Journal of
Stem Cell Research & Therapy

Faruk et al., J Stem Cell Res Ther 2018, 8:12
DOI: 10.4172/2157-7633.1000442

Research Article Open Access

Does Exosomes Derived Bone Marrow Mesenchymal Stem Cells Restore Ovarian Function by Promoting Stem Cell Survival on Experimentally Induced Polycystic Ovary in Adult Female Albino Rats? (Histological and Immunohistochemical Study)

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Abstract

Poly Cystic Ovarian Syndrome (PCOS) is increasingly reported nowadays. Recently exosomes released by Mesenchymal Stem Cells (MSCs-EX) has been a novel source with a great potential donor cell in regenerative medicine because of their low immunogenicity and easy accessibility. *Foeniculum vulgare* (FVE) is a naturally occurring estrogen compound, which is commonly used today due to the impact of the female hormones.

In this study, we evaluated the therapeutic effect of exosome released by MSCs in experimentally induced Poly Cystic Ovary (PCO). The polycystic disease was artificially induced by injecting rats daily with testosterone propionate (dissolved in propylene glycol) 1 mg/100 g body weight for 35 days. Exosomes were prepared for 3rd passage of MSCs-EX and were injected into the induced PCO rats. Another group of PCOS rats received FVE (alcoholic extract) 150 mg/kg body weight/day intra gastric for five days after induction of PCOS. The ovaries were taken for histological examination and immune-histochemical detection of Octamer-Binding Transcription Factor (OCT4), and the hormonal assay was evaluated. Both groups infused with Bone Marrow Mesenchymal Stem Cells (BM-MSCs) derived exosome and FVE have mild to moderate improvement in the histological ovarian structure by the presence of normal follicles in different stages with normal hormonal profile and highly expressive of OCT4. BM-MSCs derived exosome and FVE have moderate modulates the immunohistological structure of the PCOS ovaries, which may be a factor in the maintenance of steroid-induced PCOS.

Keywords: Polycystic ovary; BM-MSCs derived exosome; Foeniculum vulgare; OCT4

Abbreviations: PCOS: Poly Cystic Ovarian Syndrome; MSCs: Mesenchymal Stem Cells; FVE: Foeniculum Vulgare; PCO: Poly Cystic Ovary; OCT4: Octamer-Binding Transcription Factor; BM-MSCs: Bone Marrow Mesenchymal Stem Cells; OS: Oxidative Stress; PBS: Phosphate Buffer Saline

Introduction

MSCs are a varied population of multipotent stromal stem cells that could be delivered from multiple tissues [1]. Exosomes are small vesicles as products of endocytosis and can be released physiologically under ordinary circumstances by all types of cells [2]. Due to their ability for transmitting hereditary information, they have a vital role in the treatment of many diseases such as liver fibrosis, acute kidney injury and myocardial infarction [3]. PCOS is one of the famous feminine endocrine diseases in the reproductive age and the most common cause of anovulatory and infertility [4]. Chereau published the first description of the disease in 1844, as the change of ovarian morphology [5]. The European Society for Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) in 2003 were published. The diagnostic criteria of the syndrome, according to many studies established during the last decades, in what is called Rotterdam Consensus Criteria [6]. PCOS is a disease with high genetic incidence, and its clinical manifestations mainly include menstrual disorder, secondary amenorrhea, abnormal hormonal serology, loss of hair, obesity, acne, and infertility [7].

PCOS has been considered as a chronic systemic disease and it is mostly associated with hyper-androgenemia, Oxidative Stress (OS) and chronic inflammation, though the mechanism of pathogenesis has not been well-defined [8]. Many studies have proved that OS level is significantly increased in patients with PCOS, as when these OS were measured by circulating markers, such as Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) [9]. OS level is also observed to be significantly associated with obesity, hyper-androgenemia, and chronic inflammation [10], however, OS is considered as an etiological pathogenesis of PCOS [11], but it is still controversy whether the abnormal levels of OS levels of patients with PCOS derive from the disease or from its complications [12].

FVE is a Mediterranean aromatic seed plant. It is used commonly as a spice and in traditional medicine and as. It was well known that FVE used as a laxative, diuretic, analgesic, bronchodilator and antipyretic [13].

Phytoestrogen is an active biological substance content present in FVE which can act similar to estrogen [14], that, increase breast milk production, elevate libido, increase menstrual flow, relieve

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Received November 30, 2018; **Accepted** December 13, 2018; **Published** December 20, 2018

Citation: Faruk EM, El desoky RE, El-Shazly AM, Taha NM (2018) Does Exosomes Derived Bone Marrow Mesenchymal Stem Cells Restore Ovarian Function by Promoting Stem Cell Survival on Experimentally Induced Polycystic Ovary in Adult Female Albino Rats? (Histological and Immunohistochemical Study). Stem Cell Res Ther 8: 442. doi: 10.4172/2157-7633.1000442

indigestion and cough [15]. FVE has a clear protective effect against ethanol-induced gastric lesions, which is related to decreases in lipid peroxidation and antioxidant activity [16]. FVE relieves menopausal symptoms in women [17]. In a study, it was reported that FVE could be used as a safe and effective herbal drug for primary dysmenorrhea as used in comparison with mefenamic acid [18]. In Iranian folk medicine, it has been claimed that FVE improves sexual function and infertility in women, however, there is no documented study to clarify this effect.

