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A Single-Blind Randomized Controlled Trial of Intrauterine Mesenchymal Stem Cell-Derived Exosome Injection for Improving IVF Outcomes in Infertile Women With Thin Endometrium

Mahbod Ebrahimi[1], Fatemeh Davari-Tanha[1], Ghazal Sahaiyan[1,] Naghmeh Pourmand[1], Razieh Akbari[1], Fatemeh Jayervand[2], Azadeh Falahatkar[1], Maryam Hatami[1*]

Abstract

Objectives: The present study aimed to investigate the Impact of mesenchymal stem cell (MSC) therapy in women with Refractory Thin Endometrium.

Materials and Methods: In this single-blind randomized clinical trial, 60 infertile women with a history of at least one in vitro fertilization (IVF) cycle cancellation due to less than 7 mm endometrial thickness (ET) after receiving standard hormonal therapy were randomly assigned into two groups (n=30/each): the intervention group recived intrauterine injection of 5 mL exosome suspension on the 8th day of IVF cycle) and the control group received Intrauterine injection of 5 mL sterile water on the 8th day of IVF cycle). Demographic characteristics, pre- and post-treatment endometrioma size, pelvic pain, dysmenorrhea, and dyspareunia were compared in both groups.

Results: Baseline information has no significant differences between the two groups.

Despite the significant increase in the ET following hormonal therapy in both groups, the number of women with ET less than 7 mm in the control group was about twice (13 vs. 6) that of the intervention group, resulting in cycle cancellation. Of the cases who enter the embryo transfer cycle, pregnancy was detected in 3 women (12.5%) in the intervention group and 1 case (6.6%) in the control group, with no significant difference between the groups (P=0.612).

Conclusions: Finally, the results of the present study showed that although the use of MSCs can decrease the number of IVF cycle cancellations; however, larger studies is needed to confirm our findings.

Keywords: Stem-cell therapy, Exosomes, Infertility, Randomized controlled trial, Mesenchymal stem cells

Introduction

The endometrium is a flexible and highly dynamic tissue that undergoes cyclical renewal in preparation for embryo implantation (1). Cyclic endometrial remodeling depends on the proper function of several cell types, including parenchymal, endothelial, and immune cells, as well as adult stem cells that can originate from endometrial or ectopic sources (2). The ability of the endometrium to regenerate quickly and repeatedly without scarring is a unique feature of this tissue. However, if this tissue renewal process is disrupted or malfunctions, women may experience infertility due to endometrial scarring or persistent atrophic/thin endometrium. Such issues limit the treatment options for female infertility and the success of in vitro fertilization (IVF) because of the lack of therapies that specifically target the endometrium (2,3).

The pathogenesis of a thin endometrium has multiple causes, with the endometrium becoming thinner with age due to hormonal influences. Factors within the uterine cavity may affect the expression of certain genes, which can inhibit endometrial stromal cell proliferation and promoting apoptosis (4). The primary therapeutic options for endometrial thickness (ET) include pharmacological treatments (such as hormonal and vasoactive drugs), regenerative medicine, intrauterine infusion of growth factors and granulocyte colony-stimulating factor, autologous platelet-rich plasma, and complementary alternative therapies (including traditional Chinese herbal medicine and acupuncture) (5).

A growing number of studies have investigated the potential benefits of adult stem cells, including mesenchymal stem cells (MSCs), in repairing of different body organ tissues, such as ovarian dysfunction, endometrial remodeling, and uterine scars (6-8). In addition to their differentiation capacity, MSCs migrate to damaged sites where they secrete bioactive factors to aid in tissue repair (9).

Some studies using rodent models and limited human trials have demonstrated the potential benefits of MSCs in the treatment of endometrial causes of infertility (8-11).

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[1]Department of Infertility, Yas Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Gynecology and Obstetrics, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
*Corresponding Author: Maryam Hatami, Email:drmaryamhatami.gyn.115097@gmail.com

Key Messages

- ► Exosome MSCs can be applied in infertile women with thin endometrial thickness.
- ► The use of MSCs can decrease the number of IVF cycle cancellations.

However, our knowledge about the application of MSCs in this regard is limited. Hence, the present study aimed to investigate the effect of exosomes injection into the endometrial cavity on the success rate of frozen embryo transfer cycles in patients with thin endometrium.

