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Effect of Exosomes Derived from Bone Marrow Mesenchymal Stem Cells on Ovarian Granulosa Cells of Immature NMRI Mice

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Abstract

Objective: In recent years, *in vitro* maturation (IVM) has become the focus of fertility maintenance, and infertility treatment. The aim of this study is development of oocytes during folliculogenesis and oogenesis is greatly influenced by the presence of *BMP-7, BMP-15,* and *GDF-9* genes, which are present in exosomes generated from bone marrow stem cells.

Materials and Methods: In the experimental study, we investigated how exosomes obtained from bone marrow stem cells affected development and expansion of ovarian granulosa cells (GCs) in NMRI mice. In this *in vitro* experiment, bone marrow stem cells were isolated from mice's bone marrow, and after identification, exosomes were recovered. Exosome doses of 100, 50, and 25 µg/ml were applied to GCs before using MTT assay to measure survival rates and quantitative reverse-transcription polymerase chain reaction (PCR) to measure expression of the *BMP-7, BMP-15*, and *GDF-9* genes.

Results: The results showed that the GCs treated with exosomes concentrations of 25, 50, and 100 µg/ml significantly increased bioavailability, growth and proliferation and it also increased expression level of *BMP-7, BMP-15* and *GDF-9* genes compared to the controls.

Conclusion: Findings of this study indicated that exosomes derived from bone marrow stem cells improved growth of GCs in NMRI mice and they were a good candidate for further clinical studies to improve quality of the assisted reproductive techniques.

Keywords: Annexin, Exosomes, Granulosa Cells, Stem Cells

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Introduction

In the recent years, new mechanisms were proposed for cell-to-cell communications. Studies strongly showed that vesicles, such as exosomes and other microparticles cells were formed, the cells entered them into the cellular microenvironment. By sending information via vesicles, the cells might be able to influence behavior of the target cells (1). Some papers also suggested exosomes or microparticle carriers in the follicular fluid as a potential alternative mechanism for the paracrine and autocrine actions in the ovarian follicles.

The findings of this research can aid in our understanding of the many communication routes, which are crucial for early fertility and it have potential clinical implications (2, 3). In fact, extracellular carrier identification can aid in the diagnosis of reproductive disorders and offer biological indicators of oocyte quality in assisted reproductive technology (ART) (4). The Pathogenic process of many diseases, including endometriosis, which is brought on by the intercellular movement of molecules including miRNAs, RNAs, and proteins, can be described by exosomes as cell-to-cell linkers (5).

Exosomes' participation in cell-cell communications, a crucial aspect of folliculogenesis, should also be a major concern in reproductive biology (6). The primary elements of follicles are theca, granulosa cells (GCs), and oocytes (7). GCs are the most important cells in the ovary that undergo serious changes morphologically and physiologically during the processes of follicular proliferation, differentiation, ovulation, lutenization and atresia (8). GCs affect growth and maturation of oocytes. The main function of GCs is to induce production of sex hormones and various peptides required for folliculogenesis and ovulation (9).

Some reports showed that different growth factors can stimulate GCs proliferation or steroidogenesis *in vitro* (10). For example, *GDF9*- stimulated proliferation of mouse

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GCs, but prevented secretion of estradiol and progesterone by suppressing follicle-stimulating hormone (FSH). On the other hand, *BMP15*- stimulated proliferation of mouse GCs and then decreased FSH levels, while *BMP4*- and *BMP7*- both potentiated FSH-stimulating function in the production of estradiol and progesterone from mouse GCs. Growth factor *BMP15*- was secreted by oocytes (11). As two primary tissues, brain and ovary expressed *BMP15*-. After that, a research based on fertility traits was carried out, and it was discovered that *BMP15*- was crucial for growth of the early follicular phase (12). Effect of *BMP7*- on GCs proliferation and progesterone synthesis, which played an important role in controlling effects of gonadotropins and IGF-1 on follicular differentiation, was reported in the other laboratory studies (13). The present study investigated stem cell-derived exosomes in GCs of mice.

We aimed to isolate and characterize exosomes from bone marrow mesenchymal stem cells (MSCs) to examine therapeutic potential of these exosomes as effective paracrine mediators on ovarian GCs.

Materials and Methods

Exosome preparation

Preparation of bone marrow mesenchymal stem cells

In the animal compartment, NMRI mice were housed according to industry standards with 12 hours of lightness and 12 hours of darkness at a temperature of 25°C. Islamic Azad University-Mashhad Branch Institutional Research Ethics Committee guidelines were followed throughout the entire experimental process (IR.IAU.MSHD.REC.1398.194).

In this experimental study NMRI mice were sacrificed via cervical vertebra dislocation, and the femur was taken out in order to conduct the research. Two ends of the femur were then cut and the contents of the bone marrow were transferred to a cell culture flask by a syringe containing the culture medium (DMEM, Bio Idea, Iran). After changing the culture medium containing, 10% fetal bovine serum (FBS, Gibco, USA), and 5% antibiotic (Gibco, USA) several times and removing the waste material, the bone marrow stem cells began to proliferate and become pure.

Bone marrow mesenchymal stem cells identification by flow cytometry

To identify bone marrow MSCs, specific surface markers of CD 73 (antibodies, UK) and CD 105 (Antibodies, UK) and CD 45 (Antibodies, UK) and CD 34 (Antibodies, UK) and CD 31 (Antibodies, UK) stem cells were used by flow cytometry.