A study showed that the fennel seeds extract has *in vitro* antioxidant activity and this is important as agonist the oxidative mechanism of PCOS [19].

So for above condition, the aim of our study is to show the efficiency of using BM-MSCs-EX derived exosome in the restoration of hormonal profile and folliculogenesis of induced PCOS in a rat model and the therapeutic effect of MSCs-EX is comparable to FVE with determining the role of oogenesis, meiotic and apoptotic regulators as OCT4.

Materials and Methods

Materials

Testosterone propionate powder was purchased from NAMAA Pharmaceuticals (El-Monofia, Egypt). It was administered orally as a daily dose of testosterone propionate (dissolved in propylene glycol) 1 mg/100 g body weight for up to 35 days [20].

FVE seeds were bought from Egypt market. The samples were ground by an electrical mill. The aqueous extract was prepared by cold maceration of 100 g of powdered FVE in 500 ml of distilled water for 24 h. Then, the filtered extract was concentrated, dried in vacuum, and the residue was stored in a refrigerator at 2-8°C for use in subsequent experiments. The gavage extract was prepared by solving the powder in a specific volume of normal saline. Multiple doses of FVE at a dose of 150 mg/kg were administered intra-gastrically [21].

Isolation and characterization of BM-MSCs

Rat BM-MSCs were purchased from the Biochemistry Department, Faculty of Medicine, Cairo University. MSCs culture was prepared according to the method described by Yamazoe, et al. [22]. All cultures were examined using an inverted microscope: Leica DM IL LED with camera Leica DFC295 (Leica Microsystems CMS GmbH, Wetzlar, Germany). BM-MSCs were injected through intravenous route at a single dose of 3×10^6 cells suspended in 0.5 ml Phosphate Buffer Saline (PBS) [23] in which isolation of BM-MSCs was done by flushing out of BM from tibias using PBS (Grand Island, New York, USA) then centrifuged at 1,000 rpm for 5 min. The BM-MSCs were cultured with an RPMI medium (Gibco BRL, USA), 10% fetal bovine serum (FBS, Gibco BRL, USA), and maintained in a cell culture incubator containing 5% CO_2 at 37°C. At 80-90% BM-MSCs confluence, they were detached with 0.25% trypsin-EDTA (Gibco BRL, USA), then sub-cultured in new flasks. Characterization of BM-MSCs was showed in culture by the presence of spindle-shaped like cells. The flow cytometry (Beckman Coulter) also were used to phenotypeing BM-MSCs which were suspended (1×10^6 cells/ml) and stained with FITC conjugated monoclonal antibodies, CD29 (Biolegend), and CD90 (Biolegend) [24].

Isolation and identification (MSCs-EX)

MSCs-EX were delivered from supernatants of third passage BM-MSCs (5×10^6 cells/ml), which cultured in RPMI deprived of FBS and supplemented with 0.5% of Bovine Serum Albumin (BSA) (Sigma). This supernatant centrifuged at 2000 g for 20 min to remove debris, cell-free supernatants were centrifuged at 100,000 g (Beckman Coulter Optima L 90K ultracentrifuge) for 1 h at 4°C, washed in serum-free medium 199 containing HEPES 25 mM (Sigma). Analysis of BMVs was done by electron microscopy. Detection of homing of MSCs-EX into ovarian tissue in rats, they were labeled with PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) then injected into the tail vein of PCOS, 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.

Animals

In this study, 40 adult females within reproductive age (10-14 weeks) albino rats of weight 150-200 g were used. The rats exhibiting at least two consecutive 4-5 days' estrous cycles only were included in this study. The animals were obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University. Cleaning measures and strict care were used to keep the animals in a normal healthy state; the animals were kept in animal cages under the prevailing atmospheric conditions. They were kept on a normal balanced diet and tap water.

This study was done according to the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85-23, revised 1996). All animal experiments received approval from the Institutional Animal Care Committee of the Faculty of Medicine, Benha University, Egypt.