Materials and Methods

Study Design and Participants

This parallel superiority single-blinded randomized clinical trial was performed on 60 infertile women aged 20 to 45 years old, with ultrasonographic evidence of thin ET (ET less than 7 mm) referred for frozen embryo transfer in the infertility department of Yas Hospital Complex, Tehran, Iran, from December 2024 to July 2025. Exclusion criteria were a history of diabetes, liver diseases, untreated hypothyroidism, brain tumors, uterine malformations, endometrial polyps, submucosal fibroids, uterine adhesions, severe endometriosis and taking antidiabetic or corticosteroids drugs.

Interventions

For all participants, a transvaginal ultrasound was performed on the 3th day of the menstrual cycle. If there was no ovarian cyst, 6 mg of estradiol valerate tablets (2 mg, Aburaihan CO., Tehran, Iran) was started daily. On the 8th day of the cycle, 2 mg was added to the estradiol dose. At this visit time, the eligible women were randomly allocated to the intervention group or the control group with allocation ratio of 1:1. The intervention group received 5 ml of prepared MSCs (prepared by Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran) which injected using an IUI catheter one cm above the internal OS of the cervix. For the control group, 5 ml of sterile water (Iranian Parenteral and Pharmaceutical Company (IPPC), Tehran, Iran) was injected using an IUI catheter one centimeter above the internal OS.

The MCS preparation method was as follows: at first, human placenta-derived MSCs are cultured in GMP-grade specific culture medium according to GMP guidelines using an ultracentrifuge. Cells cultured at passage 3 and 80% confluence are cultured for 48 hours in GMP-grade specific culture medium containing 5% FBS plus exosomes, and the cell supernatant is collected. The collected supernatant is centrifuged according to GMP standards in the following cycles:

1) 400 g for 10 minutes to remove floating cells in the medium.
2) Centrifuge the supernatant solution from the previous step at 2500 g for 30 minutes to remove apoptotic bodies and debris.
3) Ultracentrifuge the supernatant solution from the previous step at 100,000 g for 2 hours.
4) Ultracentrifuge the supernatant solution from the previous step again at 100,000 g for 2 hours, and discard the supernatant solution.

The resulting pellet is suspended in normal saline, purified by high-performance liquid chromatography (HPLC), and finally filtered with a 0.22 µm filter (1-3). The shape and size of MSCs were measured by transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). The protein concentration on the surface of exosomes was measured using the BCA protein analysis kit. The expression of markers CD81, CD9, and CD63 was measured by western blotting, and the size of exosomes was also examined by dynamic light scattering (DLS) (4,5).

Outcomes and Data Collection

The primary outcome was ET changes, which were measured by ultrasonography on the 8th day of the cycle. Also, the clinical pregnancy rates (gestational sac observation) were assessed 12 weeks after embryo transferring was done. Patient-related variables included age, BMI, number of previous pregnancies, number of previous abortions, history of underlying diseases, and their treatment.

Sample Size

Due to the value mentioned in Hosseini Aghdam et al study (1), the sample size was estimated to be minimum of 60 (30 in each group) by considering $\alpha = 0.05$, power 80%, and acceptable difference, based on post hoc power analysis should be added, and limitations discussed.

Randomization and Blinding

For randomization allocation, we used randomized allocation rule method. At first, 30 letters A and 30 letters B were written on special papers that were not marked inside. Then all of them were placed in a bag, and for each patient, after obtaining informed consent, a paper was removed randomly and without replacement, and based on the letter written on it, the desired intervention was performed for the patient. Finally, the meaning of sheet A was the exosomes group, and the meaning of sheet B was the control group. This study was a single-blind study in which the outcome assessor was blinded and unaware of the patient grouping.

Data Analysis

All data were evaluated using descriptive statistics, including mean, standard deviation, and relative frequency. The normal distribution of data was assessed by the Kolmogorov-Smirnov. For data analysis, the chi-square test (for correlation between qualitative variables) and the t-test (for correlation between quantitative variables) were used. All analyses were performed according to the intention to treat (ITT) protocol using the Statistical Package for the Social Sciences software (SPSS, version 26.0 for Windows; SPSS Inc., Chicago, IL). A *P* value of < 0.05 was considered statistically significant.

Results

Initially, 71 women were evaluated to enter the study. Out of them, 11 women were excluded due to having underlying disorders (n = 2) and age >45 years (n = 7), also declined to continue participating in the study (n = 2). Finally, a total of 60 women with a mean age of 37.13 ± 6.45 years were assigned to two groups (n = 30/each), and their data were analyzed (Figure 1). Baseline information, including age, body mass index (BMI), infertility duration, and underlying diseases, shows no significant differences between the two groups (Table 1).

