Gene 711 (2019) 143937

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

# Effects of adipose and bone marrow-derived mesenchymal stem cells on vaginal atrophy in a rat menopause model

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ARTICLE INFO

*Keywords:*
Adipogenic derived mesenchymal stem cells
Bone marrow derived mesenchymal stem cells
Menopause
Rat
Vaginal atrophy

ABSTRACT

*Background & objectives:* Vaginal atrophy is characterized by thinning of vaginal epithelial layers and decreased local blood flow. We aimed to evaluate the regenerative effects of Adipose derived mesenchymal stem cells (ADMSC) and Bone marrow derived mesenchymal stem cells (BMDSC) on vaginal atrophy in rat menopause model.

*Materials and methods:* Rats were randomly divided into 4 (four) groups: sham, control, ADMSC, BMDSC. Vaginal epithelial thickness, structure of the lamina propria, blood vessels in the lamina propria, collagen deposition, and muscle structure were evaluated. Anti ER α, VEGF, VEGFR 1, Bax and bcl-2 antibodies were analyzed. Beta actin gene was used as endogenous control. Genetical differences among the groups were compared by using Kruskal Wallis and Mann Whitney *U* test. $p < 0.05$ was regarded as statistically significant.

*Results:* Epithelial thickness of ADMSC group was higher than control group, but less than sham group Epithelial thickness of BMDSC group was less than sham group. Lamina propria and muscle tissue of ADMSC and BMDSC groups were found to be similar to sham group. VEGFR-1, VEGF, Bax and ER-α staining levels were higher in ADMSC and BMDSC groups than control group. ADMSC group stained stronger with VEGFR-1 and VEGF than BMDSC group. Bcl-2 staining level was increased in ADMSC applied group. No statistically significant difference was detected in Bax and Bcl-2 genes and Bax-/Bcl-2 ratio.

*Conclusions:* Although genetic expression might have ended and could not be significantly demonstrated, histological and immunohistochemical results favor ADMSC application in vaginal atrophy rather than BMDSC.

## 1. Introduction

Vaginal atrophy develops due to decreases in estrogen production and leads to vaginal dryness, pruritus, irritation, and dyspareunia, which negatively affect quality of life and sexual performance in postmenopausal women (Bygdeman and Swahn, 1996). Vaginal atrophy becomes clinically apparent 4 to 5 years after menopause and is characterized by thinning of vaginal epithelial layers, increased vaginal pH, decreased local blood flow, and decreased vaginal secretions. Clinical trials have shown that these symptoms are correlated with a decrease in ovarian hormones (Labrie et al., 1997). Although systemic and local estrogen-based hormonal treatments are highly effective in symptom management (Labrie et al., 1997; Abrams et al., 1982), these treatments are not effective in some patient groups and cannot be used in women with suspicious clinical features (unknown vaginal/uterine bleeding and suspected or diagnosed endometrium and breast cancer) (The Society of Obstetricians and Gynaecologists of Canada, 2005). For these reasons, recent studies have explored the treatment of vaginal atrophy using more specific, non-hormonal alternative therapies (Ibe and Simon, 2010). Apart from these novel alternative agents, mesenchymal

*Abbreviations:* ADMSC, adipose derived mesenchymal stem cells; BMDSC, bone marrow-derived mesenchymal stem cells; MSC, mesenchymal stem cells; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; (Ang) 1 and 2, angiopoietin; VEGFR 1, vascular endothelial growth factor receptor 1; ER, estrogen receptor; hUCMSCs, human umbilical cord mesenchymal stem cells; MT, microtissue; YGW, You Gui Wan

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https://doi.org/10.1016/j.gene.2019.06.027
Received 12 February 2019; Received in revised form 3 June 2019; Accepted 13 June 2019
Available online 19 June 2019
0378-1119/ 

stem cells (MSCs) are known to have anti-inflammatory and regenerative effects (Saidi, 2012; Ishikawa et al., 2010). MSCs are found in many tissues such as bone marrow (bone marrow-derived mesenchymal stem cells, BMDSCs), umbilical cord, periodontal ligament, and adipose tissue (adipose-derived mesenchymal stem cells, ADMSCs) (Saidi, 2012). ADMSCs are used in the field of regenerative medicine because they are easier to obtain in the form of body fat (Ishikawa et al., 2010). ADMSCs have been shown to increase angiogenesis in animal model studies (Chen et al., 2015). Angiogenesis is caused by angiogenic proteins and growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and angiopoietin (Ang) 1 and 2. Moreover, in oophorectomized adult women, apoptosis of vaginal cells has been shown to increase atrophy (Nakamura et al., 2012). For this programmed cell death process, Bcl-2 family proapoptotic proteins (Bax) translocate to the outer membrane of the mitochondria, initiating apoptosis. The Bcl-2 family anti-apoptotic proteins also inhibit cell apoptosis by inhibiting the release of mitochondrial pro-apoptotic factors (Daniel et al., 2003; Martí-nez-Brocca et al., 2008). As a result, the apoptotic rate is determined by balancing anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) proteins. We hypothesized that ADMSCs and BMDSCs may have regenerative and potentially therapeutic effects on postmenopausal vaginal atrophy by decreasing apoptosis and increasing angiogenesis. To explore this hypothesis, we designed a series of studies and created a menopausal model in ovariectomized rats, which mimics estrogen withdrawal in postmenopausal women (Federici et al., 2016; Minami et al., 2016). To detect apoptotic changes in the menopausal rat model, Bcl-2 and Bax expression were investigated. In addition, the effects of ADMSCs and BMDSCs on vaginal atrophy in this menopause model were evaluated and compared histopathologically and immunohistochemically (estrogen receptor [ER], VEGF, vascular endothelial growth factor receptor 1 (VEGFR 1), Bcl-2, and Bax).