Experimental procedure

Fifty mature white albino rats were divided into two groups:

(1) Group I (the control group) included 10 rats that had no treatment

(2) Group II that was subdivided equally into four subgroups (10 rats in each group)

(a) Subgroup IIa (polycystic ovary group): included 10 rats that were administered a daily oral dose of testosterone propionate 1 mg/100 gm for up to 35 days

(b) Subgroup IIb (MVs group): included 10 rats that were administered a daily oral dose of testosterone propionate 1 mg/100 gm for up to 35 days, and then MSCs-EX (derived from 5×10^6 BM-MSCs/rat) suspended in 0.6 ml and was administered intravenously in the tail vein of rats

(c) Subgroup IIc (FVE group): included 10 rats that were administered a daily oral dose of testosterone propionate 1 mg/100 gm for 35 days, and then FVE (alcoholic extract) 150 mg/kg/day intra-gastric for 5 days after induction

Hormonal assay

Blood was collected from the tail vein and centrifuged at 4,000 rpm for 20 min to separate the serum. The level of serum testosterone, estrogen levels, Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH) were assessed with a CUSABIO Reagent Kit (USA).

Histological and immunohistochemically study

The animals were sacrificed after 6 weeks from the start of the experiment. A small specimen of the ovary was fixed in 10% formalin for 24 h. The paraffin sections were prepared and stained with H and E, Periodic Acid Schiff's reaction (PAS) and Masson's trichrome for histochemical study [25]. The specimens for Immuno-histochemical staining for OCT4 using the avidin-biotin-peroxidase complex in

which ovarian sections were incubated with mouse monoclonal antibody and counterstained with Meyer's hematoxylin. OCT4 showed a positive brown reaction in granulosa cells of the follicle and interstitial tissue of normal ovary while negative reaction observed in follicular tissue of damaged ovary its reaction was restricted to stroma only [26]. Negative controls were done by processing additional ovary specimens in the same way but skipping the application of the primary antibody.

Morphometric and statically study

The mean area % of collagen fiber deposition OCT4 expression was quantified in 10 images for ovary sections from 10 rats in each group using Image-Pro Plus program, version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA). Collagen fiber deposition and estrogen expression in groups IIb, IIc, and IId were compared with group IIa (the polycystic group) using the t-test, with a P value less than 0.05 considered as the level of statistical significance. Statistical analyses were processed using IBM SPSS Statistics Software for Windows, version 20 (IBM Corp., Armonk, New York, USA).

Results

Identification of BM-MSCs and (MSCs-EX)

BM-MSCs were identified *in vitro* by using the inverted microscope in which appeared as fibroblast-like cells (Figure 1A). They were having a positive high expression of CD 90 (98.52%) and CD29 (97.46%) (Figure 1B). MSCs-EX were identified by the appearance of spheroids MVs in electron microscopy micrographs (Figure 2A). Homing of PKH26 fluorescent labeled MSCs-EX was detected in ovarian tissue by a fluorescent microscope (Figure 2B).

Hormonal assay and ovarian size results

The mean ovarian size was 4.3+12.4 mm 3 in control ovaries while in PCOS groups they were 13.1+9.4. Macroscopic examination of the ovary of PCOS shows the presence of vascular multiple follicles (Figure 3). Serum testosterone, LH/FSH ratio, and estradiol levels were higher in PCOS than in controls and MSCs-EX groups. However, all above level was moderately higher in PCOs with FVE administration. There was a statistically significant difference between groups p=0.001 after MSCs-EX injection. FSH levels were statistically significantly higher in induced PCOS (P<0.01) (5.1+1.4 IU/liter) compared to the control group (5.8+2.7 IU/liter), while there were no statistically significant differences between the MSCs-EX treated group (P>0.01) (5.6 ± 1.5 mlU/ml) and FVE (5.3+1.9 IU/litre). Estradiol showed significant reduction after treatment with MSCs-EX and FVE (66.1+26.6 pg/ml) (77.4+21.5 pg/ml) respect compared to control (70.0+17.4 pg/ml) (P<0.001) and PCOS (97.6+21.1 pg/ml, P<0.001). Injection of MSCs-EX successfully restored the testosterone levels back to its normal values (47.6+51.1 ng/ml, P<0.01), compared to their corresponding untreated PCOS group (Table 1 and Figure 4).

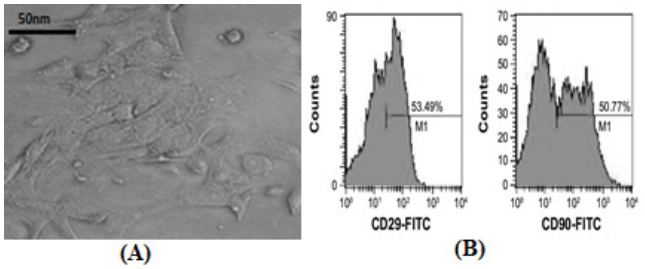

Figure 1: Identification of MSCs: **(A)** Inverted microscope showing MSCs as fibroblast like cells (spindle branched shaped (arrow) (200X); **(B)** Image analyzer showing positive expression of CD29 and CD 90.

