intracellular signaling, thus regulating multiple cell functions such as cell survival, proliferation, migration, and differentiation. *CD29* (*integrin beta-1*) gene expression is believed to be crucial for tissue repair [11].

Stra8 is a gene that is specifically expressed in mammalian germ cells before their transition from mitosis to meiosis [12]. It is a regulator of meiotic initiation in females [13] so it is considered a meiotic inducer [14] and a pre-meiosis specific marker [15].

To address stem cells' therapeutic potential in ovarian failure we compared the therapeutic efficacy between isolated hAM-MSCs (cells from fetal tissues) and human ADMSCs (cells from adult tissues) in supporting follicular development, hormonal production by the ovary and expression of pluripotent markers, germ cells markers and anchoring receptors in experimental animal model of chemotherapy induced ovarian failure.

Material and methods

This study is a prospective case control animal study performed in the Faculty of Medicine, Cairo University, at the Unit of Biochemistry and Molecular Biology. The design of this work was divided into 2 steps: *in vitro* and *in vivo* study.

In vitro study

Isolation and propagation of hAM-MSCs and AD-MSCs from human source

Placentas were freshly collected postpartum from Gynecology and Obstetric Department, Faculty of Medicine Cairo University. The amniotic membranes were mechanically separated from placentas taken from normal full-term uncomplicated elective cesarean sections after obtaining a written informed consent. They were transported in cold phosphate buffer saline (PBS; Gibco/Invitrogen, Grand Island, New York, USA) solution in a thermally insulated container on ice. The amniotic membranes were processed within 1 h and subjected to collagenase II enzymatic digestion. Collagenase II solution was prepared at 100 U/mL in DMEM and 15 mL of collagenase II solution was added to the amniotic membranes and incubated for 2 h at 37 °C in 5% CO_2 atmosphere with occasional shaking. Digested amniotic membranes were filtered through a 40 μm cell strainer to remove large tissue aggregates.

After enzyme digestion and filtration, cells were washed in phosphate-buffered saline (PBS) (Cellgro, USA) and centrifuged at 350 g for 5 min. The cell pellet was resuspended in the basal culture medium, low glucose Dulbecco's Modified Eagle's Medium (LG-DMEM; Gibco BRL, USA) containing 10% FBS (Fetal bovine serum; Gibco BRL, USA). The cells were seeded into T75 polystyrene cell culture flasks (Nunc, USA) and incubated in a humidified atmosphere with 5% CO2. The basal culture medium was changed 3 times a week and passaged once the cells reached 80–90% confluency [16].

Human adipose tissue was collected by human abdominal subcutaneous adipose tissue aspiration during surgery after obtaining an informed medical consent. It was transported on ice insulated container. The adipose tissue was washed extensively with PBS to remove contaminating debris and red blood cells then minced with scissors and enzymatic digestion was performed as described with amniotic membranes with collagenase II in PBS for 60 min at 37 °C with gentle agitation. Digested tissue was filtered through a 200-μm mesh filter to remove debris and centrifuged at 600 g for 5 min to obtain cellular pellets. Erythrocytes were removed by treatment with erythrocyte lysis buffer. The cells were cultured as described for the cells isolated from amniotic membranes [17].

Labeling the MSCs to detect their homing into the ovarian tissue in the animal model

MSCs were labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma–Aldrich) and injected into the tail vein of rats. After one month of injection, the ovarian tissue was examined with a fluorescence microscope to detect the cells stained with PKH26 dye to ensure homing and trace the injected cells in the ovarian tissue.

Characterization of isolated human hAM-MSCs and AD-MSCs

Quantitative expression of mesenchymal stem cells, amniotic membrane and adipose tissue markers was conducted with using CYTOMICS FC 500 Flow Cytometer (Beckman coulter,

Table 1 PCR primers sequence.

Oct-4 (POU class 5 homeobox 1, Pou5f1) GenBank® Accession Number NM_001009178.1	Forward primer: 5′ CCTGCAGCAGATCACTAGCAT 3′ Reverse primer: 5′ CACTCGAACCACATCCCTCT 3′
CD29 (*integrin beta-1*) GenBank® Accession Number NM_017022.2	Forward primer: 5′ GACCCCTCCGAGAGGCGGAA 3′ Reverse primer: 5′ TGGCCGGAGCTTCTCTGCCAT 3′
Stimulated by retinoic acid gene 8 (Stra8) GenBank® Accession Number XM_001067836.1	Forward primer: 5′ CAGATCATCGAGTTTTTCAAAGG 3′ Reverse primer: 5′ TCCACAGGAGGATCTGGTTC 3′
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Gene Bank® accession number NC_000019.9	Forward primer: 5′-GAAGGTGAAGGTCGGAGTCA-3′ Reverse primer: 5′-GAAGATGGTGATGGGATTTC-3′