## 2. Materials and methods

All animals were handled and euthanized in accordance with the standards of humane animal care described by the National Institutes of Health Guide for the Care and Use of Laboratory Animals, using protocols approved by the Dokuz Eylül University Ethics Committee for Animal Research (approval no: 45/2015). In this study, a total of 28 10–12-week-old virgin Wistar albino rats weighing 170–210 g were used. The rats were housed in four separate cages under 12-h light/12-h dark conditions at 20–24 °C and 50–60% humidity and were fed ad libitum with a standard rat diet. Daily vaginal smears were obtained and only rats undergoing at least two 4–5-day cycles were included in the study.

### 2.1. ADMSC and BMSC isolation

For ADMSC and BMDSC isolation, five rats were administered anesthesia at the Dokuz Eylül University Experimental Animals Laboratory, after which subcutaneous adipose tissue was excised from the inguinal regions under sterile conditions. The adipose tissue was placed in culture medium and ADMSC isolation was performed at the Manisa Celal Bayar University Faculty of Medicine Histology Laboratory. Adipose tissue was washed with sterile phosphate-buffered saline (PBS) and 5% penicillin-streptomycin to remove debris and red blood cells. It was then dissociated using a tissue scalpel and incubated for 30 min at 37 °C and 5% $CO_2$ in α-MEM containing 0.1% collagenase type 1. After at least two centrifugation steps, cells were cultured in culture media (α-MEM, 20% FCS, 1% L-glutamine) at 37 °C under a 5% $CO_2$ atmosphere (Kurt and Vatansever, 2016). The medium was changed every 2 days and the culture was continued for 10 days (Lee et al., 2015). Under sterile conditions, rat femurs and tibias were removed and placed in sterile PBS, following asepsis-antisepsis rules. The femur and tibias were carefully cleaned from the surrounding muscle tissue, the metaphyseal ends were cut, and an insulin syringe was used to fill the bone marrow cavity with primary medium (α-MEM, 15% FCS, 1% penicillin/streptomycin, 0.1% amphotericin B, 1% gentamycin). After washing the medial part of the bones, cells were centrifuged at 1000 rpm for 5 min and cultured at 37 °C and 5% $CO_2$ until confluent. After three days, hematopoietic cells and non-adherent cells were removed by changing the media and the culture was continued for 2 weeks to isolate BMDSCs. Formed stem cells were not differentiated into different tissues. The cells were stained with positive and negative MSC markers to determine the stem cell density (Kurt and Vatansever, 2016). Both ADMSCs and BMDSCs were confluent and labeled using the Brd-U labeling method before transferring to rats. Bromodeoxyuridine (5-bromo-2′-deoxyuridine, BrdU) is a synthetic nucleoside analogue used to identify dividing cells in living tissues. For this purpose, they were incubated with BrdU (11296736001, Roche, Mannheim, Germany) at a 1:1000 dilution at 37 °C for 1 h.

### 2.2. Animal studies

Rats were randomly divided into four groups, as indicated below. The number of rats in each group was minimized by request of the Dokuz Eylül University Ethics Committee for Animal Research. The treatment groups were Sham (n = 6), Control (n = 8), ADMSC (n = 8), and BMDSC (n = 8). Rats were anesthetized with intraperitoneal xylazine hydrochloride (10 mg/kg, Rompun, Bayer, Leverkusen, Germany) and ketamine (50 mg/kg, Ketalar, Eczacıbası, Istanbul, Turkey) and the surgical sites were shaved and cleared with disinfectant. One rat in the ADMSC group and one rat in the BMDSC group were sacrificed during anesthesia.

All rats were subjected to laparotomy via a 2.5-cm midline incision under anesthesia. Following uterine corn and adnexa observation, oophorectomy was performed (excluding the sham group). The skin was closed with 5/0 silk sutures. Two weeks after oophorectomy, rats in the control (n = 8), ADMSC (n = 7), and BMDSC (n = 7) groups were anesthetized and their vaginas were inspected using microforceps. A total of 20 μL of culture medium (pure, containing $1 \times 10^5$ cells/mL ADMSCs, and $1 \times 10^5$ cells/mL BMDSCs) was microinjected into the respective groups. The microinjections were targeted to infiltrate four quadrants (anterior, posterior, bilateral, and sidewall) of the rat vagina through microfolds beneath the vaginal mucous membrane. Two weeks after these microinjections, laparotomy was performed in all rats. The vaginas were anatomically detected and removed. The removed vaginal tissue was longitudinally divided into two equal parts for histological and genetic studies. Histopathological and immunohistochemical analyses were performed in the Histology Department of Manisa Celal Bayar University.