Bone marrow mesenchymal stem cells identification by osteogenic differentiation

Bone marrow MSCs were cultured in tissue culture polystyrene flasks in Dulbecco's Modified Eagle's Medium (DMEM, with 1g/l glucose and Gluta MAX; Bio Idea, Iran) supplemented with 10% FBS (Gibco, USA), as well as 1% of 100 U/ml penicillin and 100 µg/ml streptomycin (P/S, Gibco, USA) at 37°C in 5% CO_2. At 80% confluency MSCs were trypsinized (Bio Idea, Iran) for three minutes at 37°C and resuspended in DMEM with 10% FBS and 1% P/S. after passage five MSCs were seeded at 3,000 cells/ cm2 in cell culture plates. The Cells were allowed to attach for 24 hours before changing the medium to either control medium (DMEM, with 4.5 g/l glucose and Gluta MAX Bio Idea, Iran) with 10% FBS and 1% P/S, or osteogenic induction medium (additionally supplemented with 0.1 µM dexamethasone (Sigma-Aldrich, UK), 10 mM β-glycerophosphate (Sigma-Aldrich, UK) and 0.1 mM L-ascorbic acid 2-phosphate (Sigma-Aldrich, UK) (14). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to test expression levels of osteocalcin and osteopontin as key genes involved in the process of osteogenic differentiation.

Isolation and purification of exosomes

MSCs were cultivated in serum (FBS) free DMEM medium DMEM (Bio Idea, Iran) for 24 to 48 hours after removing the culture medium when their density reached to 70 to 80% of the flask (about 800,000 cells). The exosomes were separated from the supernatant using ultracentrifugation (Beckman, USA) at 100,000 g for one hour in central research laboratory of Mashhad university of medical sciences (15). Exosomes were frozen at -20°C after suspending them in phosphate-buffered saline (PBS, Sigma, USA) (16).

Exosome identification by atomic force microscopy

To prepare sample for analyzing with the atomic force microscopy (AFM, JPK, Germany), in accordance with published protocols 3 µl of the samples were taken and fixed with 100 µl of 2% paraformaldehyde solution (Sigma, Germany). After that, a little solution drop comprising exosome samples was deposited on the slide, and after 30 minutes the samples were dried and pertinent AFM investigations were carried out (17).

Exosome identification by Scanning electron microscope

To evaluate the exosome shape and size ,the purified exosomes were fixed with 2.5% glutaraldehyde and rinsed with PBS. The samples were then dewatered with ethanol, and covered with a thin layer of gold on a dry glass surface and examined by scanning electron microscope (SEM, TESCAN, USA) (18).

Exosome identification by Transmission electron microscopy

Transmission electron microscopy (TEM) was used to examine morphology of the exosomes. After incubating for 15 minutes at room temperature with a 15 µl exosome suspension on a copper grid, the samples were rinsed

with sterile distilled water and dab any remaining liquid was removed by absorbent paper. The filter paper was then removed and the samples were dried for two minutes under an incandescent light after exposing to 15 µl of 2% uranyl acetate for one minute on the copper grid. TEM was used to examine the copper mesh, and images were taken at 80 Kv (19).

Exosome identification by dynamic light scattering method

Quick and simple measurements by dynamic light scattering (DLS) are non-imaging methods used to identify exosomes. All experiments were performed in 1: 1 dilution and performed by Zetasizer (Horiba, Japan) (20).

Determination of exosome concentrations

Bradford method was used to determine the exosome concentration. To determine the concentration of an isolated exosome, its protein was determined using Bradford solution and standard diagram using successive dilutions of BCA protein (Sigma, UK) with specific concentration.

Identification of granulosa cells

FSHR and AMH immunocytochemistry

After fixing in 4% paraformaldehyde for 15 minutes, the cells were rinsed three times with PBS for a total time of three minutes. The cells were then treated in a 3% hydrogen peroxide (H_2O_2) solution while they were incubated at room temperature for 10 minutes. They were rinsed with PBS three times for five minutes to stop peroxidase. The cells were then cultured for 30 minutes at room temperature with 5% bovine serum albumin (BSA) added to the petri dish. FSHR and AMH Primary antibodies (Bioss, USA) were treated with GCs at a concentration of 1/100 in PBS. The secondary FITC goat anti-rabbit antibody (Bioss, USA) against FSHR and AMH were incubated for 30 minutes with the primary antibodies after washing with PBS. The slides were then air-dried, 90% glycerol mounted, and examined under a fluorescence microscope (21, 22).

Effects of exosomes on granulosa cells

Animals: Immature NMRI mice aged approximately 14 to 21 days were obtained from Research Center for Animal Development Applied Biology (Mashhad, Iran)

GCs extraction: Ovary follicles of 21-days-old mice were subjected to puncture with 25-gauge needle. Follicles were separated and transferred to the other petri dish (4, 24). The follicles were punctured again to release GCs. GCs were then aspirated aseptically in new media and cultured in α-MEM (Bio Idea, Iran) medium containing FBS (Gibco, USA), ITS (Gibco, USA) and FSH (Cinnal-F, Iran). After 4 days, GCs were treated with doses of 25, 50 and 100 µg / ml (23).

Cellular uptake by PKH26-labeled exosomes

Lipophilic dyes such as the PKH family have been widely used to label a range of cell types like MSCs (24). Since exosomes have a lipid bilayer structure similar to that of the cell plasma membrane, PKH dye family have been adapted for EV labeling (25).

GCs treated with bone marrow-derived exosomes were labeled with fluorescent red PKH26 (Sigma, UK). Exosomes were diluted in 1 ml of Delionnet C solution (Sigma, UK), then 1 ml of Delionnet C solution was diluted with 4 ml of PKH26. This solution was combined with the exosome suspension, and the mixture was incubated with a 1% BSA solution for five minutes. The tagged exosomes were then centrifuged at 100000 g for 70 minutes. The generated tiny stain in the tube was then gradually resuspended in PBS and recentrifuged after the supernatant had been removed (21). Finally, GCs were incubated on a slip cover with exosomes labeled PKH26 at 37°C for 24 hour and then evaluated by confocal fluorescence microscopy (26).

Effect of exosomes on the viability of granulosa cells

GCs with a density of 5×10^5 cell per well were cultured in 12 well plates at 37°C with 5% CO_2 and then the cells were incubated with exosomes for 24 and 48 hours. MTT assay was then performed to evaluate survival of GCs (23).

