Uterine Infusion With Bone Marrow Mesenchymal Stem Cells Improves Endometrium Thickness in a Rat Model of Thin Endometrium

Jing Zhao, MD[1], Qiong Zhang, MD[1], Yonggang Wang, MD[1], and Yanping Li, MD[1]

Abstract

Bone marrow mesenchymal stem cells (BMSCs) show multidirectional differentiation and possess immunoregulatory properties. Although transplantation of BMSCs has a therapeutic effect on many diseases, it is unclear whether BMSC transplantation can be used as a therapy for a thin endometrium. To explore whether transplantation of BMSCs directly into the uterine cavity can improve endometrium thickness, a thin endometrium rat model was established by infusing ethanol into the uterine cavity. In all, 48 rats with thin endometrium and 24 normal rats were divided into 3 groups: (1) normal group, (2) experimental group transplanted with BMSCs into uterine cavity, and (3) control group transplanted with saline into the uterine cavity. The morphology of the endometrium, the regeneration and receptivity of the endometrium, and the mechanisms involved in BMSC therapy were subsequently analyzed by hematoxylin and eosin staining, Western blot analysis, and reverse transcription-polymerase chain reaction throughout an observation period of 3 estrus cycles. The rats in the experimental group had a significantly thicker endometrial lining and exhibited higher expression of cytokeratin, vimentin, integrin $\alpha\gamma\beta3$, and leukaemia inhibitor factor (LIF) than that of the control group ($P < .05$). Bromodeoxyuridine -positive cells were detected in the endometrium after BMSC transplantation. Some proinflammatory cytokines, such as tumor necrosis factor α messenger RNA (mRNA) and interleukin (IL)-1βmRNA, were significantly downregulated, and anti-inflammatory cytokines, such as basic fibroblast growth factor (bFGF) mRNA and IL-6mRNA, were significantly upregulated in the experimental group compared to the control group ($P < .05$). In conclusion, BMSCs improved endometrium thickness, probably via their migration and immunomodulatory properties. Uterine perfusion with BMSCs represents a promising new tool for the currently intractable problem of an inadequate, thin endometrium.

Keywords

Introduction

Endometrium thickness is a key factor in the implantation of the embryo and in the achievement of pregnancy. There is no officially accepted definition of a thin endometrium, which results in a lower rate of full-term pregnancy. The commonly accepted cutoff is less than 8 mm on the day of luteinizing hormone surge or human chorionic gonadotropin administration.[1] Approximately 0.6% to 0.8% of patients do not have a uterus that reaches the minimum thickness.[2]

Recent studies have focused on different treatment modalities for a thin endometrium, which include the administration of exogenous estrogen, low-dose aspirin, sildenafil citrate, pentoxifylline, vitamin E, L-arginine, and cytokines as well as electroacupuncture and biofeedback therapy. Although these treatments purport to increase the implantation and pregnancy rates in assisted reproductive technology cycles, many of them have proved to be inefficient from an evidence-based medicine point of view.[3] Effective treatments are therefore urgently needed.

Bone marrow mesenchymal stem cells (BMSCs) can differentiate into skeletal myoblasts, renal parenchymal, hepatic epithelium, gut and skin epithelia, neuroectodermal cells,[4] and endometrial cells.[5] They also display immunoregulatory properties[6] that are important for the therapy of many

[1] Reproductive Medicine Center, Xiangya Hospital, Central South University, Changsha, Hunan, China

Corresponding Author:
Yanping Li, Reproductive Medicine Center, Xiangya Hospital, 87 Xiangya Road, Changsha, Hunan 410008, China.
Email: zhaojing19840427@sina.com

diseases. In rodents, BMSC treatment enhanced neoangiogenesis and tissue repair of myocardial injury after skeletal muscle ischemia by increasing the production of an angiogenic factor.[7] Preclinical studies have shown human BMSCs to be effective for the treatment of various genetic disorders, ischemic cardiomyopathy, and hematological diseases.[8] Recently, Du et al found that the bone marrow-derived cells (BMDCs) can localize to uterine endometrium, and BMDCs contribute to endometrium in 2 murine models.[5,9] So far, there has been no report evaluating the therapeutic effect of BMSCs on a thin endometrium.

Recently, several studies have reported the presence of male donor-derived bone marrow cells that could differentiate into epithelial cells[10] and compose endometrial glands in the endometrium of female recipients after bone marrow transplantation.[11] Later, other studies showed that bone marrow progenitor cells contribute to uterine epithelium thickness[12] and the formation of new blood vessels in the endometrium.[13] This has led us to explore further the use of BMSCs as a therapy for increasing the thickness of a thin endometrium. In light of the recent development of mesenchymal stem cells as a therapeutic alternative, we (1) investigated whether transplantation of BMSCs into the uterus improves endometrium thickness and (2) explored the underlying mechanisms responsible for this improvement.

Materials and Methods

Animals

Eight-week-old Sprague-Dawley (SD) rats weighing 200 to 250 g were used in all experiments. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by the Institutional Animal Care and Use Committee of the Central South University.