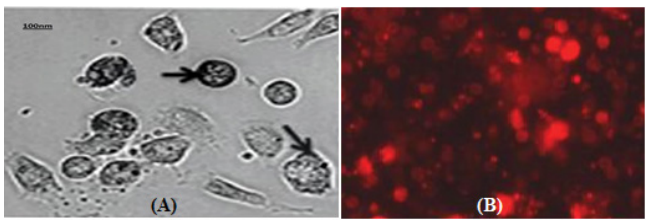

Figure 2: Identification of MSCs-EX: **(A)** electron microscopy showing spheroids MSCs-EX (arrow) (100 nm); **(B)** Homing of PKH26 fluorescent labeled MSCs-EX was detected in ovarian tissue by fluorescent microscope (arrows) (1000X).

Histological results

Hematoxylin and eosin-stained sections: The control group showed the normal ovarian histological architecture, in which ovary covered with simple cubical germinal epithelium and has highly cellular cortex, it contains primordial, primary, secondary, antral follicles, corpora lutea and corpora albicans where medulla contain veins and arteries (Figure 5A).

After induction of the PCOS model, their group showed bulged surface epithelia with multiple cysts as well as degenerated cortical and medulla areas. Also, ovary tissues showed the absence of developing follicles, corpus lutea with congested blood vessels (Figure 5B) In contrast, after FVE treatment ovarian sections showed mild

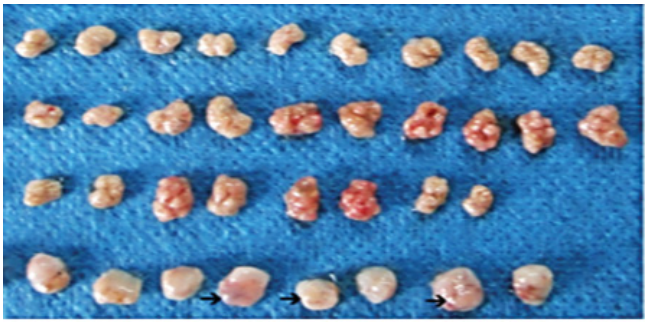

Figure 3: Macroscopic pictures of ovaries in different groups in which ovary showing multiples highly vascularized follicle and corpus luteum.

Groups/Hormonal level	Control group	PCOS group	MSCs-EX group	FVE group
LH (IU/liter)	8.4 ± 20.2	9.3 ± 5.3	7.5 ± 3.3	6.5 ± 3.3
FSH (IU/liter)	5.8 ± 2.7	5.1 ± 1.4	5.6 ± 1.5	5.3 ± 1.9
LH/FSH ratio	1.2 ± 0.5	1.7+0.6[a]	1.3[a] -0.8	1.4[d]+0.9
Estradiol (pg/ml)	70.0 ± 17.4	97.6+21.1[b]	66.1[b]+26.6	77.4[e]+21.5
Testosterone (ng/dl)	42.7 ± 15.6[c]	78.4+43.6	47.6[b]+51.1	58.4+65.2
Overian size (mm)	4.3 ± 12.4	13.1 ± 9.4[b]	7.2 ± 12.7	9.1 ± 8.4

Data are shown as mean+SD. Analysis was performed by ANOVA followed by the LSD test for multiple comparisons
[a]P-0.05 PCOS vs. MSCs-EX PCOS and P-0.01 PCOS vs. control
[b]P-0.05 PCOS vs. control and P-0.05 PCOS vs. MSCs-EX-PCOS
[c]P-0.001 control vs. MSCs-EX-PCOS and P-0.001 control vs. PCOS
[d]P-0.001 PCOS vs. FVE-PCOS
[e]P-0.01 PCOS vs. control and P-0.05 PCOS vs. FVE-PCOS

Table 1: Hormonal findings and ovarian size in all groups: PCOS, PCOS with MSCs-EX, PCOS with FVE, and Control.

improvement in tissue architecture. The primordial follicles and developing surface epithelium well observed. Some areas still show degenerated dilated cystic growing and antral follicles (Figure 5C).

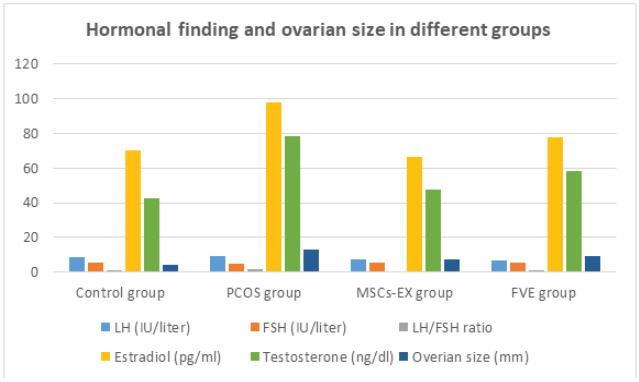

Figure 4: Hormonal findings and ovarian size in all groups: PCOS, PCOS with MSCs-EX, PCOS with FVE, and Control.