Tracking and quantifying apoptosis and necrosis

Annexin-V-PI diagnostic kit (Abcam, UK) was used to track and quantify GCs that underwent apoptosis and necrosis (27). For this purpose, the cells were cultured, and treated using a flow cytometer (BD, USA) and Annexin-V-PI kit according to the manufacturer's instructions.

Evaluation of changes in *BMP7*, *BMP15* and *GDF9* expression levels by quantitative reverse transcription polymerase chain reaction

qRT-PCR was used to evaluate the expression levels of *GDF-9*, *BMP-15*, and *BMP-7* as key genes in the process of folliculogenesis 48 hours after the treatment of GCs at concentrations of 25, 50, and 100 g/ml exosome. Table S1 (See Supplementary Online Information at www.celljournal.org) lists the primers used in the current study.

According to the manufacturer's instructions (Scientific Thermo Fisher, USA), total RNA was extracted from the treated and untreated groups. Using a cDNA synthesis kit from Scientific Thermo Fisher (USA), the cDNA was created. qRT-PCR was performed using (BIORAD CFX 96 PCR instrument (BIORAD, USA) (29). This method made use of the Sybergreen fluorescent stain (Pars Tous, Iran).

Statistical analysis

The trials were run at least three times, and all data

were provided as mean ± standard deviation (SD). One-way ANOVA and the Tukey post hoc tests were both used to analyze variations means in each trial. GraphPad Prism 8 software (GraphPad Software, USA) was used for the statistical analysis. The statistically significant level was set at $P<0.05$.

Results

Confirmation of the cultured bone marrow mesenchymal stem cells by flow cytometry

Positive CD73 and CD105 markers, and negative CD45 and CD34 and CD31 markers, were investigated. According to the findings, CD73 was expressed by more than 99.9% of the cells, CD105 was expressed by more than 92.4%, but CD45 was only present in 6.92% of these cells. CD34 was only present in 0.2% and CD31 was only present in 3%. Consequently, the stem cells were verified (Fig.1A-E).

Results of osteocalcin and osteopontin expression in bone marrow mesenchymal stem cells

In the groups treated with osteogenic induction medium, the osteocalcin and osteopontin expression levels were increased significantly ($P<0.001$) compared to the control group. (Fig.1F, G)