Bone Marrow Mesenchymal Stem Cells s From Male Rats

Bone marrow mesenchymal stem cells were isolated from adult, male/female, SD rats as described previously.[14] In brief, the femurs and tibiae were collected from rats killed by cervical dislocation. Bone marrow cells were harvested by flushing the marrow cavity with Dulbecco modified Eagle's medium (DMEM; Biowest, France). The suspended cells were then collected by centrifugation at $200g$ for 5 minutes. The cells were resuspended and cultured in L-DMEM media with 10% fetal calf serum and 1% penicillin–gentamicin at 37°C in a humidified incubator with 5% CO_2. The nonadherent hematopoietic cells were removed after 48 to 72 hours. The culture medium was changed every 3 days. Adherent BMSCs that reached 90% confluence were harvested using 0.05% trypsin–EDTA (Life Technoogies, Gibco). The BMSCs cultured to the third passage were used for transplantation. Seventy-two hours prior to transplantation, BMSCs

were supplemented with 2 μmol/L bromodeoxyuridine (BrdU; Sigma, St Louis) to label dividing cells. Cells were harvested with trypsin–EDTA, washed twice with L-DMEM, and resuspended at a concentration of 50 000 cells/μL in L-DMEM.

Before performing the in vivo experiments, cells were characterized for their ability to differentiate into adipocytes and osteoblasts as reported previously. Their mesenchymal phenotype was also assessed by fluorescence-activated cell sorting (FACS).

Groups and Treatment

The rats were kept under standardized laboratory conditions in an air-conditioned room with free access to food and water. A thin endometrium rat model was set up by injecting anhydrous ethanol into the uteruses of rats according to the method described in our preliminary study.[15] A preliminary experiment was done to characterize the extent of endometrial cell injury when BMSCs were infused (n = 3). Seventy-two rats were randomly assigned to 3 groups: (1) an experimental group directly injected with BMSC into the uterine cavity 6 to 8 hours after modeling (n = 24), (2) a control group directly injected with saline into the uterine cavity 6 to 8 hours after modeling (n = 24), and (3) a normal group with no treatment (n = 24). In experimental/control group, the specific approach was: experimental rats received reoperation after developing model. Clipping one end of the uterus, inject BMSCs suspension/saline into the uterine cavity from another end with syringe. At 3 estrus cycles after BMSC transplantation, the rats were killed and uteri were excised. Portions of the uteri were sectioned and preserved in formalin and/or liquid nitrogen for further examination.

Hematoxylin and Eosin Staining

Sections on slides were immersed in xylene (10 minutes, twice) and rehydrated in a decreasing ethanol series in distilled water (100%, 100%, 95%, 95%, 75%, and 0%, 1 minute each). The sections were rinsed in deionized water, stained in hematoxylin for 45 seconds, rinsed in deionized water, and finally stained in eosin for 1 second. After the color reaction, sections were dehydrated through an ethanol series into xylene and mounted using Permount mounting medium (Fisher Scientific). Endometrial thickness was measured in cross-section of the uterus as the vertical distance between the endometrial–myometrial interface and the endometrial surface. The endometrial thickness was measured with image-processing software (Image-ProPlus 6.0) on the photos taking from slides. The thickness and the morphology of endometrium were evaluated and compared between the groups.

Immunohistochemistry

After fixation in 4% paraformaldehyde, the uterine horns were embedded in paraffin and about 6-μm serial sections were prepared and placed on SuperfrostPlus microscope slides (VWR International Ltd, Mississauga, Ontario, Canada). Sections were deparaffinized in xylene, rehydrated through a

series of ethanol washes, and rinsed in water. Endogenous peroxidase activity was blocked by incubating sections in 0.3% H_2O_2 in methanol for 40 minutes at room temperature. Slides were blocked for 1 hour in phosphate-buffered saline supplemented with 10% normal goat serum. The expression of cytokeratin, vimentin integrin β3, LIF, and BrdU proteins was determined by incubating sections of rat uteri with rabbit polyclonal antibodies against cytokeratin (Santa Cruz, California), vimentin (Sigma), integrin β3 (Santa Cruz), LIF (Santa Cruz), and BrdU (Sigma) overnight at 4°C. After further incubating the sections with 1:3000 horseradish peroxidase -conjugated goat antirabbit immunoglobulin G (IgG) in 10% goat serum for 1 hour at room temperature, the sections were then briefly counterstained (10 seconds) with hematoxylin solution (Gill no. 3, Sigma) and examined under a Nikon microscope (Eclipse 90i, Japan). For each protein studied, immunohistochemical staining was repeated twice at each time point on sections obtained from different rats.

Western Blotting

The tissues were homogenized in solubilization buffer. The homogenate was centrifuged at 10 000*g* for 10 minutes at 4°C. The supernatant was removed. The protein concentration was determined using a detergent-compatible protein assay using bovine serum albumin as the standard. For the detection of cytokeratin, vimentin, integrin αγβ3, and LIF, 20 μg of protein from each sample was loaded onto an 8% sodium dodecyl sulfate -polyacrylamide gel and then transferred to a polyvinylidene fluoride membrane. The blots were blocked with 5% milk in Tris-buffered saline (TBS) buffer and then incubated with the primary antibody overnight at 4°C with anticytokeratin antibodies (Santa Cruz, 1:500), anti-vimentin antibodies (Sigma, 1:500), anti-integrin αγ antibodies (Santa Cruz, 1:300), anti-integrin β3 antibodies (Santa Cruz, 1:500), or anti-LIF antibodies (Santa Cruz, 1:300) according to the manufacturers' recommendations. The membrane was washed with TBS and incubated with antirabbit IgG (1:3000; Cell Signaling Technology; Danvers, Massachusetts). Immunoreactivity was detected using enhanced chemiluminescence (ECL, Amersham). Equal protein loading was verified by reprobing the membrane with anti-β-actin antiserum (Sigma) and by staining the membrane with Coomassie Blue.