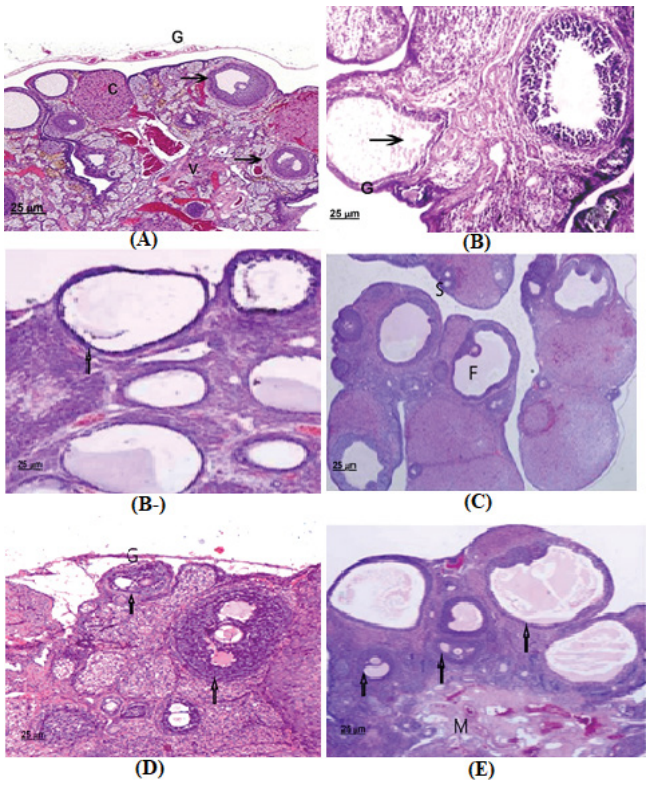

Figure 5: Photomicrographs of H and E stained ovaries sections: **(A)** control group showing normal histological ovarian appearance: bulged highly cellular cortex that covered by germinal epithelium (G) and contained follicles; primordial follicles, secondary and Graafian follicles (arrows), the medullary region was clearly differentiating by its vascularity (V). Note that presence of Corpora lutea and albicans (C); **(B)** PCOS group showing degenerated germinal epithelium with many dilated cystic follicles that bulge from cortex (arrow) increasing layers of genulosa cells (G); **(B-)** PCOS group showing degenerated medulla by dilated multiple cystic follicles (arrow); **(C)** FVE treated group showing well observed primordial follicles with well-developed mature follicle (F). Note presence of secondary follicle (S); **(D)** MSCs-EX treated group showing well developed germinal epithelium (G). Note presence of many well developing follicles in different stages (arrows); **(E)** MSCs-EX treated group showing well developed medulla (M). Note presence of well-developed follicles (arrows) (H and E, 400X, scale bar=25 µm).

However, after MSCs-EX treatment, a moderate improvement of ovarian architecture and appearing of all types of follicles including primary, secondary, antral follicles and corpus luteum were apparently normal (Figure 5D and 5E).

Histochemical results

Polysaccharides stain in control and MVS groups showed a moderate positive reaction in oocytes and strong reaction in the *Zona pellucida* surrounding the oocyte (Figure 6A and 6D). PCOS groups showed that Polysaccharides were poorly stained in lining epithelium of oocytes of the ovaries (Figure 6B). However, mildly increased stain affinity of PAS +ve materials were detected in those treated with FVE (Figure 6C). Ovarian sections of the control group showed some little collagen fibers within the cortical stroma in between the cortical follicles (Figure 7A). Ovarian sections of PCOS group showed increased collagen deposition in the cortical stroma among the few maintained follicles, that extending towards the medulla (Figure 7B). On the other hand, decreased collagen fiber content through the whole ovary tissue and well detected healthy cortical stroma between follicles were observed in FVE and MSCs-EX treated group (Figure 7D).

Immunohistochemical results

OCT4-immunostained sections: Control ovarian sections showed positive OCT4 immunostaining in the cytoplasm of almost all oocytes and in the medulla and cortex regions. Stromal cells showed no OCT4 immunostaining (Figure 8A). PCOS group showed decreased OCT4 immunostaining and limited to the undamaged cells (Figure 8B). Moderate OCT4 immunostaining was seen with FVE treated group (Figure 7C) where the cytoplasm of most cells of the ovary tissues showed high staining of OCT4 in MSCs-EX (Figure 8C and 8D).