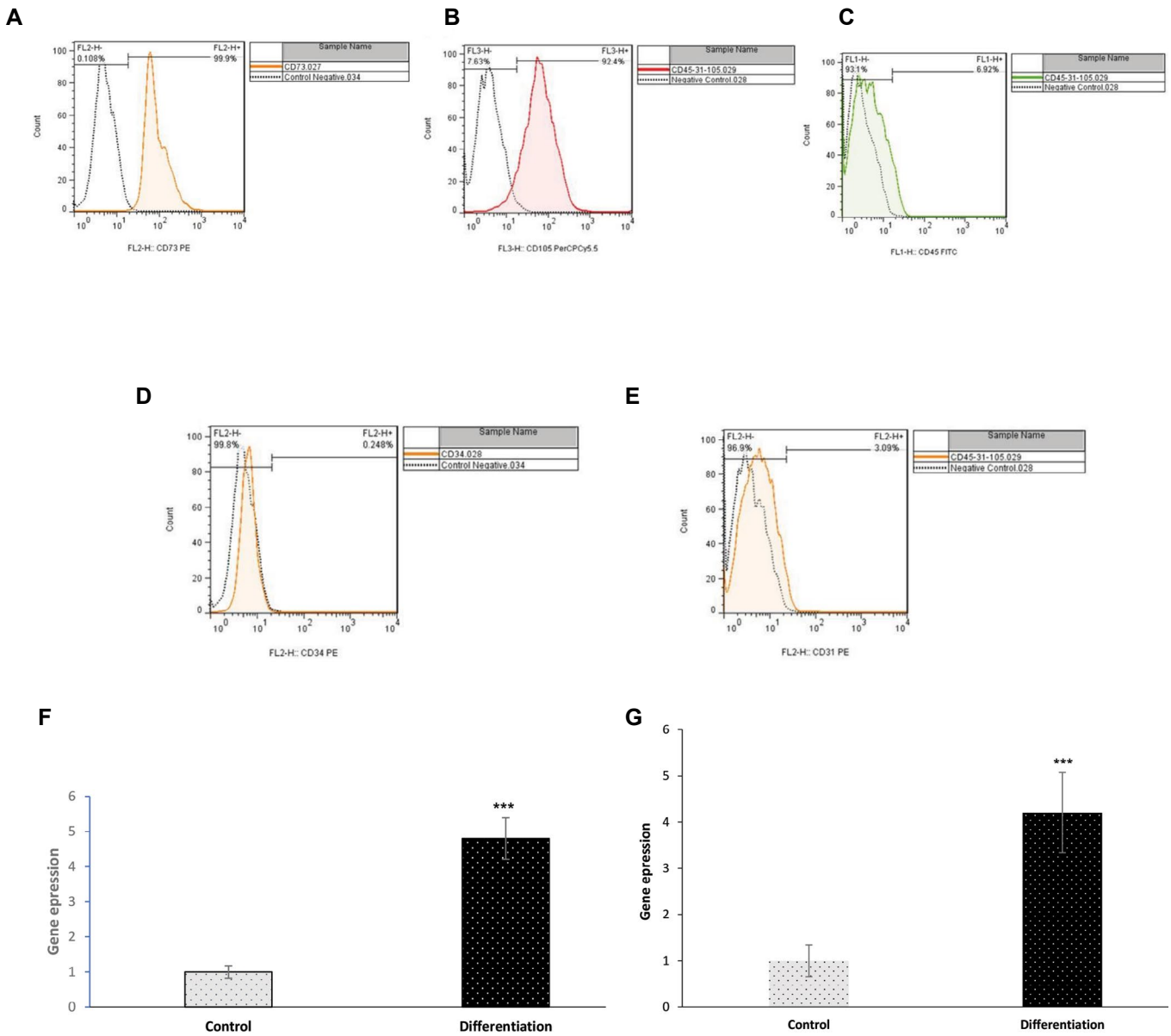

Fig.1: Confirmation of bone marrow mesenchymal stem cells (MSCs). **A.** CD73 surface marker diagram. about 99.9% of the cells expressed CD73 marker. **B.** CD105 surface marker diagram. About 92.4% of the cells expressed marker CD105. **C.** CD45 surface marker diagram. A total of 6.92% of the cells expressed marker CD45. **D.** CD34 surface marker diagram. only about 0.2% of the cells expressed marker CD34. **E.** CD31 surface marker diagram. About 3% of the cells expressed marker CD31. Results of **F.** Osteocalcin and **G.** Osteopontin expression in MSCs. As is observed, there is a significant increase in the expression of osteocalcin and osteopontin in the differentiation group compared to the control group. All experiments were repeated three times, each in triplicate. One-way ANOVA test was used for statistical analysis. ***; $P<0.001$ compared to the control.

Results of exosomes isolated from bone marrow mesenchymal stem cells by dynamic light scattering

The results of this experiment confirmed that the particles extracted using ultracentrifugation from bone marrow stem cells had a diameter from 60-170 nm and the exosome was extracted (Fig.2A).

Results obtained from the atomic force microscopy of exosomes

According to the reports published in many articles, the average dimeter of exosomes is about 20-200 nm. The results of AFM showed presence of the exosomes with an average diameter of approximately 170 nm (Fig.2B, C).

Electron scanning microscope results

SEM images showed presence of the exosomes with an approximate diameter of 50 nm (Fig.2D, E).

Transmission electron microscopy results

Morphology of the exosomes was examined using TEM. Findings showed spherical membrane vesicles with less than 100 nm width (Fig.2F).

Results of granulosa cell confirmation

Considering GCs have FSH receptors, AMH specific staining was performed by immunohistochemistry to confirm the presence of GCs. The results confirmed presence if these cells (Fig.3).

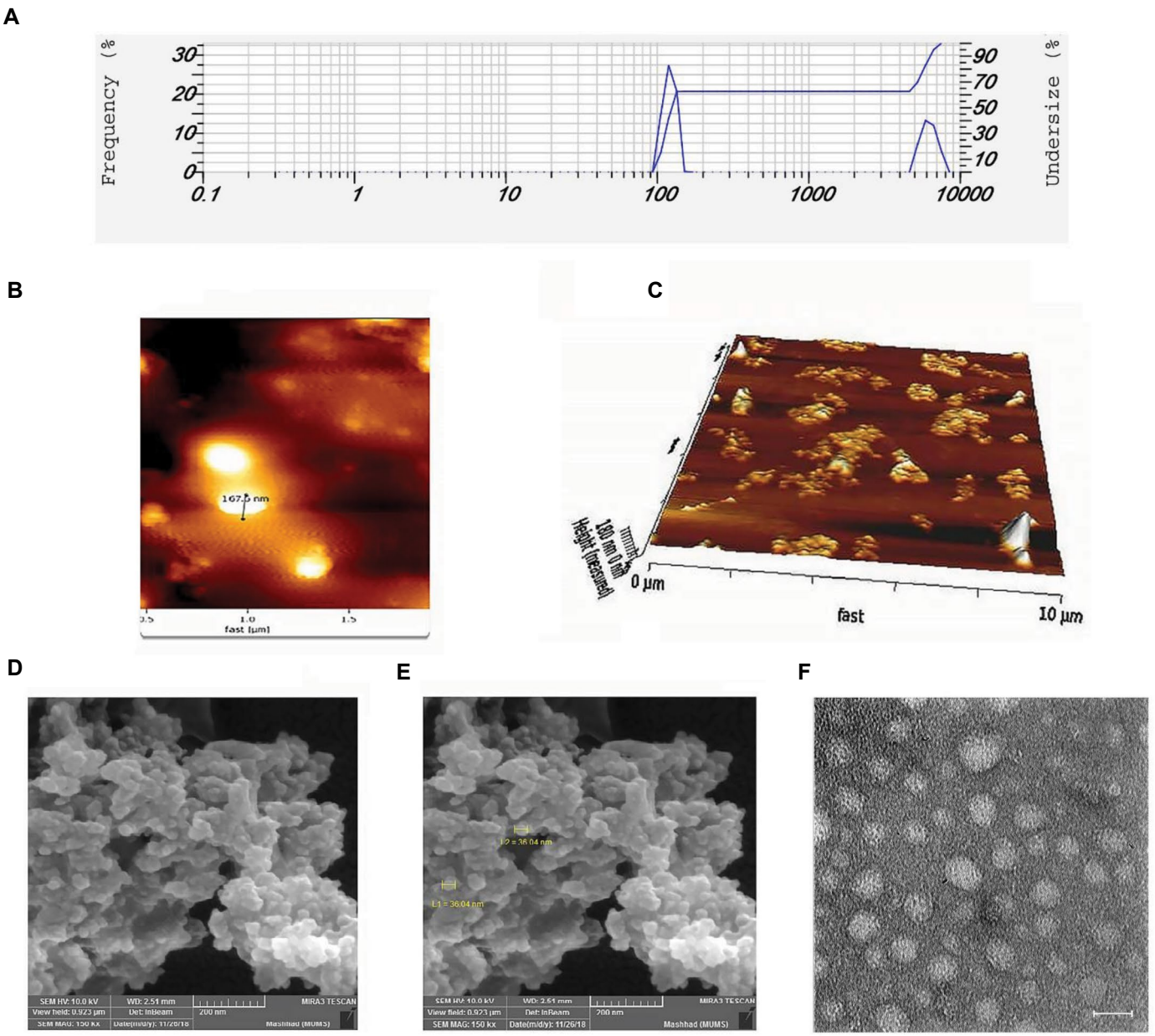

Fig.2: Characterization of exosomes. **A.** Exosome diameter measurement by DLS. **B.** The exosome with size of 167-nm is visible in an AFM image. **C.** An exosome with a diameter of around 170 nm is depicted in the AFM output diagram. **D.** SEM image that shows a mass of exosomes. **E.** The same image whereby the approximate dimensions of exosome are specified. **F.** TEM micrograph of the isolated exosomes derived from bone marrow MSCs describes spherical membrane vesicles with the diameters less than 100 nm (scale bar: 50 µm). DLS; Dynamic light scattering, AFM; Atomic force microscopy, SEM; Scanning electron microscopy, TEM; Transmission electron microscopy, and MSCs; Mesenchymal stem cells.