Reverse Transcription-Polymerase Chain Reaction

Total RNA was collected using TRIzol (Invitrogen, Carlsbad, California), and complementary DNA was synthesized using the SuperScript first-strand synthesis system (Invitrogen). Complementary DNA was amplified by the polymerase chain reaction (PCR) using the AmpliTaq Gold Kit (Applied Biosystems, Foster City, California). The PCR was performed using primers for proinflammatory factors, interleukin (IL) 1β, and tumor necrosis factor α (TNF-α), anti-inflammatory factors, basic fibroblast growth factor (bFGF) and IL-6, along with primers for glyceraldehyde-3-phosphate dehydrogenase as an internal control. The following conditions were used for amplification: denaturation at 95°C for 9 minutes, followed by 38 (IL-1β and TNF-α) or 35 (bFGF and IL-6) cycles at 94°C for 45 seconds, 57°C for 45 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. Polymerase chain reaction products were separated on 2% agarose gels.

The PCR primers used are as follows:

> Tumor necrosis factor α:
> Sense 5′-CCA CGT CGT AGC AAA CCA CCA AG-3′
> Antisense 5′-CAG GTA CAT GGG CTC ATA CC-3′, 316 bp
> Interleukin 6:
> Sense 5′-GGA GTT CCG TTT CTA CCT GG-3′
> Antisense 5′-GCC GAG TAG ACC TCA TAG TG-3′, 275 bp
> Interleukin 1β:
> Sense 5′-CAC CTT CTT TTC CTT CAT CTT TG-3′
> Antisense 5′-GTC CTT GCT TGT CTC TCC TTG TA-3′, 268 bp
> Basic fibroblast growth factor:
> Sense 5′-GGA GAA GAG CGA CCC ACA CG-3′
> Antisense 5′-TGC CCA GTT CGT TTC AGT GC-3′, 237 bp
> Glyceraldehyde 3-phosphate dehydrogenase:
> Sense 5′-CTC AAG ATT GTC AGC AAT GC-3′
> Antisense 5′-CAG GAT GCC CTT TAG TGG GC-3′, 404 bp

Data Analysis

Data are presented as mean ± standard error. Within-group comparisons were analyzed using the 1-sided *t* test. Multiple groups were analyzed with a 1-sided analysis of variance and Bonferroni post hoc testing, using the statistical software SPSS Version 16.0 (IBM, Chicago, Illinois). For Western blot (WB) analysis, the ECL-exposed films or gel images were digitized, and densitometric quantification was carried out using UN-SCAN-IT gel (version 5.3, Silk Scientific Inc., Orem, Utah). Relative protein levels were obtained by comparing the respective specific band to the β-actin control from the same membrane or gel. For animals that were subject to repeated testing, analysis of variance with repeated measures was used. A *P* value of <.05 was considered statistically significant.

Results

Bone Marrow Mesenchymal Stem Cells Phenotype

Bone marrow mesenchymal stem cells were grown in culture as reported previously.[16] The FACS analysis showed the BMSCs expressed CD90 and CD73 but did not express CD45 or CD34. The BMSCs were able to differentiate into adipocytes and osteoblasts (not shown).

Histopathological Observations

Endometrial thickness, superficial epithelia of the endometrium, and the number of endometrial glands were all significantly different between control rats and those transplanted with BMSCs. Histologic evaluation of the uterine in the experimental group showed that the structure of endometrium was intact and characterized by an increased endometrial thickness and a higher number of endometrial glands and capillaries, whereas the uterine horn in the control group was completely destroyed and showed extensive necrosis.

The endometrial thicknesses of the normal group, experimental group, and control group were as follows: 359.13 ± 49.70 μm, 330.54 ± 56.31 μm, and 187.53 ± 34.38 μm, respectively. The endometrial lining of rats in the experimental group was significantly thicker than that of rats in the control group ($P < .01$), but there was no significant difference between the experimental group and the normal group ($P > .05$; Table 1 and Figure 1).

Bone Marrow Mesenchymal Stem Cell Treatment Promotes the Regeneration of Endometrial Cells

To explore the effect of BMSC transplantation on a thin endometrium, we assessed the expression of cytokeratin, vimentin, integrin β3, and LIF by performing immunohistochemical and WB analyses. Immunohistochemical staining showed that expression of cytokeratin, integrin β3, and LIF was mainly localized in the cytoplasm of the endometrial epithelium and that vimentin was mainly expressed in the cytoplasm of endometrial stroma cells. The expression of cytokeratin, vimentin, integrin β3, and LIF in the experimental group was significantly higher than that of the control group ($P < .01$) and was slightly weaker than that of the normal group ($P > .05$; Figure 2).

The results of WB analysis showed that the expression of cytokeratin, vimentin, integrin αvβ3, and LIF was significantly higher in the experimental group than in the control group ($P < .05$). However, there was no significant difference between the experimental group and the normal group (Figure 3).