Morphometrical results

PCOS showed a highly significant decrease in the number of primordial, primary, secondary, and mature graafian follicles with no

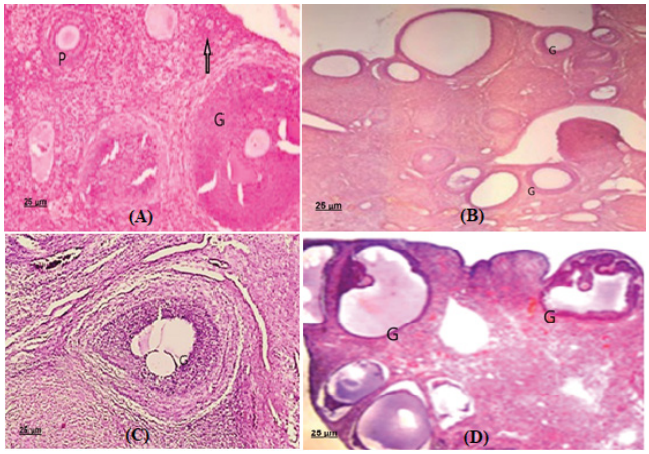

Figure 6: A photomicrograph of a section in rat ovary stained PAS: **(A)** from the control group showing the cortical region of an ovary with strong positive PAS reaction (arrow) and the granulosa cells lining the growing follicle (G). Note that a strong reaction in the zona pellucida (P) surrounding the oocyte; **(B)** from the PCOS group a showing cortex with week PAS reaction of the granulosa cells lining the growing follicle (G); **(C)** from the group FVE showing cortex with mild to moderate PAS reaction of the granulosa cells lining the growing follicle (G); **(D)** from the MSCs-EX showing cortex with moderate strong PAS reaction of the granulosa cells lining the growing follicle (G) (PAS, 400X, scale bar=25 µm).

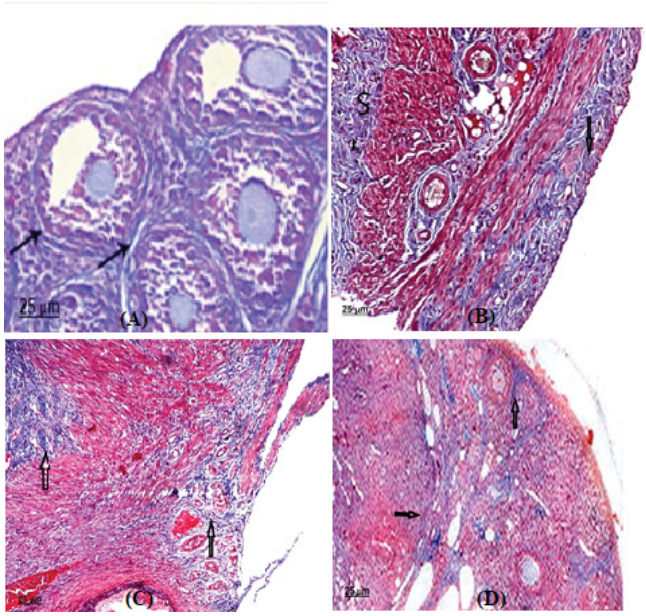

Figure 7: A photomicrograph of a section in rat ovaries stained PAS: **(A)** from the control group showing collagen fibers (arrows) around the ovarian follicles. Note the collagen fibers within the ovarian stroma in between the follicles (S); **(B)** from the PCOS group a showing increase collagen fibers surrounding ovarian follicle (arrow), others appear within the ovarian stroma (S); **(C)** from the group FVE showing mild to moderate increase in the amount of collagen fibers surrounding the ovarian follicles and within the ovarian stroma (arrow); **(D)** from the MSCs-EX showing mild collagen fibers (arrow) surrounding the ovarian follicle (Masson, 400X, scale bar=25 µm).

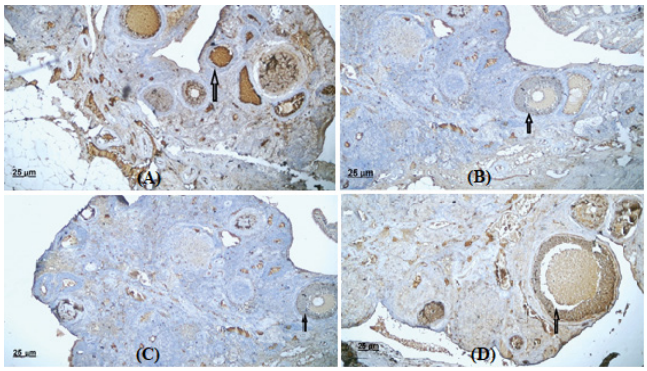

Figure 8: A photomicrograph of a section in rat ovaries has OCT4 immuno-stain: **(A)** from the control group showing positive showing positive OCT4 immuno-staining in cytoplasm of all germ cells and oocyte (arrow); **(B)** from the PCOS group a showing faint OCT4 immuno-staining in the cytoplasm of all cells of the ovarian tissue (arrow); **(C)** from the group FVE showing mild to moderate OCT4 immuno-staining in the cytoplasm of all cells of the ovary(arrow); **(D)** from the MSCs-EX showing moderate and high OCT4 immuno-staining in the cytoplasm of all cells of the ovary(arrow) (OCTA immune-stain, 400X, scale bar=25 µm).