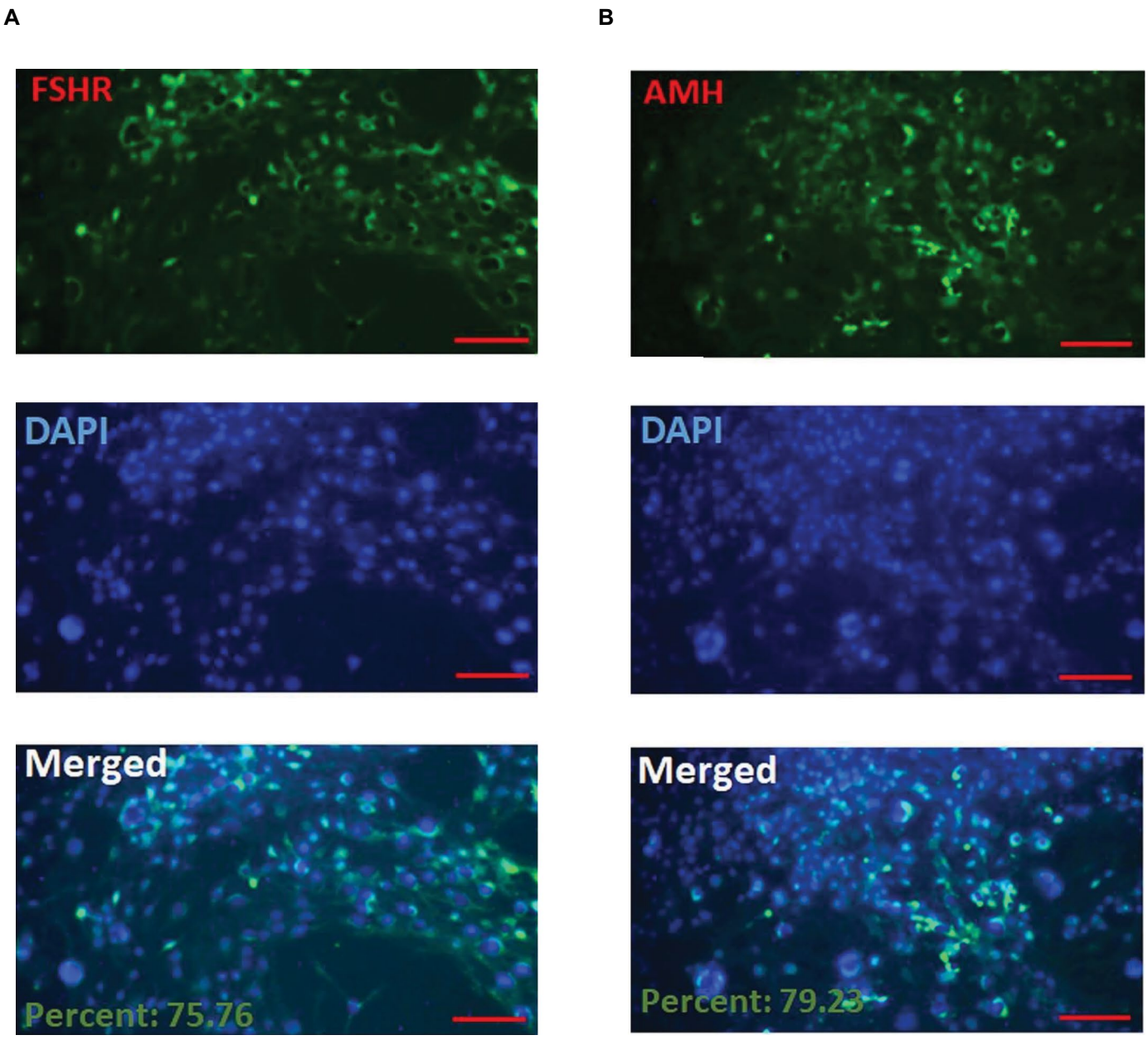

Fig.3: Immunofluorescence staining for granulosa cells. **A.** FSHR (green color) and **B.** AMH (green color) and nuclei staining by DAPI (blue color) (scale bar: 100 µm).

Results of exosome cellular uptake by granulosa cells

PKH26 staining was used to ensure exosome cellular uptake by GCs. The results showed exosome uptake by GCs. The figure displays how tagged exosomes are absorbed by cells. Presence of fluorescent red light in the cytoplasm of GCs shows that they have taken up significant quantities of exosomes from bone marrow-derived stem cells (Fig.4A).

Results of viability assay

By increasing quantity of exosomes from 25 µg/ml to 50 and 100 µg/ml, viability assay results showed a substantial increase in the cell treated for in 24 hours and 48 hours compared to the control group ($P<0.001$, Fig.4B).

Results of Annexin-V assay on granulosa cells

Following the exosome treatment at dosages of 25, 50 and 100 µg/ml, GCs were assessed using the annexin kit in accordance with the corresponding protocol to determine rate of necrosis and apoptosis. Findings demonstrated that, increasing the exosome dose caused dropping GCs levels in the apoptotic treatment groups compared to the control group. Cell viability was roughly 96, 97, and 99.6 in the 25, 50 and 100 µg/ml treated groups, respectively, while it was roughly 90% in the control group (Fig.5).