Bone Marrow Mesenchymal Stem Cells Were Localized to the Endometrium

Immunohistochemical staining showed the presence of BrdU-labeled cells in the endometrium of rats in the experimental group, whereas no BrdU-labeled cells were detected in the endometrium of rats in the control group or in the normal group. Labeled BMSCs were localized predominantly around blood vessels (Figure 2).

Bone Marrow Mesenchymal Stem Cell Modulated the Expression of Cytokines

To test whether BMSCs treatment resulted in the regeneration of the endometrium via modulation of the expression of

Table 1. Endometrial Thickness of Groups (x ± s).[a]

Groups	Thickness of Endometrium, μm
Normal group	359.13 ± 49.70[b,c]
Experiment group	330.54 ± 56.31[b,d]
Control group	187.53 ± 34.38[c,d]

[a] There was significant difference between the groups ($P < .05$).
[b] $P > .05$.
[c] $P < .05$.
[d] $P < .05$.

pro- and anti-inflammatory cytokines, we examined the expression of bFGF messenger RNA (mRNA), TNF-αmRNA, IL-1βmRNA, and IL-6mRNA using reverse transcription-PCR (RT-PCR). The results showed that the expression of proinflammatory cytokines, such as TNF-αmRNA and IL-1βmRNA, was significantly downregulated and that the expression of anti-inflammatory cytokines, such as bFGFmRNA and IL-6mRNA, was significantly upregulated in the experimental group compared with the control group ($P < .05$; Figure 4).

Discussion

Mesenchymal Stem Cells (MSCs) have shown a strong propensity to ameliorate tissue damage in response to injury and disease. MSCs have demonstrated efficacy as therapeutic vectors in animal models of lung injury,[17] kidney disease,[18] diabetes,[19] graft versus host disease,[20] and myocardial infarction.[21] However, there has been no evaluation of the therapeutic effect of BMSCs on a thin endometrium until now.

Our results showed that rats whose uterine cavities had been injected with BMSCs had a thicker endometrium and a higher expression of cytokeratin and vimentin, a marker protein for endometrial cells, than that of rats that had not been injected with BMSCs. This indicates that the direct infusion of BMSCs into the uterine cavities of these rats with a thin endometrium protected against cell damage and/or promoted the regeneration of endometrial cells.

Another novel finding of the present study was that the expression of integrin αγβ3 and LIF was significantly higher in rats after BMSC transplantation, and the rats had similar levels of expression of integrin αγβ3 and LIF to that of normal rats. It is well known that integrin and LIF, which are both regulators of endometrial function, are markers for endometrial receptivity and have an important role in embryo implantation.[22] Our data, which showed higher expression of cytokeratin, vimentin, integrin αγβ3, and LIF in the BMSC-transplanted rats, suggest that stem cell therapy may not only promote endometrial regeneration but may also improve endometrial receptivity.