Despite the significant increase in the ET following the mentioned therapy in both groups, the number of women with ET less than 7 mm in the control group was about twice (13 vs. 6) that of the intervention group, resulting in cycle cancellation. The ET was not statistically different on the day of embryo transfer between the two study groups (7.60 ± 1.21 vs. 7.12 ± 1.30 mm, *P* = 0.147). However, ET delta increasing in the intervention group was more (2.47 ± 1.59 vs. 1.95 ± 1.04 mm, *P* = 0.142).

Embryo transfer was done for 24 women of the interventional group while this was only in 15 cases of the control group. Of the cases who enter the embryo transfer cycle, pregnancy was detected in 3 women (12.5%) in the intervention group and 1 case (6.6%) in the control group, with no significant difference between the groups (*P* = 0.612) (Table 2).

We did not observe any significant adverse effects or any exosome-related reactions in our patients. At the same time, one case of the control group reported vaginal discharge after injection. Also, we did not need any interim analyses and stopping rules for safety or futility of the participants.

Discussion

Today, many new methods are used to treat male and female infertility. Assisted reproductive technology (ART) includes all infertility treatments in which an ovum or embryo is managed outside the body (13). The main type of ART is IVF. IVF is the most common form of ART. The steps of this method are that the ovum and sperm are combined in the laboratory to create an embryo. After about 3 to 5 days, the embryo (or embryos) is transferred to the woman's uterus. Embryos can also be frozen for future use (13,14).

Endometrial thickness is one of the important factors in the success of IVF treatments. The appropriate ET for embryo implantation and pregnancy success is in the range of 8 to 12 mm. The lower this thickness, the lower the chance of fertility (14). Thin or damaged endometrium remains an unsolved problem in the treatment of infertility patients. Empirical preference for ET among physicians is greater than 7 mm, and a thin and refractory endometrium that does not respond to standard medical treatments can be the cause of frequent embryo implantation failure (15).

Francés-Herrero et al showed that to provide an optimal environment for embryo implantation and growth, the

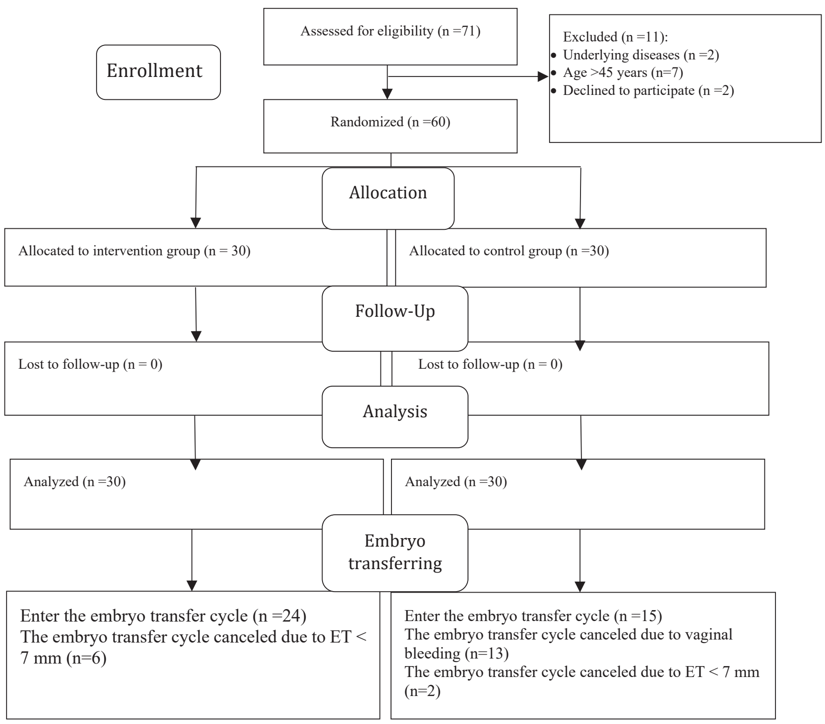

Figure 1. The Flow Diagram of the Study.