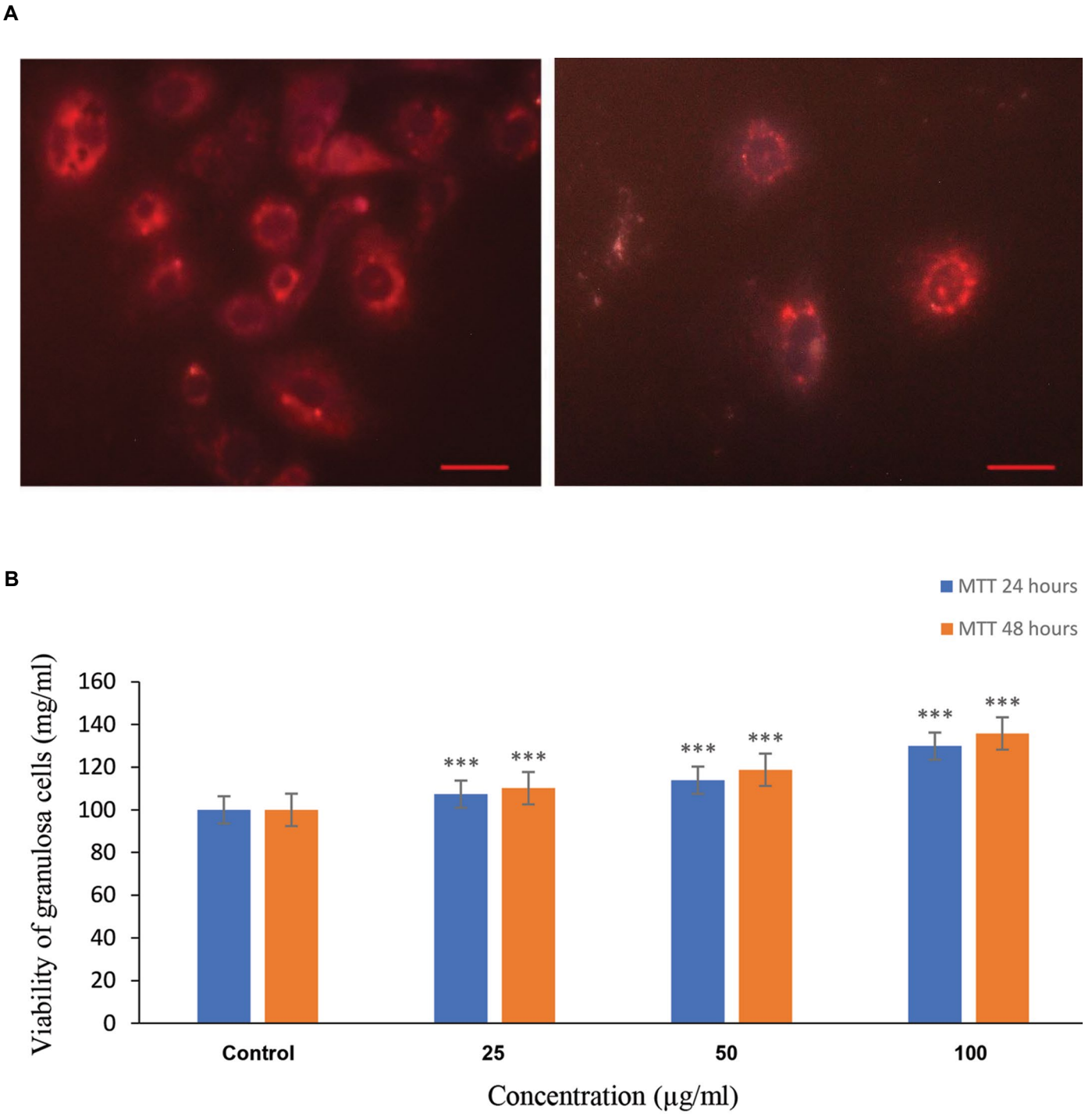

Fig.4: Cellular internalization of exosomes and viability of granulosa cells (GCs). **A.** Uptake of the PKH26-labled exosomes (red) in GCs (scale bar: 100 µm). **B.** Viability diagram of the exosome-treated GCs at 24 and 48 hours. Exosomes were treated in three concentrations of 25, 50 and 100 µg/ml. All three treatment groups are significantly different from the control. Data are represented as the mean ± SD. All experiments were repeated three times, each in triplicate. Statistical One-way ANOVA was used (***; P<0.001).

Results of *GDF-9 BMP-15 and BMP-7* expression levels in granulosa cells

In the groups treated with 50 and 100 µg/ml exosome, *BMP-15* expression levels were increased significantly (P<0.001) compared to the control group. There was no significant change in 25 µg/ml treated group compared to the control group. *BMP-7* gene expression level in GCs showed that in the groups treated with 50 and 100 µg/ml exosome, expression levels of this gene were increased significantly (P<0.001) compared to the control group. There was no significant change in the 25 µg/ml treated group compared to the control group. The results showed that in the groups treated with 50 and 100 µg/ml exosome, *GDF-9* expression level was significantly increased (P<0.001) compared to the control group. There was no significant change in the 25 µg/ml treated group compared to the control group (Fig.6).