FL, USA) and analyzed using CXP Software version 2.2. Monoclonal antibodies used were *CD45 FITC, CD34 PE, CD 29 PE, CD 44 PE, CD 90 PE, and CD 73 PE.*

In vivo study

Experimental animals

The study included fifty mature white albino female rats of proven fertility from an inbred colony (Curl: HEL1) of matched age and weight at the Kasr Al-Aini animal experimental unit, Faculty of Medicine, Cairo University. Their median age at the beginning of the experiment was 6 months with a range from 5–7 months and their median weight was 200 g with a range 170–230 g. The rats were inbred in the Experimental Animal Unit, Faculty of Medicine, Cairo University. *All animals were maintained according to the standard guidelines of Institutional Animal Care and Use Committee*. They were maintained in an air-conditioned animal house with specific pathogen-free conditions. They were kept 5 per cage. Animals were fed a semi-purified diet that contained the following (g/kg): 200 casein, 530 sucrose, 100 cellulose, 100 fat blends, 35 vitamin mix, and 35 mineral mix and a free access to water was allowed. Rats were subjected to a 12:12-h daylight/darkness. They were observed for 15 days prior to commencing treatment to ensure adequate adaptation.

Induction of ovarian failure and injection of stem cells

Group I includes 10 rats, which were chosen randomly as a negative control group (normal healthy rats) while the other 40 rats were included in the study group which in turn, were administered a single intraperitoneal injection of 35 mg/kg of Cyclophosphamide (CTX) (Endoxan, Baxter, Germany). CTX destroys the existing pre- and post-meiotic germ cell pools [18]. Three weeks after CTX, ovaries from two rats were dissected from the anterior end of the uterus and the surrounding adipose tissues were carefully removed. Ovarian failure was confirmed after histopathological examination. One week after confirming induction of ovarian failure, the remaining rats in the study group ($n = 38$) were randomly divided into 4 groups:

Group II (IOF) comprised 8 rats with induced ovarian failure that did not receive any more injections (a positive control group).

Group III rats (IOF + PBS, $n = 10$) were injected once phosphate buffer saline (PBS) only.

Group IV rats (IOF + hAM-MSCs, $n = 10$) were injected intravenously once via the tail vein by hAM-MSCs (5×10^6 cells) in a volume of 0.3 mL of 0.1 M PBS (pH 7.4).

Group V rats (IOF + AD-MSCs, $n = 10$) were injected intravenously once via the tail vein by AD-MSCs (5×10^6 -cells) in a volume of 0.3 mL of 0.1 M PBS (pH 7.4).

No transplant-related deaths were reported.

MSCs labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma–Aldrich) were injected into the tail vein of all relevant rat groups.

Estimation of serum FSH and E2

Serum FSH and E2 levels were assessed by ELISA kits [CUSABIO, USA] every 15 days to assess ovarian function till the end of the study (30 days after injection of stem cells). Blood samples were collected in the morning from retro-orbital vein of all rat groups; serum was separated and stored at -20 °C until analyzed.

Quantitative real time PCR for OCT4, Stra 8 and Integrin genes expression in ovarian tissue

The 2 ovaries of each rat were harvested: one for histopathological examination and the other was made into frozen sections and then further processed for RNA extraction using SV Total RNA Isolation system (Promega, Madison, WI, USA) followed by Reverse Transcriptase for cDNA synthesis (#K1621, Fermentas, USA) and qRT-PCR for *OCT4, Stra 8 and Integrin beta-1* gene expression in ovarian tissue. Histopathology was assessed by hematoxylin and eosin stained tissues prepared paraffin impeded sections 30 days after stem cells transplantation.

Real-time quantitative analyses

The relative abundances of the mRNA species were assessed by the SYBR® Green method using StepOnePlus™ Real-Time PCR Systems (Applied Biosystems, Foster City, CA, USA). The PCR primers used were designed with Gene Runner Software (Hastings Software Inc., Hastings, NY, USA) from RNA sequences in GenBank (Table 1). All of the primer sets had a calculated annealing temperature of 60 °C. Quantitative RT-PCR analyses were performed in duplicate in a 25-μL reaction volume consisting of 2 × SYBR Green PCR Master Mix (Applied Biosystems, USA), 900 nM of each primer, and 2–3 μL of cDNA. The amplification conditions were 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at

60 °C for 10 min. Data from the real-time assays were calculated by Software version 1.7 (PE Biosystems, Foster City, CA, USA). The relative expression levels of *Oct-4, Stra8 and integrin beta-1* were calculated by the comparative Ct method as stated by the manufacturer recommendations (Applied Biosystems, USA).