### 2.3. Histopathological analysis

Half of the vaginal tissues were fixed in 10% formalin solution for 48 h and routine paraffin embedding procedure was performed. From paraffin blocks, 5-μm sections were cut and stained using routine hematoxylin and eosin staining protocol. Vaginal epithelial thickness, structure of the lamina propria, blood vessels in the lamina propria, collagen deposition, and muscle structure were evaluated under light microscopy (400×, Olympus).

### 2.4. Immunohistochemical analysis

After deparaffinization of the paraffin sections, the distributions of ER-α, VEGF, VEGFR-1, Bax, and Bcl-2 were analyzed using routine indirect immunoperoxidase staining technique. Binding sites of the primary antibodies were determined using secondary antibodies conjugated with horseradish peroxidase and 3,3′ diaminobenzidine substrate. Negative controls were obtained with antibody primer. Antibody intensities were evaluated as negative (−), weak (+), moderate, (++)

or strong (+++) by two histologists.

### 2.5. RNA isolation and qPCR analysis

Research was performed at the Muğla Sıtkı Koçman University Medical Faculty Medical Biology Laboratory. The vaginal tissue used to detection mRNA expression was stored at −80 °C in RNAlater (Thermo AM7020 RNAlater Stabilization Solution). RNA was extracted from tissues using a total RNA isolation kit (Thermo PureLink 96 total RNA Purification Kit, Catalog number: 12173011A). cDNA was isolated from the obtained RNAs using the reverse transcriptase method with the RevertAid First Strand cDNA Synthesis Kit (Catalog number: K1621). The obtained cDNAs were amplified by real-time PCR using the Taqman Rat Bcl-2 gene expression assay (Cat. 4,331,182), Rat Bax gene expression assay (Cat. 4,331,182), and Rat beta-actin gene expression assay (Cat. 4352340E). Gene expression levels of Bax and Bcl-2 were determined, with the beta-actin gene as an endogenous control. The $2^{-\Delta\Delta Ct}$ method was used to quantify expression.

### 2.6. Statistical analysis

SPSS for Windows version 15.0 was used for all statistical analyses. Genetic variables between the groups were analyzed using the Kruskal Wallis and Mann-Whitney *U* tests. $p < 0.05$ was considered significant. In the analyses, Bax and Bcl-2 gene expression were compared among the four groups. The difference between the Bax/Bcl-2 ratios among the four groups was also examined. The Kruskal-Wallis test, a non-parametric test, was used for comparisons. Gene expression values from six samples in the sham group, seven samples in the control group, six samples in the ADMSC group, and four samples in the BMDSC group were used.

## 3. Results

### 3.1. Histopathological results

Cells were cultured to maintain the adipogenic stem cell lineage after being processed from rat adipose tissue. Although epithelioid cells adhered to the culture chamber in some places (Fig. 1A) on the first day of culture, they accumulated and preserved their epithelioid features in the absence of adipogenic cells (Fig. 1B) during the culture period. Although BMDSCs were not adhered to the culture chamber on the third day of culture (Fig. 1C), we showed that the number of adhered cells was greater than the number of fusiform cells, and they multiplied through ongoing culture in the absence of floating cells (Fig. 1D).

Sham group vaginal specimens were evaluated histologically and found to have non-keratinized stratified squamous epithelium, as well as connective tissue cells with collagen fibers in the lamina propria and normal vaginal muscle structure (Fig. 2A, B). In the menopause model control group specimens, the vaginal epithelial thickness was thinner, the lamina propria had fewer cells, and collagen deposition was more common (Fig. 2C, D) than in the sham group. Epithelial thickness in the ADMSC group was higher than in the control group, but lower than in the sham group (Fig. 2E, F). However, the lamina propria and muscle tissue of ADMSC group were similar to the normal vaginal structure observed in the sham group (Fig. 2E, F). The vaginal epithelial thickness of the BMDSC group was less than that in the sham group, but the muscular tissue and lamina propria maintained normal histological features as observed in the sham group (Fig. 2G, H). Overall, the epithelial layer damage created after oophorectomy in the control group was more regenerated in the ADMSC group than in the BMDSC group. Although changes in the lamina propria and connective tissues were obvious in both the ADMSC and BMDSC groups, the histopathological characteristics of the groups were similar.