corpus luteum but a highly significant increase in the number of cystic follicles as compared with control rats. Rats treated with MSCs-EX and FVE showed a significant increase in the number of primordial, primary, and secondary follicles but a non-significant decrease in the number of cystic follicles compared with control rats as shown in Table 2 and Figure 9 where Table 3 and Figure 10 showed Mason trichrome and PAS staining scores, the OCT4 Immuno-scores of the different

Groups/ Mean ± SD	Primordial follicles	Primary follicles	Secondary follicles	Mature follicles	Corpus leuteum	Cystic follicles
Control	21.1 ± 2.4	4.8 ± 0.9	4.5 ± 2.1	1.6 ± 0.1	1.7 ± 2.1	0.0 ± 0.0
PCOS group	3.4 ± 1.9[a]	1.6 ± 2.4[a]	1.3 ± 0.1[a]	0.5 ± 2.1[a]	0.2 ± 0.0[a]	12.3 ± 6.1[a]
FVE group	20.3 ± 2.1	3.9 ± 2.1	3.8 ± 2.0	1.3 ± 0.7	1.3 ± 5.1	9.3 ± 2.1
M S C s - E X group	21.5 ± 1.1[b]	4.5 ± 0.1[b]	4.3 ± 0.8[b]	1.4 ± 1.4[b]	1.6 ± 0.9[b]	7.2 ± 7.1[b]
P>0.05 means 'nonsignificant'; [b]P<0.05 means 'significant'; [a]P<0.01 means 'highly significant'						

Table 2: Mean ± SD of the count of primordial, primary, secondary, mature Graafian, corpus luteum, and cystic follicles in different groups.

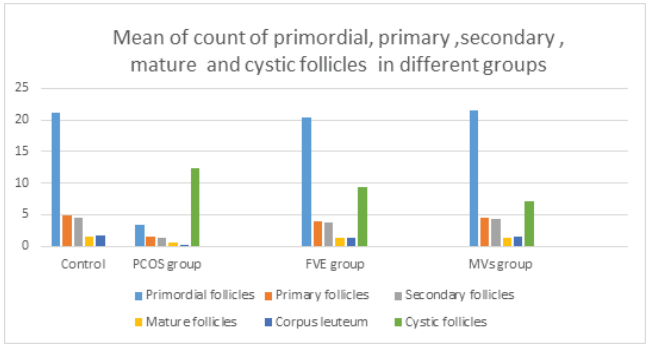

Figure 9: Mean ± SD of the count of primordial, primary, secondary, mature Graafian, corpus luteum, and cystic follicles in different groups.

Groups/Mean ± SD	Controls	PCOS group	FVE group	MSCs-EX group
OCT4	5.03 ± 0.29	1.21 ± 0.27*	5.11 ± 0.39*	3.75 ± 0.42*
PAS	7.52 ± 0.41	0.52 ± 0.07*	5.40 ± 0.47*	6.38 ± 0.38*
Mason Trichrome	0.17 ± 0.03	5.42 ± 0.59*	0.28 ± 0.08*	0.18 ± 0.07*
*Significance at P<0.01 compared to controls, and=Significance at P<0.01 compared to corresponding POF group				

Table 3: The mean area%, SD of masson trichrome, PAS staining and OCT4 immuno-expression in different study groups.

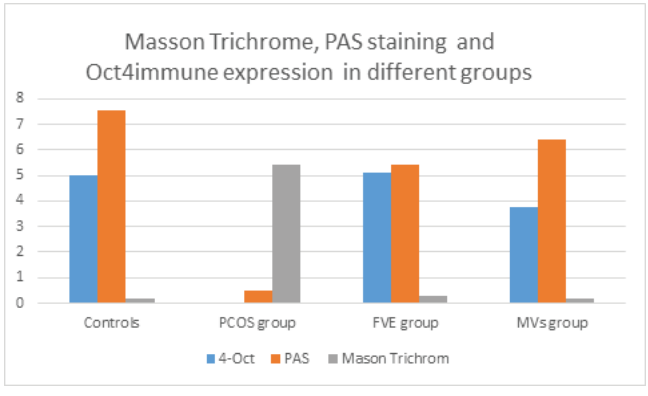

Figure 10: The mean area%, SD of Masson Trichrome, PAS staining and OCT4 immuno-expression in different study groups.

study groups, in which PCOS showed marked reduction of OCT4, marked collagen and mucopolysaccharides expression compared to controls, MSCs-EX and FVE treated groups (P<0.01). In the mean-time, no significant variation between controls and MVSC treated group indicating the success of MVs treatment to restore the ovarian

structure of the rat's model of PCOS.