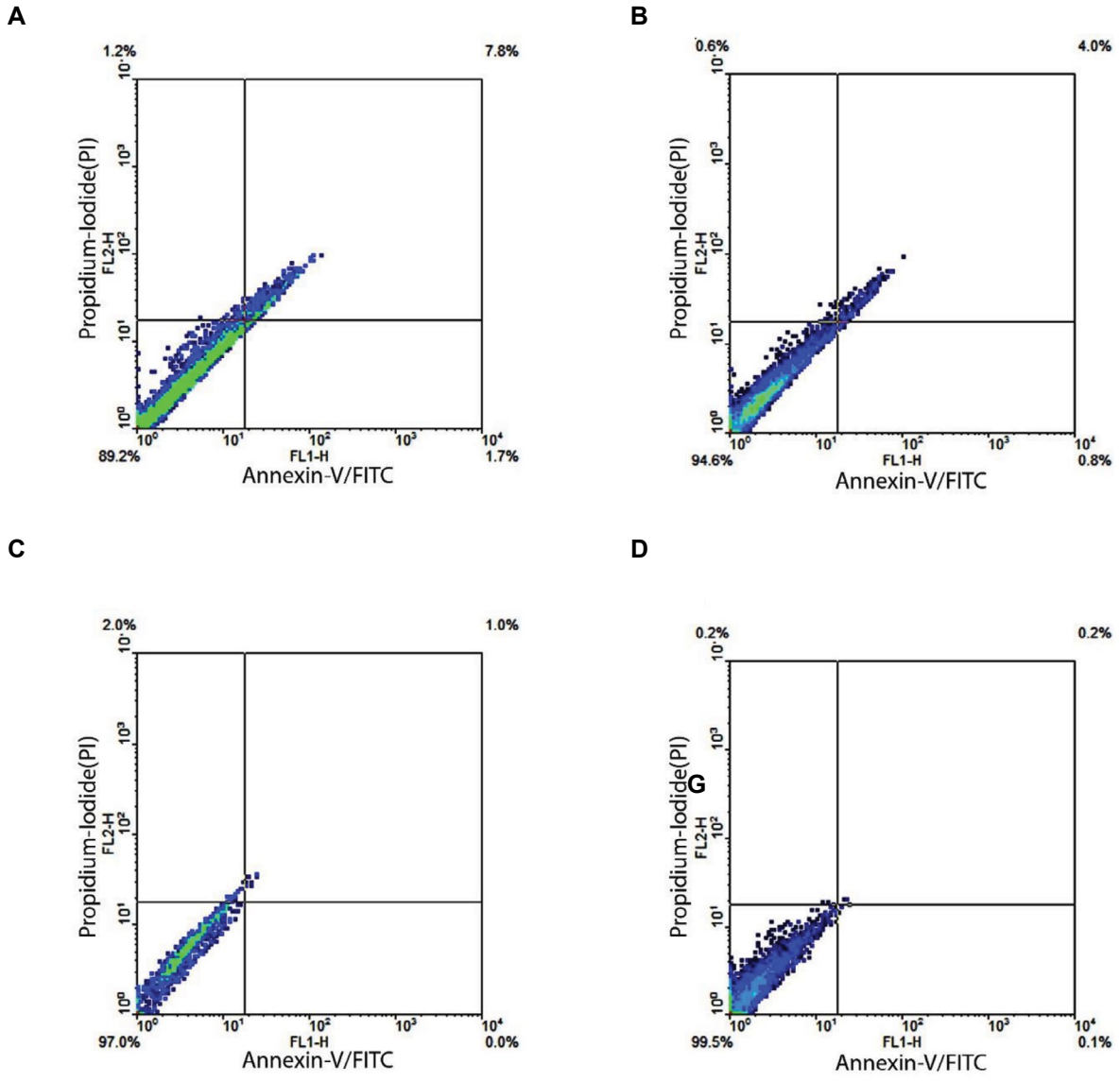

Fig.5: Impact of exosomes on granulosa cell (GC) death. Proportion of apoptosis was measured by flow cytometry and Annexin V/PI. **A.** Control group: about 90% of GCs are still alive. **B.** GCs treated group 25 µg/ml: 94.6% of cells are alive. **C.** GCs treated group 50 µg/ml: 97% of cells are alive. **D.** GC treated group 100 µg/ml: 99.5% of cells are alive.

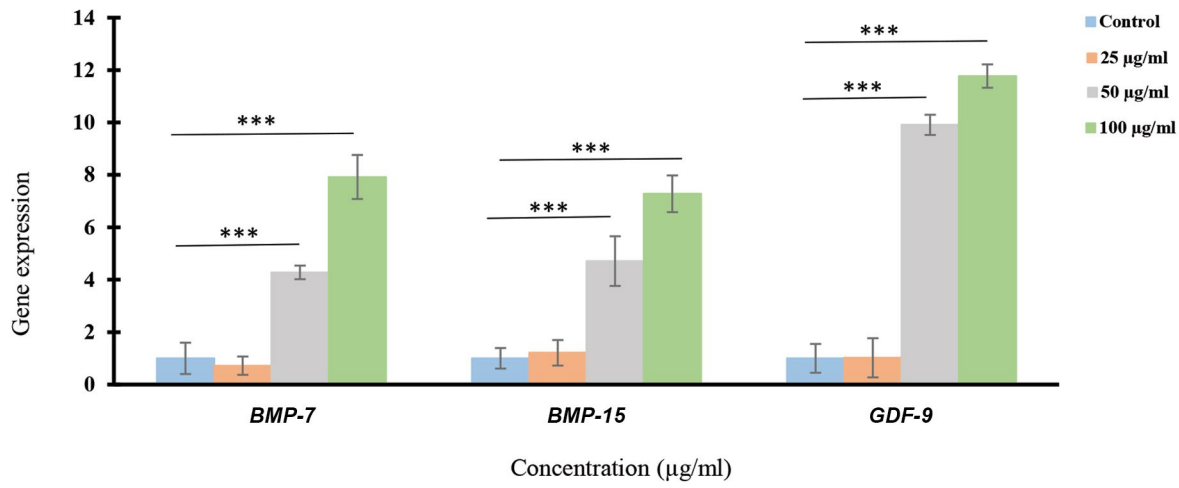

Fig.6: Evaluation of *GDF-9 BMP-15 BMP-7* genes expression in granulosa cells (GCs). Results of *BMP-7, GDF-9* and *BMP-15* expression levels in GCs. All experiments were repeated three times, each in triplicate. Statistical One-way ANOVA test was used. ***; P<0.001 compared to the control.