### 3.2. Immunohistochemical results

Brd-u labeled cells were followed after transfer into rats. VEGFR-1 immunoreactivity was strongly positive (+++), especially in the epithelium (Fig. 3, A1) in the sham group, whereas the control group showed decreased VEGFR-1 immunoreactivity that stained with moderate intensity (++) (Fig. 3, A2). Epithelial VEGFR-1 immunoreactivity increased and stained strongly (+++) in the ADMSC and BMDSC groups, similar to what was observed in the control group (Fig. 3, A3 and A4) (Table 1). In addition, VEGFR-1 immunoreactivity was strongly positive in some connective tissue regions in the ADMSC group (Fig. 3, A3) (Table 1). Whereas VEGF immunoreactivity was negative in the control group (Fig. 3, B2), it was moderately positive (++) in the sham group (Fig. 3, B1), weakly positive (+) in the epithelium and connective tissue of the ADMSC group (Fig. 3, B3), and was focally moderate (++) in the BMDSC group (Fig. 3, B4) (Table 1). Although VEGF requires VEGFR binding for activation, VEGFR may be present in the absence of VEGF expression. Therefore, the co-occurrence of VEGF and VEGFR may induce angiogenesis. This finding is consistent with our histopathological findings. Negative Bcl-2 immunoreactivity in sham, control and BMDSC groups (Fig. 3, C1, C2, and C4) and weak (+) Bcl-2 immunoreactivity in the ADMSC group (Fig. 3, C4) (Table 1) suggest that ADMSCs increased Bcl-2 expression. Although Bax immunoreactivity was weakly positive (+) in the sham group (Fig. 3, D1), it was negative in the control group (Fig. 3, D2), and weakly (+) similar to the control group in the ADMSC and BMDSC groups (Fig. 3, D3 and D4) (Table 1). Because we observed both Bax and Bcl-2 immunoreactivity in the ADMSC group, ADMSCs may have increased Bcl-2 expression to maintain the Bcl-2:Bax ratio to preserve cell viability in the vaginal epithelium. ER-α immunoreactivity was moderately positive (++) in the sham, ADMSC, and BMDSC groups (Fig. 3, E1, E3, and E4), but it was reduced and stained weakly (+) in the control group (Fig. 3, E2) (Table 1). VEGFR levels decreased in the control group, but this decrease was completely reversed in the ADMSC group (epithelial and connective tissue) and partly reversed in the BMDSC group (only in the epithelial tissue). Although we did not detect VEGF staining in the control group, VEGF levels in the ADMSC and BMDSC groups partially improved. VEGF staining was not detected in every high-power field in the BMDSC group, but in focal fields, the staining level was similar to that in the sham group.

### 3.3. mRNA expression levels of Bax and Bcl-2 genes

There were no significant differences in Bax and Bcl-2 and Bax/Bcl-2 ratios among the four groups ($p = 0.231$, $p = 0.176$, and $p = 0.277$, respectively; Kruskal-Wallis test), and there were no significant differences between Bax and Bcl2 gene expression levels and Bax/Bcl2 ratios between any two groups when compared using the Mann-Whitney U Test individually for each group (Table 2).

## 4. Discussion

ADMSCs and BMDSCs had positive effects on vaginal changes in a rat menopause model. Because there was a stronger increase in VEGFR and Bcl-2 immunoreactivity and greater improvement in vaginal mucosal epithelial thickness after ADMSC administration than after BMDSC administration, we suggest that ADMSCs may have more favorable results based on histopathological changes.

In a recent study, women with menopausal symptoms received transdermal estrogen therapy. This treatment increased the adipogenic stem cell potential in subcutaneous fat of the femoral region, but did not genetically inhibit apoptosis (Cox-York et al., 2017). In our study, both ADMSC and BMDSC treatment reduced the decrease in menopausal ER-α levels, and enhanced ER-α levels to pre-menopausal levels, but did not alter the expression of apoptotic genes. Based on our results, stem cell treatment combined with estrogen therapy, or the isolation of