Statistical analysis

Statistical Package for Social Studies (SPSS) program version 16.0.1 (SPSS Inc., Chicago, IL, USA) was used. Numerical data were expressed as mean ± standard deviation. For comparisons between treatment groups, the null hypothesis was tested by a single-factor ANOVA. Multiple comparisons were conducted using Tukey–Kramer test post hoc tests. Comparisons were considered statistically significant if $p < 0.05$.

Results

In vitro study

Identification, homing and characterization of hAM-MSCs and AD-MSCs

hAM-MSCs and AD-MSCs were identified in culture by their fusiform fibroblast like-structure (Fig. 1).

Homing of PKH26 fluorescent labeled hAM-MSCs and AD-MSCs was detected in ovarian tissue by fluorescent microscope (Fig. 2).

Flow cytometric characterization analyses of hAM-MSCs and AD-MSCs showed that hAM-MSCs were negative for *CD34-* and were uniformly positive for $CD29^+$ $CD44^+$. AD-MSCs were negative for $CD45^-$ and were uniformly positive for $CD90^+$ $CD73^+$ (Fig. 3).

In vivo study

E2 levels were assessed 15 days and 30 days after injection of hAM-MSCs and AD-MSCs. Results showed that there was a significant decrease in E2 levels in IOF and in IOF + PBS groups versus control (15.5 ± 0.78 and 15 ± 0.79 after 15 days versus 50.35 ± 21.8 in control rats, $p < 0.001$ and 13.1 ± 1.6 and 13.4 ± 1.4 after 30 days versus 54.1 ± 15.8 in control rats respectively, $p < 0.001$). The use of either hAM-MSCs or AD-MSCs led to a significant elevation in E2 levels with more superior therapeutic effects with hAM-MSCs as compared to AD-MSCs (61.2 ± 11.5, $p < 0.05$ and 30.5 ± 12.6, $p < 0.01$ after 15 days versus 50.35 ± 21.8 in control rats and 64.1 ± 7.9; non-significant difference and 38.9 ± 10.2, $p < 0.01$ after 30 days versus 54.1 ± 15.8 in control rats respectively) (Table 2 and Fig. 4).

FSH levels were assessed 15 days and 30 days after injection of hAM-MSCs and AD-MSCs. Results showed that there was a significant elevation of FSH levels in POF and in POF + PBS groups versus control group (82.2 ± 19.8 and 83.6 ± 20.1 versus 36.6 ± 8.0 in control rats, $p < 0.001$ after 15 days, 89.3 ± 20.3 and 90 ± 20.4 versus 39.6 ± 7.4 in control rats after 30 days respectively, $p < 0.001$). The use of hAM-MSCs led to a significant decrease in FSH levels as compared to POF and POF + PBS groups (62.3 ± 21.7 after 15 days, $p < 0.05$ and 58 ± 9.4 after 30 days, non-significant difference versus 36.6 ± 8.0 and 39.6 ± 7.4 in control rats). The use of AD-MSCs did not lead to decrease in FSH levels as compared to control and IOF rat groups. (78.6 ± 15.9 after 15 days, $p < 0.01$ and 82.1 ± 9.5 after 30 days, $p < 0.01$ versus 36.6 ± 8.0 and 39.6 ± 7.4 in control rats, $p < 0.05$) (Table 2 and Fig. 5).

As regards gene expression, results showed that there was a significant downregulation of *OCT-4, Stra8 and integrin beta-1*