Discussion

FVE is a famous spice that used in traditional medicine for its antioxidant and anti-inflammatory effects. Nowadays, FVE is used for treating PCOS treatment due to their phytoestrogens compounds (Sadrefozalayi). Previously, the use of stem cells as a new approach therapy for treatment of ovarian failure and infertility, a large number of these studies suggested direct differential of the injected stem cells into granulosa cells, latterly supported by hormones that enhance oocyte maturation [27]. Others were not accepting the ability of stem cells to differentiate into oocytes but they have paracrine effects maybe lead to reactivation of the host oogenesis [28].

In this study, we demonstrate that infusion of BM-MSCs derived MVs (MSCs-EX) could restore ovarian function in a rat model of the polycystic ovary and compare its effect with FVE.

Our results revealed that there is a marked increase in the number of growing pre-antral and antral follicles, and this result supports that there is a decrease in primary follicles in PCOS. This result supports the hypothesis that primary follicles in PCOS ovaries are growing slower than normal. Therefore, there is abnormal folliculogenesis in PCOS. Our results were supported by Webber, et al., [29] as they explained that the ratio of early growing (primary) follicles is higher in anovulatory and ovulatory women with PCOS ovaries compared with that in normal ovaries. Also, excessive ovarian androgen production may play an important role in pathogenesis in PCOS as explained [30]. The mechanism is not known but appears to be mediated by androgen receptors expressed in the granulosa cells [31]. Increased LH secretion can enhance the growth of primary follicles. It is well recognized that the rate of LH release is increased in women with PCOS and that increased plasma LH leads to increased androgen production by the theca-interstitial cells [32]. In our study, androgens have been shown to significantly increase FSH receptor mRNA abundance in granulosa cells. These results were supported previously by Castro, et al., [33] as increased androgen and LH level were a reflection of these histological abnormalities.

Stem cell therapy for ovarian failure was studied previously using different types of stem cells including; embryonic, bone marrow, amniotic fluid, adipose tissue, skin, and menstrual blood-derived stem cells. Many of these studies indicated that direct different ion of the injected stem cells into granulosa cells and this in need of hormonal support for oocyte maturation [34]. Our results showed a complete differential of stem cells into ovarian cells as these cells may be trapped in the intertie and may have paracrine action within the oocyte microenvironment leading to reactivation of the rat's oogenesis. Also, stem cells may have anti-apoptotic, anti-inflammatory, providing growth and renewal of germline stem cells.

Extracellular vesicles released by stem cells defined as membranous structures loaded with bioactive molecules that have a role in cell to cell communication. Our study showed that the use of BM-MScs derived MVs for treatment of PCOS moderately successfully to restore the hormonal level and restore the ovarian histological structure as follows: increased number of ovarian follicles at different developmental stages and decrease the number of dilated follicles compared to those untreated PCOS. This was done as MSCs-EX may promote folliculogenesis and/or by inhibiting follicular atresia.

OCT4 played an important role in the oogenesis in both prenatal and postnatal [35]. OCT4 expressed in adult at the time of growth phase of oocytes. The lower level of OCT4 expression leads to the death of the primordial germ cells and increase in antral follicles in adult ovaries [36]. This is in line with our model of PCO in which lower OCT4 expression and reduction of follicles at different growth stages. In the mean-time, after MSCs-EX therapy regenerative process was achieved is probably related to the essential role of OCT4 in maintaining germline stem cells and in inducing and establishing the pluripotency of many cell lineages [37]. It reported that overexpression of OCT4 in undifferentiated ovarian follicles may lead to increase the efficiency of their differentiation into oocyte-like cells [38]. The increased expression of OCT4 on stem cell transplantation in PCOs model has been reported by Lee, et al. and Fouad, et al. [39,40].

In the present study, FVE has created a significant increase in the numbers of graffian and multilaminar primary follicles and improved folliculogenesis in rat PCOS. This may have been due to the same estrogenic effects of this plant and these estrogenic effects of FVE have been demonstrated by Oktay, et al. [19]. The researchers did not study the histological changes of the ovaries. Here, we have shown the effect of FVE on the histology of rat's ovaries. This study showed that MVs infusion moderately alleviated the induced PCOS pathological changes in rats in a similar way to that was achieved by FVE based therapy, explaining the potential role of exosome as a novel therapeutic agent for treatment of PCOS. Similarly, the study by da Silveira, et al. [5] showed that exosomes isolated from the follicular fluid can regulate members of the TGFB/BMP signaling pathway in granulosa cells, and may this possibly play an important role in regulating follicle maturation.

Conclusion

This study showed for the first time that BM-MSCs derived exosome (MSCs-EX) infusion in induced PCOS rat model showed structural and functional reparative properties. Further studies are suggested for understanding the exact mechanisms underlying these actions.

Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

All authors agreed to publish this manuscript.

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