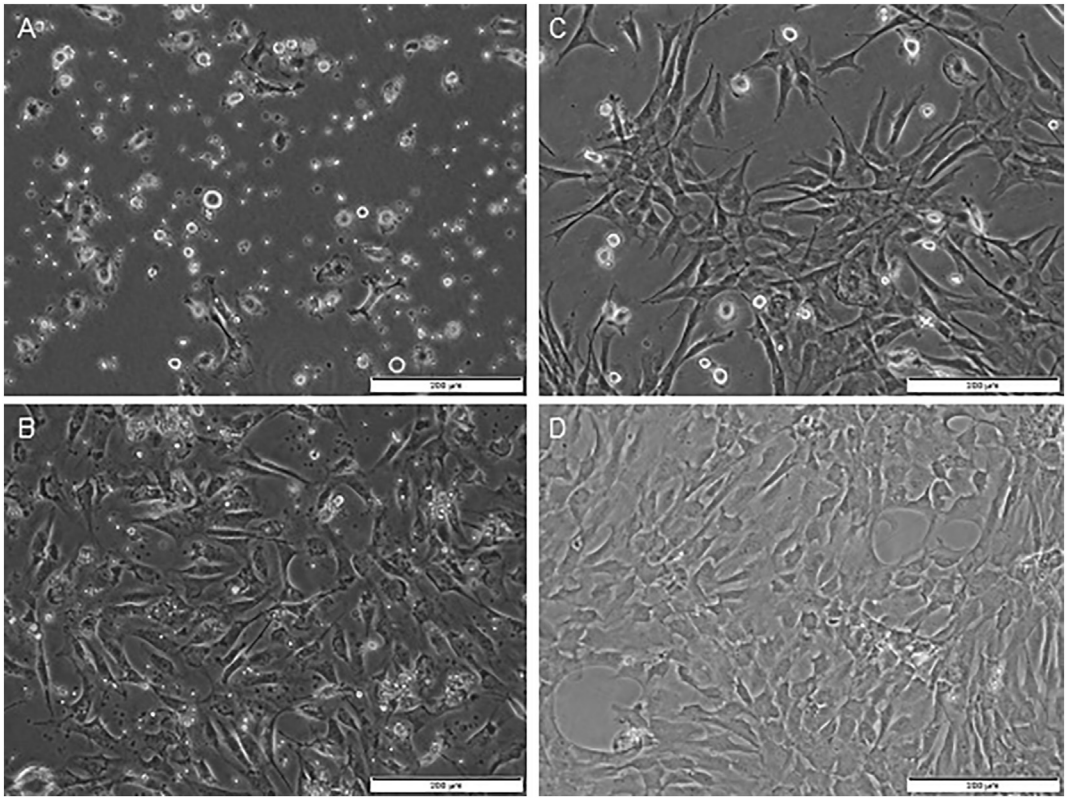


**Fig. 1.** Adipogenic (A, B) and bone marrow stromal (C, D) stem cells after 3. (A, C) and 7. (B, D) days in culture. Scale bars: 200 μM.

stem cells after estrogen therapy for the treatment of vaginal atrophy should be explored in future studies.

In a stress urinary incontinence model created by simulating menopause via oophorectomy combined with preceding child birth injury in rats, it was determined that microtissue obtained using ADMSCs improved voiding function and histopathological findings more effectively than ADMSCs alone (Li et al., 2016a). It has been reported that more VEGF and TNF-α-stimulated gene/protein 6 are produced in rats using microtissue. This previous study is similar to our study in terms of the use of MSCs. In our study, we showed that vaginal atrophy was reduced by ADMSCs and BMDSCs. Based on our results, future studies in which microtissues are formed and used for treatment with ADMSCs are required.

In a study in patients with vulvar dystrophy, adipocytes were obtained from the abdominal regions of patients and ADMSC isolation was performed by cultivating these cells (Giuseppina Onesti et al., 2016). The vaginal and paraurethral tone of patients was restored by injecting these ADMSCs into dystrophic tissues, and hypotrophy-induced genitourinary channel symptoms improved.

A review article (Caplan, 2015) emphasized that exogenous or endogenous MSCs have both scientific and clinical use, and that pilot studies in terms of women's health and randomized controlled clinical trials are required. In an experimental study, MSCs injected intravenously in an experimental vaginal delivery model established in rats were found to be effective through paracrine factors in urethral recovery and elastogenesis (Dissaranan et al., 2014). However, in the rat menopausal model, vaginal surgical wounds were introduced and MSCs were injected into the subepithelial tissue to compare wound healing with local estrogen. Based on our results, the histological structure of MSCs, collagen content, and biomechanical properties of the damaged vagina were the same as those in the placebo group (Ripperda et al., 2017).

In an experimental rat study conducted by Zhang et al. (2018), small intestinal submucosa (SIS) was used to reconstruct a neovagina in rats. They seeded the SIS with BMDSCs and analyzed the effect in rat neovaginas. They reported that microvessel density and VEGF were higher in the neovaginas of the BMDSC-seeded group. In our study, VEGF expression increased in our BMDSC group, which supports our hypothesis of improved angiogenesis through stem cell application.

In an experimental rat study, granulosa cells were collected from perimenopausal rats, and apoptosis of the follicles was induced via cisplatin exposure. The apoptotic follicles were cultured with BMDSCs, and the reduction in apoptosis after BMDSC application was supported by the reduction in Bax mRNA expression in vivo and in vitro (Guo et al., 2013). Our results were consistent with two previous studies on the restorative effect of MSCs in ovaries (Fu et al., 2008; Takehara et al., 2013). In our study, Bax levels were lower in the ADMSC group than in the BMSC group, but this decrease was not significant when compared between groups. Immunohistochemically, Bcl2 levels were higher in the ADMSC group than in the BMDSC group, although this increase was not significant at the gene expression level.