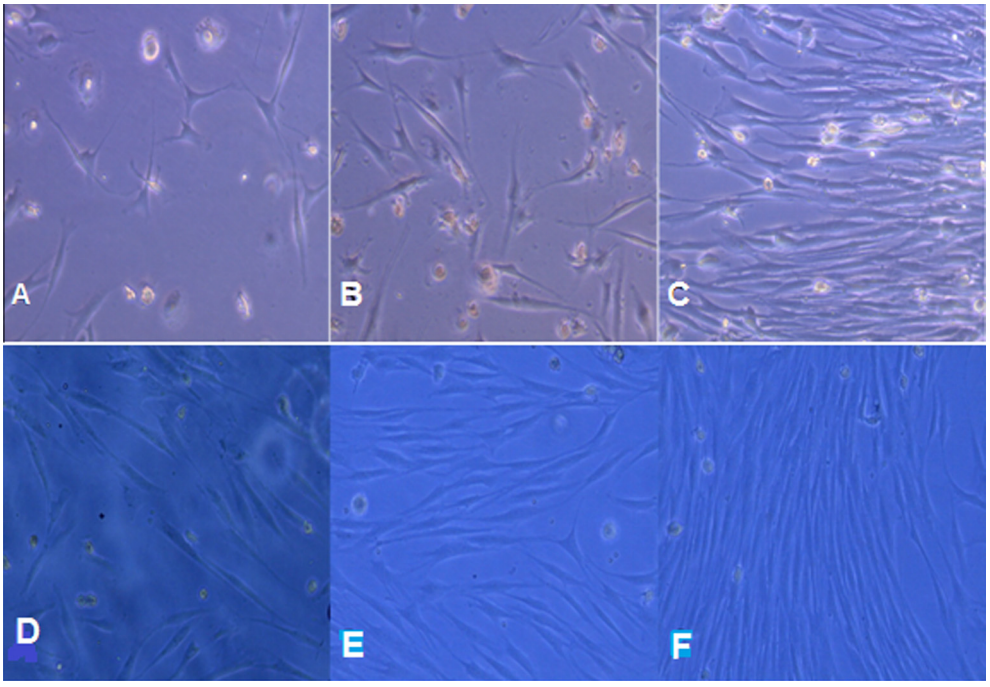

Fig. 1 Isolated and cultured hAM-MSCs (plates A, B, C) and AD-MSCs (plates D–F): (A and D) Stem cells propagated for 2 days, (B and E) Stem cells reached 40–50% confluence at 7 days, (C and F) Stem cells reached 80–90% confluence at 14 days.

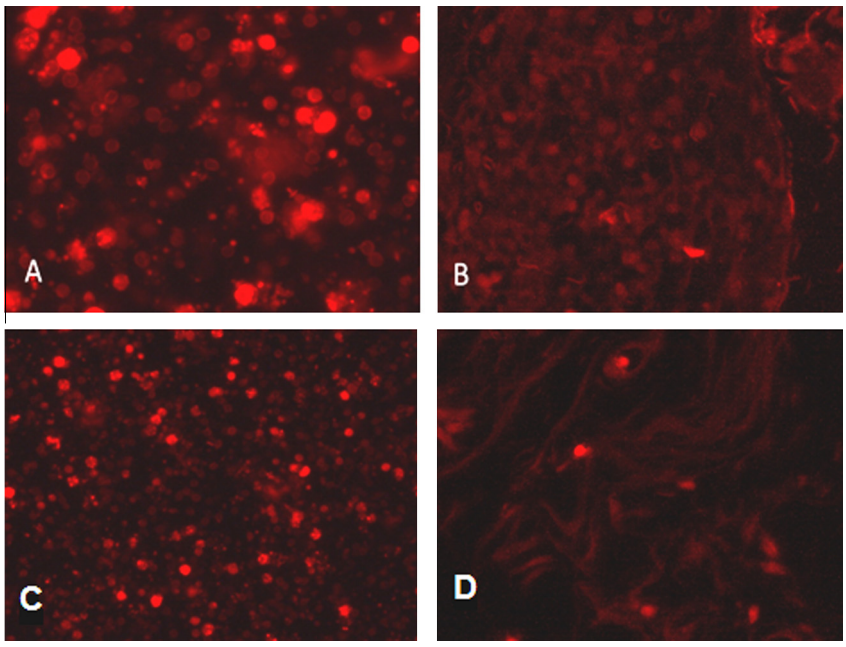

Fig. 2 Homing of PKH26 fluorescent labeled hAM-MSCs (plates A and B) and AD-MSCs (plates C and D) in ovarian tissues of rat.