Table 1. The Demographic and Clinical History of the Women, Separately for the Two Groups of Patients

Variable	Intervention group (n=30)	Control group (n=30)	*P* value
Age (years), mean ± SD	37.96 ± 6.57	36.06 ± 6.36	0.260[a]
BMI (kg/m^2), mean ± SD	25.52 ± 2.71	25.63 ± 1.80	0.854[a]
Mean time of infertility (years), mean ± SD	5.03± 3.80	4.33± 3.17	0.442[a]
Underlying diseases, No. (%)	19 (63.3)	19 (63.3)	0.605[b]

[a] Independent t-test; [b] Chi-square test.

Table 2. The Infertility History and Therapeutic Measures of Patients

Variable	Intervention group (n=30)	Control group (n=30)	*P* value
ET on the day of embryo transfer, mean ± SD	7.60 ± 1.21	7.12 ± 1.30	0.147[a]
ET diff, mean ± SD	2.47 ± 1.59	1.95 ± 1.04	0.142[a]
ET <7 mm, No. (%)	6 (20)	13 (43.3)	0.052[b]
Clinical pregnancy, No. (%)	3 (10.0)	1 (3.3)	0.612[b]

[a] Independent t-test; [b] Chi-square test.

female reproductive system must coordinate uterine dynamics with appropriate hormones produced by the ovaries. One of the most important programs of this coordination is to adjust the thickness of the endometrium for ovum implantation. In animal studies and in vitro, exosomes have been able to largely cause the creation of appropriate ET and successful ovum implantation and pregnancy (16).

Wang et al showed that the use of exosome-derived MSCs can largely improve problems related to thin ET and increase the chances of fertility, but this effect depends on various factors such as the patient's age, underlying diseases, and uterine manipulation (17).

Both of the above studies are largely consistent with our study in terms of the successful effect of exosomes on changing ET and the success of embryo transfer (16, 17).

Stem cell exosomes are small vesicles secreted by stem cells and contain various substances, including growth factors, proteins, and RNA. These substances can play a role in intercellular communication and the regulation of biological processes, and are of interest in various fields, including tissue repair (6-8).

The success of IVF depends on a set of events and factors related to the endometrium and the embryo. One of the most important factors of the endometrium is its thickness. Factors that can be effective in regulating the thickness of the endometrium can increase the success of IVF. Exosomes can be effective in this field, although their role is not yet well defined (18).

In a study by Liang et al, it was found that exosomes derived from human umbilical cord MSCs can repair damaged endometrial epithelial cells, reduce cell death, and increase the anti-inflammatory properties of the endometrium (19).

Exosome MSCs influence biological processes such as fibrosis, angiogenesis, cell proliferation, and inflammatory responses, and play a key role in dynamic physiological homeostasis and disease development. These cells can be effective in repairing endometrial tissue, especially thin endometrium, and have a significant impact on increasing fertility by IVF (20).

Today, laboratory studies have shown that stem cell-based therapies have been able to improve the therapeutic frontiers of endometrial insufficiency and inject new hope into infertile couples using IVF (21). The current understanding and future directions for research and treatment of infertility related to endometrial structural defects based on stem cells will provide a broad perspective of new scientific findings in this regard (22).

Limitations of the Study

Limitations of the study include a small sample size, a single-center study design, the low follow-up period, which did not allow us to draw definitive conclusions.

Conclusions

Finally, the results of the present study showed that although the use of MSCs can decrease the number of IVF cycle cancellations, it did not have a significant effect on the pregnancy rates.

Authors' Contribution

Conceptualization: Mahbod Ebrahimi, Fatemeh Davari-Tanha.
Data curation: Ghazal Sahaiyan, Fatemeh Jayervand.
Formal analysis: Naghmeh Pourmand.
Investigation: Maryam Hatami.
Methodology: Razieh Akbari.
Project administration: Maryam Hatami, Mahbod Ebrahimi.
Supervision: Mahbod Ebrahimi.
Writing–original draft: Maryam Hatami, Fatemeh Jayervand, Azadeh Falahatkar.
Writing–review & editing: Mahbod Ebrahimi, Fatemeh Davari-Tanha, Ghazal Sahaiyan, Naghmeh Pourmand, Razieh Akbari, Fatemeh Jayervand, Azadeh Falahatkar, Maryam Hatami.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues
The study proposal was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (Code: IR.TUMS.MEDICINE.REC.1403.221) and registered in the Iranian Registry of Clinical Trials (identifier: IRCT20150105020558N8). All participants signed a written informed consent form before entering the study..

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