Severe uterine damage was established in an experimental rat study, and scaffold-free cell sheets of ADMSC seed cells were applied to these rats. Regeneration and angiogenic stimulation were detected in the basal layer of the endometrium 21 days after transplantation in rats receiving ADMSC sheets. These findings were supported by an increase in VEGF expression levels (Sun et al., 2018). Human umbilical cord mesenchymal stem cells (hUCMSCs) were also administered through the venous route in a study on natural aging in perimenopausal rats. In these rats, ovarian VEGF expression was measured based on immunohistochemical analysis and western blotting after hUCMSC administration. This study showed therapeutic benefits of hUCMSCs in perimenopausal rats via paracrine mechanisms (Li et al., 2017). In another study conducted in a menopausal rat model, microtissue with ADSCs or ADSCs alone (without microtissue) was applied to the external urethral region. According to the results of this study, in the microtissue with ADMSCs group, VEGF expression was increased compared to that in the ADMSCs-only group. The external and internal

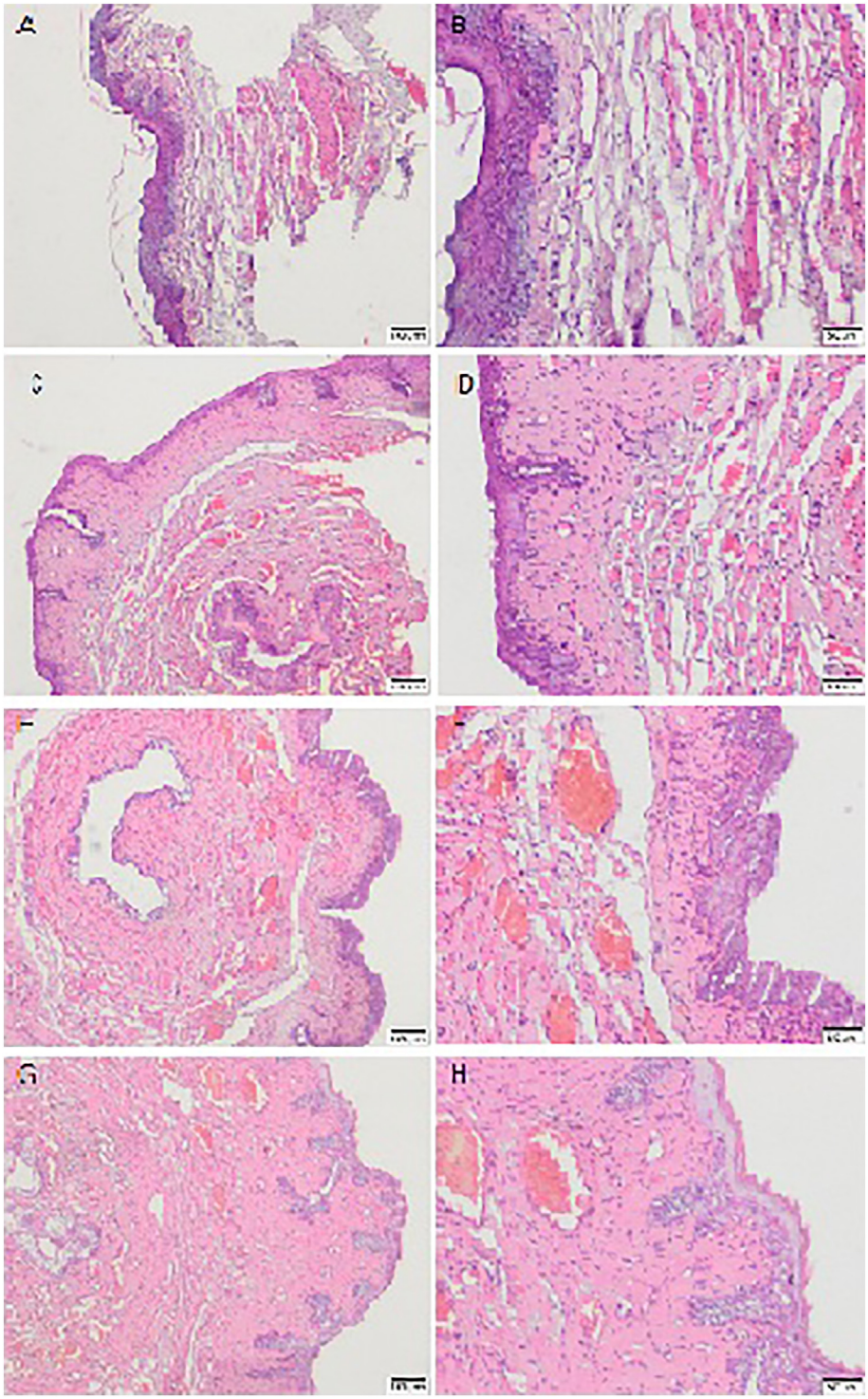


**Fig. 2.** Light microscopical evaluation of vaginas from sham group (A, B), control group (C, D.) ADMSC group (E, F), BMDSC group (G, H) scale bars: 50 μM (A, C, E, G), 100 μM (B, D, F, H).

urethral sphincter functions improved in the group administered ADMSCs with microtissue (Lee et al., 2015). In our study, VEGFR-1 levels were lower in the menopausal group than in the sham group, and VEGF levels decreased to undetectable levels in the menopausal group. After administration of ADMSCs, both epithelial and connective tissues showed increased VEGFR-1 levels. Meanwhile, the increase was only at the epithelial level in the BMDSC group. VEGF levels also increased at both the epithelial and connective tissue levels after administration of ADMSCs, but only showed focal increases in the BMDSC group. Studies in menopause model rats have shown that estrogen replacement therapy improves vaginal atrophy, but does not alter ER-α distribution and expression levels (Li et al., 2016b).