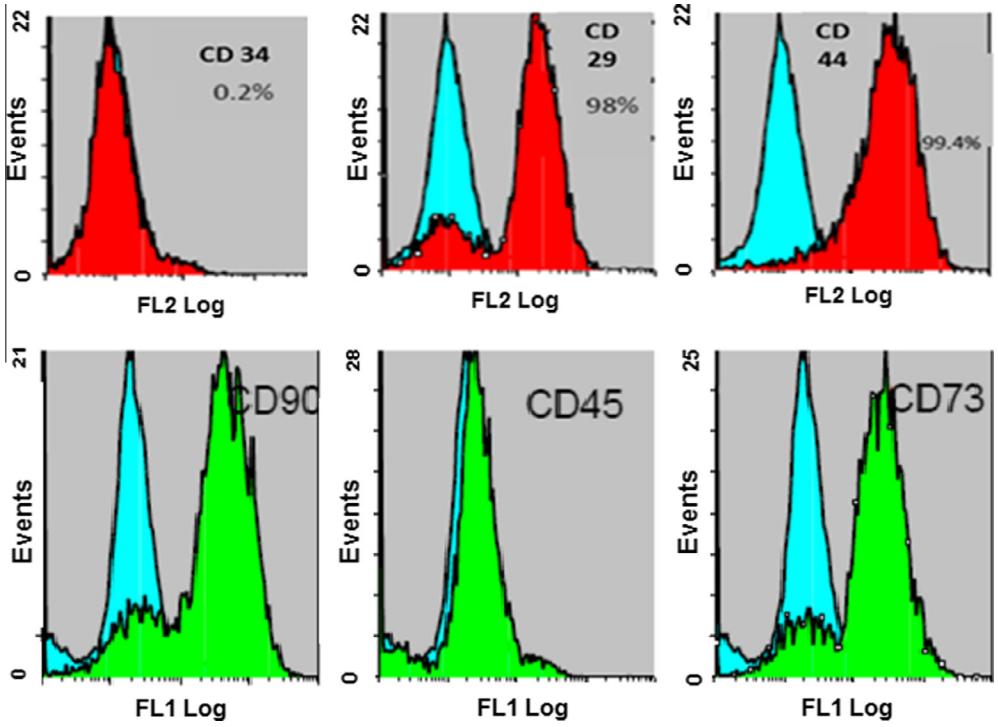

Fig. 3 Flow cytometric characterization analyses of hAM-MSCs (upper plates) and AD-MSCs (lower plates). hAM-MSCs were negative for *CD34*- and were uniformly positive for $CD29^{+}$ $CD44^{+}$. AD-MSCs were negative for $CD45^{-}$ and were uniformly positive for $CD90^{+}$ $CD73^{+}$.

in IOF (0.17 ± 0.09, 0.14 ± 0.03, 0.16 ± 0.02 respectively) and in IOF + PBS (0.21 ± 0.04, 0.14 ± 0.03, 0.16 ± 0.03) groups versus control (0.25 ± 0.06, 0.38 ± 0.1, 0.87 ± 0.18, non-significant changes with *OCT-4*, $p < 0.05$ for *Stra8* and $p < 0.01$ for *integrin beta-1*). The use of either hAM-MSCs or AD-MSCs led to a significant elevation of all genes with more superior effect with hAM-MSCs (0.59 ± 0.09, $p < 0.01$, 0.69 ± 0.09, $p < 0.01$, 2 ± 0.4, $p < 0.001$) versus AD-MSCs regarding *integrin beta-1* gene only (0.39 ± 0.07, 0.43 ± 0.08, 0.45 ± 0.07, $p < 0.01$ for all genes) (Table 2 and Fig. 6).

As regards the total number of ovarian follicles and corpora lutea in a cross section of the right ovarian in each of the studied rat groups, results showed that there was a

Table 2 E2 levels, FSH levels and gene expressions of OCT-4, STRA-8 and Integrin beta-1 in the studied rat groups.

	Control ($n = 10$)	IOF ($n = 8$)	IOF + PBS ($n = 10$)	IOF + AM-MSCs ($n = 10$)	IOF + AD-MSCs ($n = 10$)
FSH (mIU/mL)	36.6 ± 11.8 on 15 days	82.2 ± 30.4 on 15 days	81.5 ± 28.8 on 15 days	62.3 ± 30.4 on 15 days	78.6 ± 24.1 On 15 days
	39.6 ± 11.7 on 30 days	89.3 ± 30.8 on 30 days	88.5 ± 23.9 on 30 days	58 ± 14.6 on 30 days	82.2 ± 15.9 on 30 days
E2 (pg/mL)	50.4 ± 21.8 on 15 days	15.6 ± 0.78 on 15 days	15.03 ± 0.79 on 15 days	62.2 ± 11.5 on 15 days	30.5 ± 12.6 on 15 days
	54.1 ± 15.8 on 30 days	13.2 ± 1.6 on 30 days	13.9 ± 1.4 on 30 days	64.1 ± 7.9 on 30 days	38.7 ± 10.2 on 30 days
Oct-4	0.25 ± 0.06	0.17 ± 0.09	0.21 ± 0.04	0.59 ± 0.09	0.39 ± 0.07
Stra-8	0.38 ± 0.1	0.14 ± 0.03	0.18 ± 0.03	0.69 ± 0.09	0.43 ± 0.08
Integrin beta-1	0.87 ± 0.18	0.18 ± 0.02	0.16 ± 0.03	2 ± 0.4	0.45 ± 0.07
Number of ovarian follicles	15 ± 4	4 ± 2	4 ± 2	18 ± 5	13 ± 3

significant decrease in their numbers in IOF and in IOF + PBS rat groups as compared to control group (5 and 5 versus 15 in control, $p < 0.001$). The use of either hAM-MSCs or AD-MSCs led to a significant elevation in ovarian follicles and corpora lutea numbers when compared to IOF rat groups (19, $p < 0.001$ and 12, $p < 0.01$ versus 5 in IOF groups) and normalization of their numbers when compared to control rat group (Table 2 and Fig. 7).

As regards histopathology examination, results showed that IOF and IOF + PBS exhibited a decrease in number of ovarian follicles with no oocytes inside, evidence of fibrosis and cystic changes (Fig. 8).

The use of either hAM-MSCs or AD-MSCs led to an evident increase in numbers of maturing follicles and corpora lutea with apparent oocytes inside (Fig. 9).