To date, BMDCs have been shown to regenerate the endometrium in a single patient with apparent Asherman syndrome[23] and in a newly established mouse model of Asherman syndrome. In the case study,[23] the administration of autologous BMDCs into the uterine cavity immediately
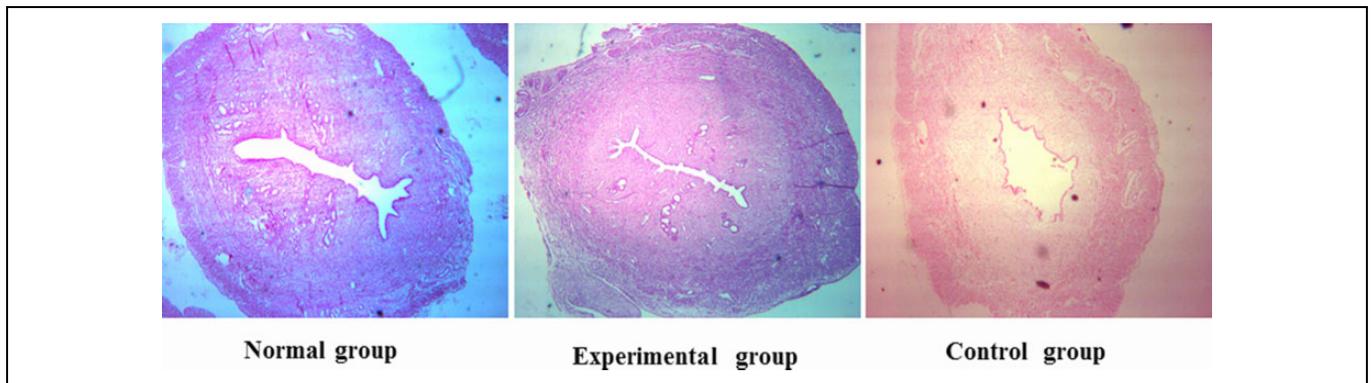
Figure 1. A, Normal group, (B) experimental group, and (C) control group. Endometrium morphology in the 3 groups was examined by H&E staining (H&E, ×40). The endometrial lining was significantly thicker in the experimental group than in the control group (*P* < .01), and there was no significant difference between the experimental group and the normal group. H&E indicates hematoxylin and eosin.
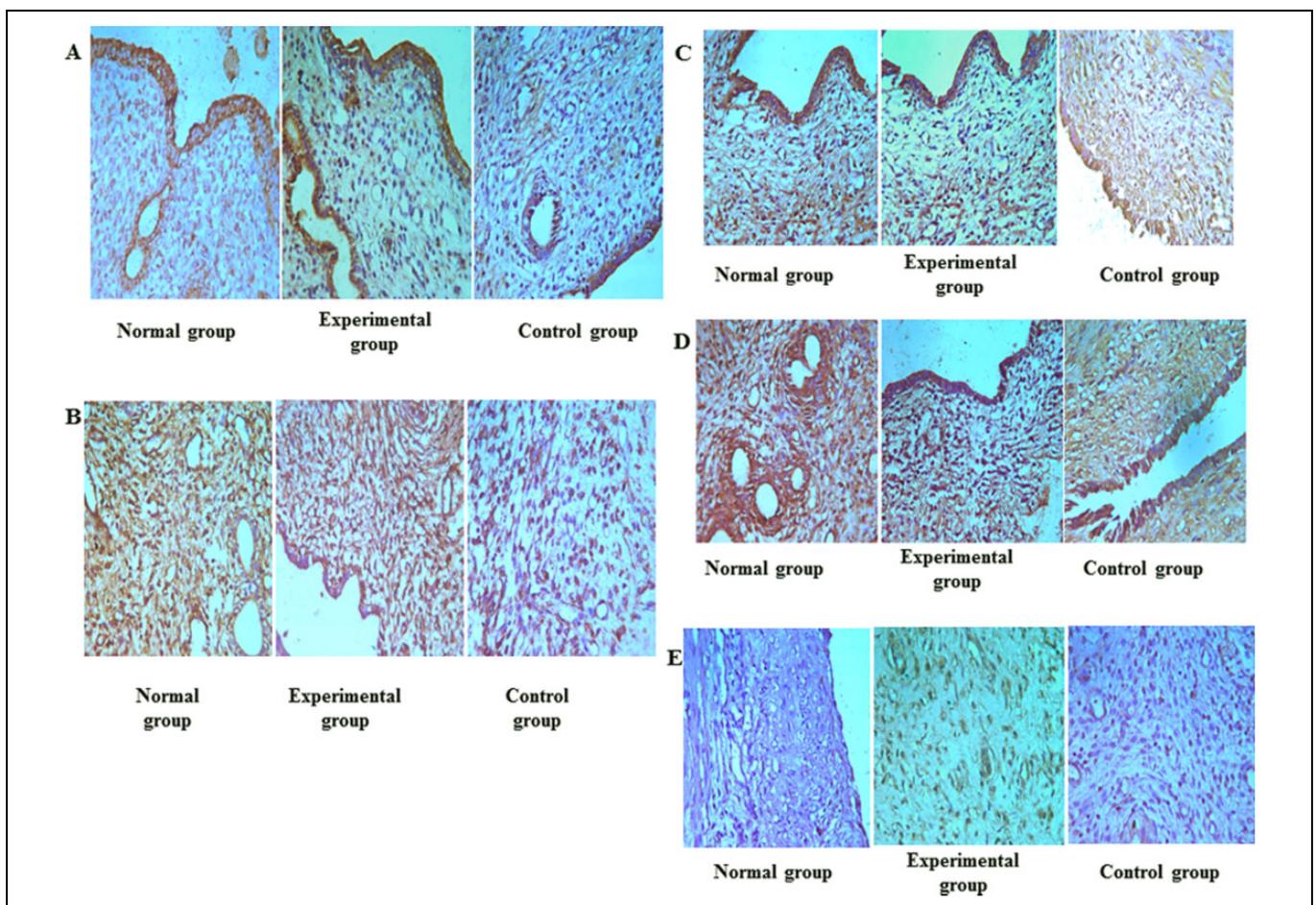

Figure 2. Expression of vimentin, cytokeratin, integrin β3, LIF, and BrdU labeling. A-E, Expression of cytokeratin, vimentin, integrin β3, LIF, and BrdU labeling. A-D, The expression of cytokeratin, vimentin, integrin β3, and LIF was significantly higher in the experimental group than that of the control group (*P* < .05), and there was no significant difference between the experimental group and the normal group (*P* > .05). E, BrdU-positive cells only detected in the experimental group, and the number of BrdU-positive cells was significantly different compared with the normal group and the control group (*P* < .05). BrdU indicates bromodeoxyuridine.