In another experimental rat study, You Gui Wan, a classical herbal formula, was explored as an alternative to hormone replacement therapy for the treatment of menopausal vaginal atrophy, and it was shown that the symptoms of vaginal atrophy were reduced by increasing ER expression in menopause model rats (Hu et al., 2011).

In other studies, intravaginal dehydroepiandrosterone

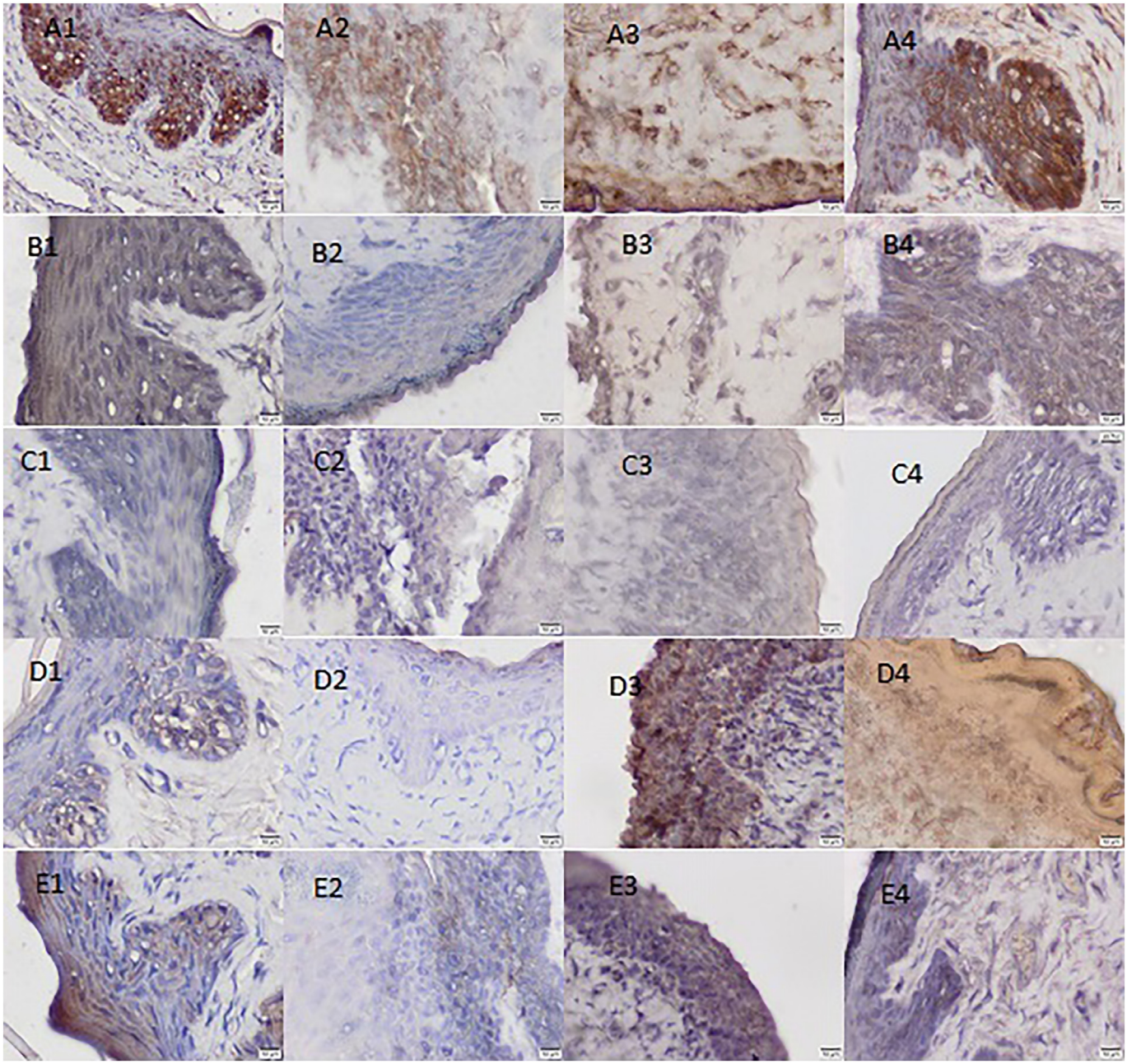


**Fig. 3.** Immunoreactivity of VEFGR-1 in sham group (A1), control group (A2), ADMSC group (A3), and in BMDSC group (A4). Immunoreactivity of VEFG in sham group (B1), control group (B2), ADMSC group (B3), and in BMDSC group (B4). Immunoreactivity of Bcl-2 in sham group (C1), control group (C2), ADMSC group (C3), and in BMDSC group (C4). Immunoreactivity of Bax in sham group (D1), control group (D2), ADMSC group (D3), and in BMDSC group (D4). Immunoreactivity of ER-α in sham group (E1), control group (E2), BMDSC group (E3), and in ADMSC group (E4) Scale Bar: 10 μM.