Discussion

Cyclophosphamide is an alkylating agent that induces the formation of intra chain and inter chain DNA cross linking resulting in interference with DNA replication specially in rapidly dividing cells. It is well known that cyclophosphamide massively eliminates granulosa cells [19] which are needed for oocyte survival and follicle development. In the present study cyclophosphamide was used to induce experimental model of ovarian failure in rats. Ovarian failure was confirmed in our study by histopathological examination, the significant decrease in E2 levels, the significant increase in FSH levels and the significant decrease in numbers of ovarian follicles and corpora lutea in ovarian tissues. These findings were also reported in a recent study [20] that confirmed the exhaustion of primordial follicles and follicular atresia with severe impairment in ovarian function which resulted in POF after cyclophosphamide treatment in mice.

In this study, we demonstrated that both of hAM-MSCs and AD-MSCs significantly increased the number of ovarian follicles and corpora lutea and restored certain fertility parameters such as E2 and FSH levels in a rat model of POF induced by cyclophosphamide. However, hAM-MSCs showed more significant therapeutic effects than AD-MSCs regarding all the assessed parameters. Similar studies reported that amniotic stem cells have great potential to differentiate into primitive oocyte and participate in folliculogenesis within ovaries of induced ovarian failure [20]. As regards the superior therapeutic effects of hAM-MSCs versus AD-MSCs, this finding can be explained by the multilineage potentials of amniotic membrane stem cells and also the presence of side population cells (SP cells) in human amniotic membranes. SP cells have multilineage potentials to several cell lineages with unique immunological characteristics such *as HLA I* (-)/*II* (-) *or HLA I*+/*II* (-) [21].

Previous studies demonstrated that follicular atresia is mainly caused by apoptosis of follicular cells, particularly granulosa cells which are essential for follicular development [22]. The therapeutic effects of hAM-MSCs on ovarian function resulted from paracrine secretion of some trophic factors such as TGF-β [23], vascular endothelial growth factor [24], and glia cell derived neurotrophic factor [25], that are essential for follicular development and can inhibit follicular cell atresia and follicles apoptosis [26,27]. These findings agreed with our results that demonstrated the significant increase in number of

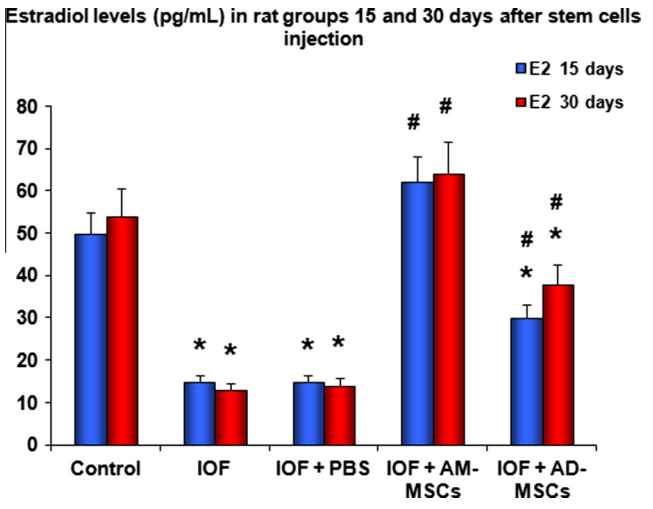

Fig. 4 Serum E2 levels 15 days and 30 days after stem cells injection in the studied groups. * significant p value < 0.05 versus control group. # significant p value < 0.05 versus IOF group. Y axis represents E2 levels in pg/mL.

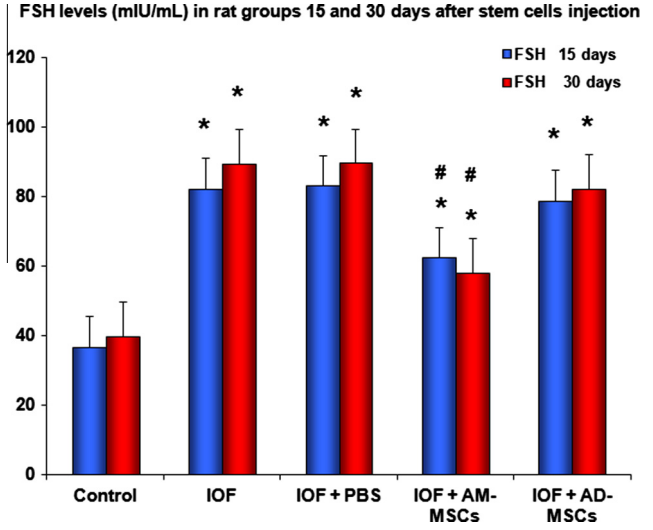

Fig. 5 Serum FSH levels 15 days and 30 days after stem cells injection in the studied groups. * significant p value < 0.05 versus control group. # significant p value < 0.05 versus IOF group. Y axis represents FSH levels in mIU/mL.

ovarian follicles, the significant increase in E2 levels and the significant decrease in FSH levels in IOF rat groups that received either hAM-MSCs or AD-MSCs when compared to untreated IOF rat groups. However, the assessment of the paracrine trophic factors was not evaluated in our study.