following curettage resulted in endometrial regeneration to a thickness of 7 mm over several months of estrogen treatment. Subsequent in vitro fertilization procedures resulted in a clinical pregnancy. However, it is difficult to determine whether the curette-induced damage provided a portal of entry for BMSCs into the residual endometrium or whether it stimulated previously refractory resident stem/progenitor cells to generate a thicker endometrium.[23]
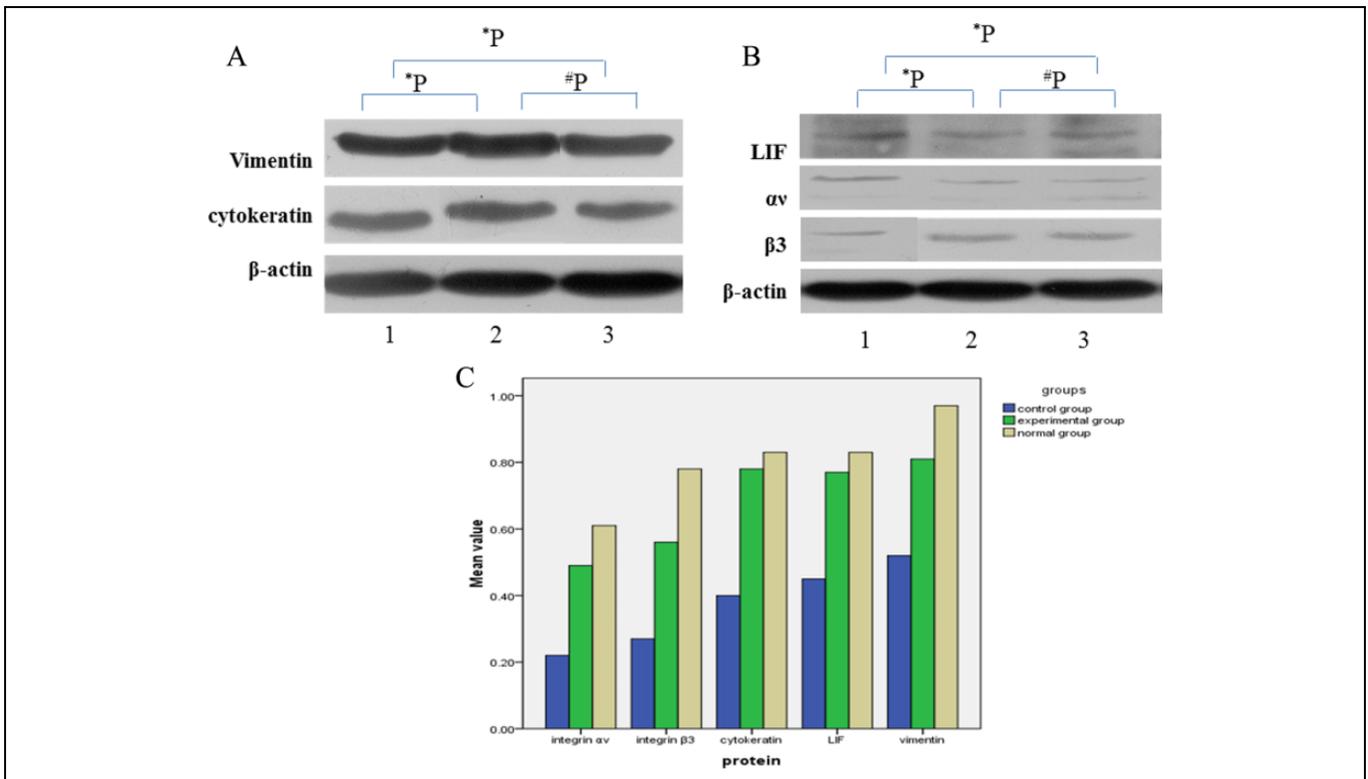
Figure 3. Western blot analysis of the expression of cytokeratin, vimentin, integrin $\alpha\gamma\beta3$, and LIF. Groups 1-3, normal group, experimental group, and control group, respectively. A and B, The results of Western blot analysis. C, The relative protein levels expressed as the Gray value ratio (% Adj Vol). All the results showed that the expression of cytokeratin, vimentin, integrin $\alpha\gamma\beta3$, and LIF was significantly stronger in the experimental group than in the control group ($P < .05$), and there was no significant difference between the experimental group and the normal group ($P > .05$).

In the present study, we not only showed that BMSCs were effective in improving endometrium thickness but also explored the possible mechanisms underlying the therapeutic effects of BMSCs.

The exact mechanism of how BMSCs improve endometrium thickness is not known. One possibility is that some BMSCs introduced into the uterine cavity differentiated into endometrial cells, thus repairing the thin endometrium. In agreement with other studies employing human MSCs in a rat model of myocardial infarction,[24] we also detected BrdU-positive cells in the endometrium after BMSC transplantation. Although BrdU-positive cells were present in very low numbers, they may have been incorporated into organs where they transdifferentiated into cells to generate new tissue or they may have contributed to angiogenesis as shown previously in other organ injury treatment studies.[25,26]

An alternative explanation is that BMSCs function through their immunomodulatory properties. For instance, BMSCs upregulate anti-inflammatory cytokines, such as IL-2, IL-4, and IL-10, and downregulate proinflammatory cytokines such as TNF-α and IL-17 in animal disease models.[27,28] In one animal model where MSCs were introduced into infarct (ischemia) lesions in the heart,[29] the MSCs did not act by differentiating into cardiac myocytes but rather acted by secreting molecules that increased angiogenesis and decreased scarring or fibrosis.

In our study, we detected gene expression of bFGFmRNA, TNF-αmRNA, IL-1βmRNA, and IL-6mRNA using RT-PCR. The results demonstrated upregulation of anti-inflammatory cytokines (bFGF and IL-6) and downregulation of proinflammatory cytokines (TNF-α and IL-1β) after BMSC transplantation. Interleukin-2, IL-3, IL-6, IL-8 and bFGF are all known to stimulate and mobilize BMSCs.[30,31] The regulation of cytokines by BMSCs may stimulate endometrial cell proliferation and exert a powerful inhibitory action on endometrial cell necrocytosis induced by ethanol. Thus, the immunomodulatory action of BMSCs may be one of the mechanisms via which the BMSCs in our study exerted a therapeutic effect on the thin endometrium.