Discussion

The most common causes of infertility are ovulation disorders, male factor infertility, and fallopian tube disease. Infertility can indicate a related underlying chronic disease (29). Oocytes, GCs, and theca cells are the main components of follicles (7). Proliferation, differentiation, ovulation, luteinization, and atresia that occur during follicular processes, which comprise these follicular processes, affect GCs, one of the most significant ovarian cells, may result in physiological and morphological alterations. Oocyte development and maturation are impacted by GCs (4). The main function of GCs is to induce production of various sex hormones, and peptides required for folliculogenesis and ovulation (9). The most commonly employed cell phenotype for treatment is MSCs. Numerous secreted molecules have been identified as factors influencing the MSCs purely biological effects. It is thought that paracrine signals play a major role in mediating the effects MSCs. Numerous studies have demonstrated that microvesicles removed from MSCs' culture medium can mimic the regenerative effects of these cells (30). Exosomes are the most significant type of these microvesicles (31). Exosomes are used as carriers for various cellular cargoes. Exosomes' primary job is to transport RNA, miRNA, hormones, proteins, carbohydrates, and other intracellular materials from one cell to another (5). The target cell's behaviour and function may be regulated and altered by the transfer of these chemicals (3).

Findings of the current *in vitro* investigation demonstrated that exosomes are present in the bone marrow MSCs' (BMSCs') supernatant as they develop. Vitality of the GCs was improved by these exosomes, which decreased the incidence of apoptosis in the treated groups. These exosomes were demonstrated to have a favourable impact on the expression of genes related to folliculogenesis.

Flow cytometry was used to prove presence of stem cells in order to investigate cell surface markers. Surface markers that are responsible to identify MSCs include CD44, CD90, CD73 and CD105, while CD11b, CD31, CD34, CD45 markers are not expressed in these cells (32). Results of the present study showed that, CD73 was expressed by more than 99.9% of the cells, CD105 was expressed by more than 92.4%, but CD45 was only present in 6.92% CD34 was only present in 0.2% and also CD31 was only present in 3% of the indicated cells.

Chuo et al. (32) demonstrated to identify various microvesicles secreted by cells, such as exosomes, SEM is an appropriate and important method. In this study, presence of exosomes in the supernatant of stem cells was confirmed using SEM. In the study aimed to identify exosomes, van der Pol et al. (33) showed that one of the approaches to identify exosomes was the DLS method. In the present study, DLS method showed that the approximate exosome diameter is about 50-170 nm.

Results of this study's showed that the exosome extracted from bone marrow cells caused proliferation of GC cells so that bioavailability of GCs at exosome dosage of 25, 50 and 100 µg/ml was increased compared to the control group and there was reduction in the incidence rate of the treated GCs compared to the control group. Yang et al. (34) used Annexin-V apoptosis assay, and showed that T24-derived exosomes of bladder cancer cells could significantly inhibit apoptosis in T24 and 5637 of patients with bladder cancers in a dose-dependent manner.

PKH26 fluorescent dye was used to ensure that the exosomes were absorbed by the GCs and the results showed that this dye was adsorbed by these cells. Salek et al. (21) showed exosome uptake by spermatogenic cells. They labeled the exosomes with the PKH-26 fluorescent dye, and then confirmed that the exosomes entered to cells. The results of this study confirmed the uptake of exosomes by GCs.

According to Maumus et al. (35), these effects of MSCs on immune responses and tissue repair are attributable to the nature and delivery of paracrine signals. These cells transport therapeutic elements and transfer them to the site of injury, which makes them implicated in a variety of physiological and pathological processes (30). They participate in a number of biological activities, including angiogenesis, blood clotting, tissue generalization, immunity, inflammation, and pregnancy (3). It is generally recognized that the chemicals transported by exosomes have the ability to mediate specific physiological pathways and functions in cells. Exosomes, in particular, mediate the interaction between endocrine, paracrine, and juxtacrine glands for cell growth, maintenance, and regeneration (31). Many studies were conducted to find factors that improve the growth and differentiation of follicles cultured *in vitro* in the recent years, and the use of growth-promoting compounds *in vitro* maturation (IVM) has attracted much attention. For example, interleukin-1 (IL-1) regulated the proliferation of bovine and rat GCs *in vitro*. IL-1 also stimulated ovarian cell proliferation and suppresses apoptosis and follicular growth (36). IL-1 beta acted for the synthesis and regulation of steroids and ovulation in GCs and theca cells of rats (21). This cytokine also improved germinal vesicle breakdown (GVBD) in rabbit ovaries and stimulated meiosis and oocyte maturation in female horses (26).

Pashoutan Sarvar et al. (37) reported that exosomes acted as biological mediators produced under physiological and pathological conditions and it included mRNAs, siRNAs, lipids, ribosomal RNAs. In fact, it was shown that they have a supportive function like mesenchymal cells and suppressed inflammatory responses. They also have tissue repair factors in order to repair tissue damage (27). FSH stimulated estradiol production, and researchers reported when the antral follicle phase occurs naturally in the body, it had a significant effect on oocyte meiosis. FSH was proposed to stimulate GC proliferation, while estradiol increased cell size (28). Based on the results of the previous studies, estradiol can have a positive effect

on the growth of preantral follicles (29). The results of qRT-PCR test showed an increase in *BMP-15*, *BMP-7* and *GDF-9* expression levels of GCs treated with exosome dosages of 25, 50 and 100 µg/ml. Similarly, Ghorbani et al. (38) found that treatment of GCs with Barijeh plant extract increased *BMP-15, BMP-7* and *GDF-9* expression levels that are effective in folliculogenesis.

Conclusion

Results obtained from this study indicated that BMSCs derived exosomes of mice had positive effects on the bioavailability of GCs and reduced apoptosis in these cells. Furthermore, since exosomes increased expression levels of *BMP-15*, *BMP-7* and *GDF-9* genes, they had a positive effect on improving the folliculogenesis and GC growth, Nevertheless, further clinical studies are recommended.

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Author's Contributions

S.F.; Formal analysis, Writing - the original draft, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, and Statistical analysis. J.B.; Formal analysis, writing – the original draft, Study concept and design, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content, and Statistical analysis. A.E.; Critical revision of the manuscript for important intellectual content, Statistical analysis critical revision of the manuscript for important intellectual content. N.H.R.; Formal analysis, Analysis and interpretation of data, Critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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