Other studies reported similar findings, and Wang and his colleagues [28] used hAECs to restore ovaries of chemotherapy-induced ovarian failure in mice. The transplanted hAECs homed to mice ovaries and survived, differentiated into granulosa cells. The restored ovarian cells developed follicles at all stages. Lai et al. [2] suggested that the transplanted hAFCs differentiated into granulosa, not germ cells and that hAFCs directed follicle formation and may enhance folliculogenesis via indirect tropism on ovarian

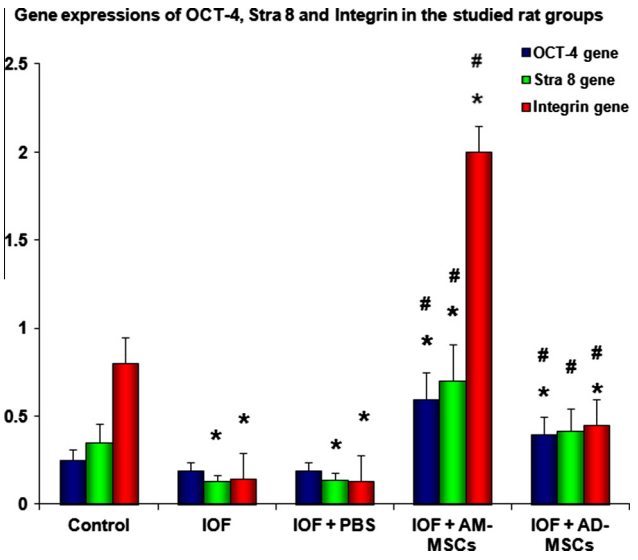

Fig. 6 Relative gene expressions of Oct-4, Stra 8 and Integrin-beta-1 in the studied rat groups. * significant p value < 0.05 versus control group. # significant p value < 0.05 versus IOF group. Y axis represents relative gene expression values.

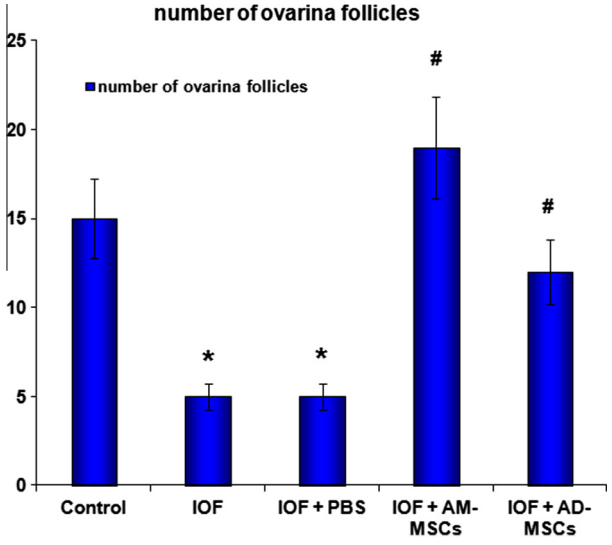

Fig. 7 The total number of ovarian follicles and corpora lutea in a cross section of the right ovary in each studied rat groups. * significant p value < 0.05 versus control group. # significant p value < 0.05 versus IOF group. Y axis represents the number of ovarian follicles in each studied rat group.

tissue in the chemically damaged mice ovary as evidenced by increased expression of AMH after hAFCs transplantation in the ovaries of sterilized mice. Conversely, AMH expression is negative in the positive control groups.

As regards gene expressions in the present study, there was a non-significant decrease in *OCT4* levels in IOF groups. However, there was a significant elevation in *OCT4* levels in IOF rat groups that received hAM-MSCs and AD-MSCs. Gene expression of *OCT4* is one of the molecular triggers involved in the commitment to meiosis [29]. This fact is also supported by Bahmanpour et al. [30] who thought that a