**Table 1**
Staining levels of VEGFR-1, VEGF, Bcl-2, Bax, ER-α in experimental groups.

| | Sham | Control | ADMSC | BMDSC |
|---|---|---|---|---|
| VEGFR-1 | + + + | + + | + + + epithelial<br>+ + + connective tissue | + + + epithelial tissue<br>(−) connective tissue |
| VEGF | + + | − | + epithelial and connective tissue | Focal + + |
| Bcl-2 | − | − | + | − |
| Bax | + | − | + | + |
| ER-α | + + | Focal + | + + | + + |

administration improved menopausal atrophy but did not alter ER levels (Berger et al., 2008). Oral administration of lasofoxifene, a novel selective estrogen receptor modulator (SERM), has been shown to correct vulvar-vaginal atrophy at the cellular and molecular levels. In addition, raloxifene, tamoxifen, and 17α-ethinylestradiol treatments have been associated with increased levels of vaginal estrogen receptor beta protein (Wang et al., 2006). No previous studies have investigated atrophy and ER-α levels in stem cell applications. Our study is the first to explore stem cell applications in vulvovaginal atrophy. We showed that α-α levels decreased focally in the control group, and it was determined that this focal reduction was corrected in both the ADMSC and BMDSC groups.

We investigated the histopathological, immunohistochemical, and genetic effects of ADMSCs and BMDSCs on vaginal atrophy in a

**Table 2**
Comparison of BAX, BCL2 mRNA expression levels and Bax/Bcl2 ratio among experimental groups. The minimum, maximum, and median values for each group of Bax, Bcl2, and Bax/Bcl2 values are shown. In the middle column of the table, the p-values obtained from the Kruskal-Wallis test and in the rightmost column of the table, the p-values obtained from the Mann-Whitney *U* Test are included. $p < 0.05$ was considered statistically significant.

| | Groups | Min | Median | Max | p-Value | Comparison | p-Values |
|---|---|---|---|---|---|---|---|
| Bax | Sham | 0.466 | 0.782 | 1.044 | 0.231 | Sham vs Control | 0.95 |
| | Control | 0.279 | 0.808 | 2.190 | | Sham vs ADMSC | 0.13 |
| | ADMSC | 0.683 | 0.952 | 6.220 | | Sham vs BMDSC | 0.11 |
| | BMDSC | 0.692 | 1.784 | 4.118 | | Control vs ADMSC | 0.37 |
| | | | | | | Control vs BMDSC | 0.23 |
| | | | | | | ADMSC vs BMDSC | 0.61 |
| Bcl2 | Sham | 0.066 | 0.410 | 1.056 | 0.176 | Sham vs Control | 0.07 |
| | Control | 0.480 | 1.056 | 1.702 | | Sham vs ADMSC | 0.18 |
| | ADMSC | 0.065 | 1.381 | 1.915 | | Sham vs BMDSC | 0.48 |
| | BMDSC | 0.125 | 0.888 | 0.995 | | Control vs ADMSC | 0.73 |
| | | | | | | Control vs BMDSC | 0.16 |
| | | | | | | ADMSC vs BMDSC | 0.35 |
| Bax/Bcl2 | Sham | 0.744 | 2.247 | 7.091 | 0.277 | Sham vs Control | 0.10 |
| | Control | 0.263 | 0.744 | 2.111 | | Sham vs ADMSC | 0.59 |
| | ADMSC | 0.357 | 1.695 | 18.686 | | Sham vs BMDSC | 0.91 |
| | BMDSC | 0.695 | 2.946 | 19.664 | | Control vs ADMSC | 0.29 |
| | | | | | | Control vs BMDSC | 0.23 |
| | | | | | | ADMSC vs BMDSC | 0.48 |

menopause model. Both ADMSCs and BMDSCs triggered protein expression for cell renewal, which was more effective with ADMSC application. However, expression of these proteins persisted for 2–4 weeks (during the procedure), whereas changes in protein levels through cDNA and mRNA is much more rapid. For this reason, genetic demonstration of this positive effect was not possible. To evaluate our results, early stages of the tissues should be collected for gene expression analysis. Nevertheless, our results provide a basis for future human studies.

## Declaration of Competing Interest

The authors declare no competing interest.

## Acknowledgment

This paper was granted by the Muğla Sıtkı Koçman University Research Projects Coordination Office (Project Grant Number 15/208). The statistical analysis was carried out and checked by Dr. Kürşad Tosun.

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