Conclusion

Bone marrow mesenchymal stem cells may be useful for the regeneration of endometrium thickness by differentiating into multiple cell phenotypes and by exercising immunomodulatory effects. These findings suggest a potential cell therapy for the treatment of a thin endometrium.
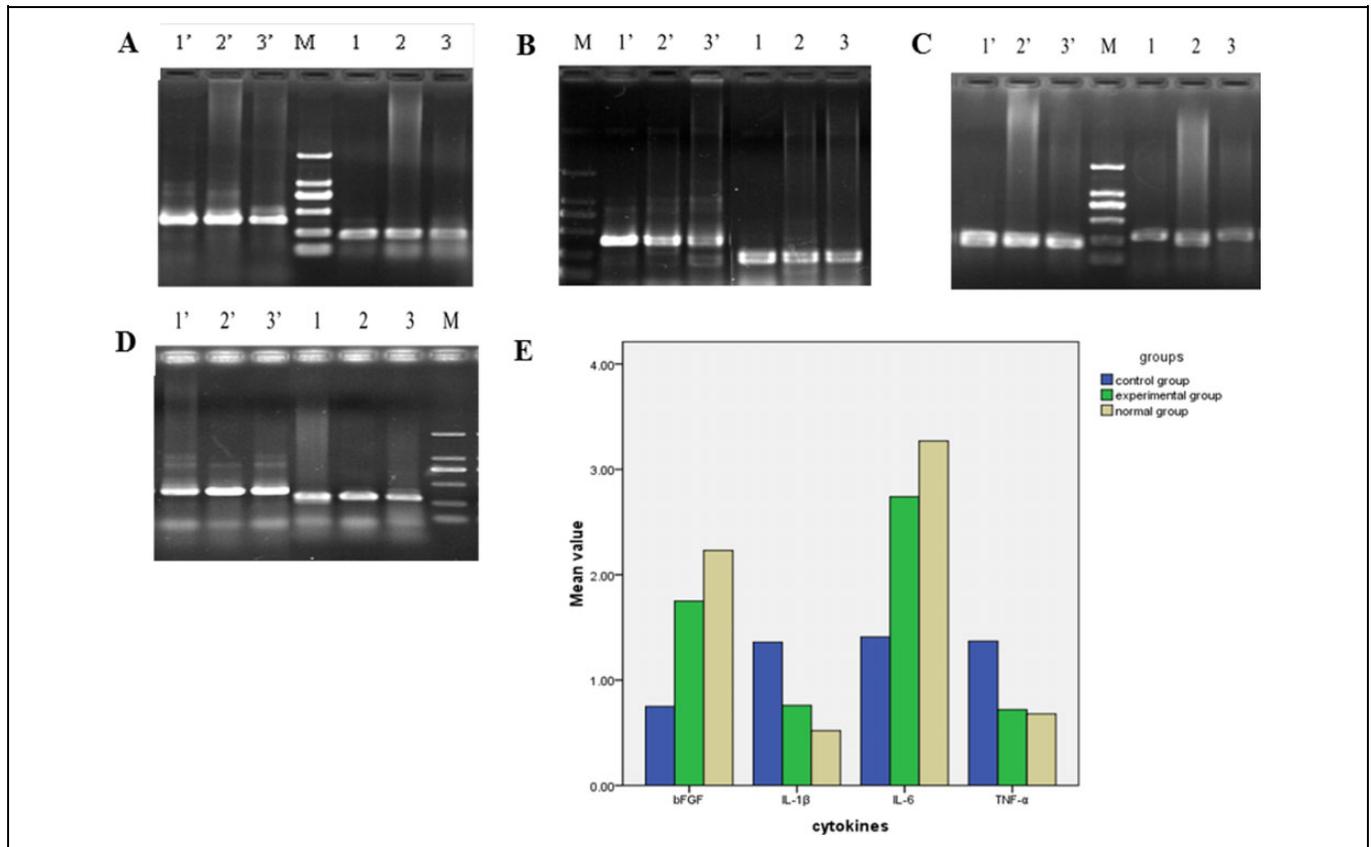
Figure 4. RT-PCR analysis of the expression of bFGFmRNA, IL-6mRNA, IL-1βmRNA, and TNF-α mRNA. A-D, Expression of bFGFmRNA, IL-6mRNA, IL-1βmRNA, and TNF-αmRNA. E, The relative mRNA content expressed as the Gray value ratio (% Adj Vol). Groups 1-3 (1'-3'), normal group, experimental group, and control group. Groups 1'-3': Internal reference GAPDH. The results showed that the expression of bFGFmRNA and IL-6mRNA was significantly upregulated in experimental group compared with the control group ($P < .05$), and the expression of IL-1βmRNA and TNF-αmRNA was significantly downregulated in the experimental group compared with the control group ($P < .05$). RT-PCR, reverse transcription polymerase chain reaction; bFGF, basic fibroblast growth factor; mRNA, messenger RNA; IL, interleukin; TNF, tumor necrosis factor α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Authors' Note

Dr Jing Zhao contributed to designing the study, to acquiring the data, and to the analysis and interpretation of the data, and participated in the drafting and final approval of the article. Dr Qiong Zhang and Dr Yonggang Wang contributed to designing the study and to interpreting the data, and participated in the revision and final approval of the article. Dr Yanping Li contributed to designing of study, to supervising the experiments and to interpreting the data, and participated in the revision and final approval of the article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultra-sonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review. *Hum Reprod Update*. 1996;2(4):323-335.
2. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. *Reprod Biol Endocrinol*. 2008;6:37.
3. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. *Curr Opin Obstet Gynecol*. 2008;20(3):221-228.
4. Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. *Stem Cells*. 2004;22(4):487-500.

5. Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*. 2007; 25(8):2082-2086.

6. Yagi H, Soto-Gutierrez A, Parekkadan B, et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cells Transplant*. 2010;19(6):667-679.

7. Kinnaird T, Stabile E, Burnett MS, et al. Local delivery of marrow derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation*. 2004;109(12):1543-1549.

8. Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: An update on clinical trials with mesenchymal stem cells. *J Cell Physiol*. 2007;211(1):27-35.

9. Du H, Naqvi H, Taylor HS. Ischemia/reproduction injury promotes and Granulocyte-Colony Stimulating Factor inhibits migration of bone marrow-derived stem cells to endometrium. *Stem Cells Dev*. 2012;21(18):3324-3331.

10. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA 2004;292(1):81-85.

11. Ikoma T, Kyo S, Maida Y, et al. Bone marrow-derived cells from male donors can compose endometrial glands in female transplant recipients. *Am J Obstet Gynecol*. 2009;201(6):608.e1-e8.

12. Bratincsák A, Brownstein MJ, Cassiani-Ingoni R, et al. CD45-positive blood cells give rise to uterine epithelial cells in mice. *Stem Cells*. 2007;25(11):2820-2826.

13. Mints M, Jansson M, Sadeghi B, et al. Endometrial endothelial cells are derived from donor stem cells in a bone marrow transplant recipient. *Hum Reprod*. 2008;23(1):139-143.

14. Munoz-Elias G, Woodbury D, Black IB. Marrow stromal cells, mitosis, and neuronal differentiation: stem cell and precursor functions. *Stem Cells*. 2003;21(4):437-448.

15. Zhao J, Gao H, Li YP. Development of an animal model for thin endometrium using 95% ethanol. *J Fert In Vitro*. 2012;2(4):109.

16. Morigi M, Introna M, Imberti B, et al. Human bone marrow mesenchymal stem cells accelerate recovery of acute renal injury and prolong survival in mice. *Stem Cells*. 2008;26(8):2075-2082.

17. Ortiz LA, DuTreil M, Fattman C, et al. Interleukin 1 receptor antagonist mediates the anti-inflammatory and anti-fibrotic effect of mesenchymal stem cells during lung injury. *Proc Natl Acad Sci U S A*. 2007;104:11002-11007.

18. Kunter U, Rong S, Djuric Z, et al. Transplanted mesenchymal stem cells accelerate glomerular healing in experimental glomerulonephritis. *J Am Soc Nephrol*. 2006;17(8):2202-2212.

19. Lee RH, Seo MJ, Reger RL, et al. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci U S A*. 2006; 103(46):17438-17443.

20. Ringden O, Uzunel M, Rasmusson L, et al. Mesenchymal stem cells for the treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006;81(10):1390-1397.

21. Minguell JJ, Erices A. Mesenchymal stem cells and the treatment of cardiac disease. *Exp Biol Med (Maywood)*. 2006;231(1):39-49.

22. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*. 2006;12(6):731-746.

23. Nagori CB, Panchal SY, Patel H. endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. *J Hum Reprod Sci*. 2011;4(1):43-48.

24. Hou M, Yang KM, Zhang H, et al. Transplantation of mesenchymal stem cells from human bone marrow improves damaged heart function in rats. *Int J Cardiol*. 2007;115(2):220-228.

25. Deng J, Petersen BE, Steindler DA, Jorgensen M, Laywell ED. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells*. 2006;24(4):1054-1064.

26. Wislet-Gendebien S, Hans G, Leprince P, Rigo JM, Moonen G, Rogister B. Plasticity of cultured mesenchymal stem cells: switch from nestin-positive to excitable neuron-like phenotype. *Stem Cells*. 2005;23(3):392-402.

27. Mao F, Xu WR, Qian H, et al. Immunosuppressive effects of mesenchymal stem cells in collagen induced mouse arthritis. *Inflamm Res*. 2010;59(3):219-225.

28. Bai L, Lennon DP, Eaton V, et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia*. 2009;57(11):1192-1203.

29. Imanishi Y, Saito A, Komoda H, et al. Allogenic mesenchymal stem cell transplantation has a therapeutic effect in acute myocardial infarction in rats. *J Mol Cell Cardiol*. 2008;44(4): 662-671.

30. Yagi H, Soto-Gutierrez A, Parekkadan B, et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. *Cell Transplant*. 2010;19(6):667-679.

31. JY Oh, KM Kim, MS Shin, et al. The anti-inflammatory and anti-angiogenic role of mesenchymal stem cells in corneal wound healing following chemical injury. *Stem Cells*. 2008;26(4): 1047-1055.