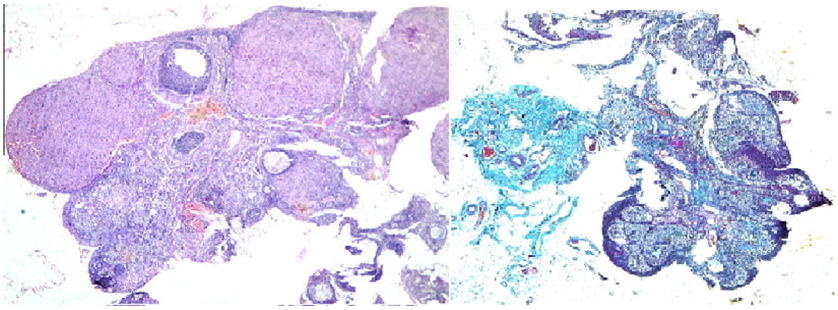

Fig. 8 A cross section in the right ovary of a rat from the IOF group showing only 6 follicles, 3 of them are cystic with no oocyte inside (H & E) (left plate) and a cross section in the right ovary of a rat from the IOF + PBS group showing no follicles or corpora lutea; fibrosis is evident with Masson's trichome (right plate).

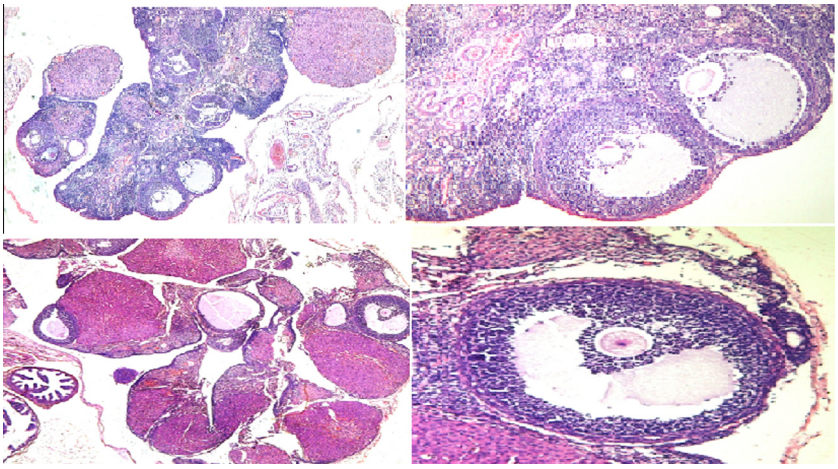

Fig. 9 A cross section in the right ovary of a rat from the IOF + AM-MSCs group (upper plates) and IOF + AD-MSCs group (lower plate) showing many mature follicles and corpora lutea with apparent oocytes inside them (upper plates).

decrease in the number of primordial germ cell and an increase in atretic and antral follicles could be attributed to the lower expression of *OCT4*. On the same way Abdel Aziz et al. [31] proved increased expression of *Oct4* in the rats with azoospermia when they received BM-MSCs. Moreover, the authors stated that MSCs could transdifferentiate into premeiotic and postmeiotic cells when transplanted in a suitable microenvironment niche for germ cells development [31]. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice [13].

More noteworthy, the observed significant increase in the *OCT4* gene expression in hAM-MSCs and the AD-MSCs rat groups means completion of the prophase I of meiotic division and oocyte growth. These concepts were supported by Pesce et al. [29].

As regards *integrin beta-1 (CD29),* there was a significant decrease in its levels in IOF rat groups. There was a significant increase in hAM-MSCs and AD-MSCs rat groups when compared to IOF groups. The observed significant increase in *intergrin beta-1* in hAM-MSCs and AD-MSCs rat groups may suggest that stem cell transplantation may induce differentiation of the oogonia to produce more mature oocytes. Similar to our findings, Phillips et al. [32] detected increased expression of *β1 integrin* when the human spermatogenetic stem cells from azoospermia patients were induced to make sperm-like cells (became more differentiated). Also Abdel Aziz et al. [31] proved increased expression of *integrin β1* in the rats with azoospermia when they received BM-MSCs.

Stra8 is a specifically expressed gene in mammalian germ cells. Germ cells undergo transition from mitosis to meiosis during the activation of transcription of *Stra8 and Rec8*, which is required for meiotic DNA replication and the subsequent processes of meiotic prophase [12,13,33].

In the present study, *Stra8* gene expression showed a significant decrease in IOF rat groups versus control group, whereas there was a significant increase in its levels in hAM-MSCs and AD-MSCs rat groups when compared to IOF rat groups indicating increased entry in meiotic division to generate oocytes with subsequent improvement in the oogenesis. These results agree with Niikura et al. [34] who stated that increased *Stra8* gene expression is related to neo-oogenesis induction in young female mice. Similar to our results Abdel Aziz et al. [31] proved increased expression of *Stra8* in the rats with azoospermia when they received BM-MSCs.

Conclusions

Administration of either hAM-derived MSCs or AD-MSCs exerts a significant therapeutic efficacy on chemotherapy induced ovarian insult in rats. hAM-MSCs exert higher therapeutic efficacy as compared to AD-MSCs.

Conflict of Interest

The authors have declared no conflict of interest.

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