Case Report

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The First Case Report Of A Pregnancy After Exosome Therapy In Diminished Ovarian Reserve Patient

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1. Abstract

Diminished ovarian reserve (DOR) is one of the causes of infertility. Some new research focuses on mesenchymal stem cells (MSCs) in ovarian regeneration. MSCs exert their effect by secreting exosomes. Evidence has shown that secreted exosomes from MSCs have the same effect as MSCs. This study investigated safety and efficacy of autologous exosomes derived from menstrual blood mesenchymal stem cells (EXO-MensMSCs) injection into the ovary of an DOR patient. To assess the safety and efficacy, some clinical symptoms and incidence of natural pregnancy were investigated after injection, respectively. After injection, no suspicious signs were attended during follow-up. Also, in the monthly ultrasound examinations, a natural pregnancy occurred. As well as the number of ovarian follicles increased. However, there was no significant change in Anti Mullerian Hormone (AMH). In conclusion, EXO-MensMSCs can be used as promising candidates to improve the function of ovaries in DOR patients.

2. KeyWords:

Diminished ovarian reserve; Cell free therapy; Exosomes; Assisted reproduction

3. Introduction

Diminished ovarian reserve (DOR) is a common cause of infertility that occurs when the ovary loses its natural fertility potential due to a low number or poor quality of the remaining ova [1-3]. Pregnancy difficulties are more likely to occur in infertile women who have undergone high-tech infertility treatments like in vitro fertilization (IVF), intrauterine insemination (IUI), and intracytoplasmic sperm (ICSI) injection [4]. IVF clinical practice still faces a major difficulty in handling patients diagnosed with DOR. DOR is frequently linked to suboptimal ovarian stimulation response, a heightened likelihood of cycle cancellations, and a notable decrease in conception rates during IVF cycles. In recent years, there has been a surge in research efforts aimed at enhancing ovarian function, leading to novel perspectives on the treatment of patients diagnosed with DOR. Numerous treatment interventions have been explored to enhance ovarian function in women diagnosed with DOR. However, their utilization in clinical practice is limited due to a lack of substantial evidence about their safety and effectiveness [5,6].

One of these hopeful strategies is the usage of mesenchymal stem cells (MSCs) derived exosomes, which are mediators and messengers in reproductive biology. The existence of exosomes throughout reproductive bio-fluids, such as follicular and ovarian fluid, indicates their involvement in the intercellular communication processes that are crucial for the optimal operation of the reproductive system [7,8]. Exosomes derived from different sources have been applied in different animal experiments to restore ovarian function. These exosomes influenced the signaling pathways, growth and proliferation, control of gene expression, and apoptosis via their cargo and induced the regeneration of ovarian function [9,12]. Here, we employed the intraovarian injection of autologous exosomes derived from menstrual blood mesenchymal stem cells (EXO-MensMSCs) as an alternative approach to IVF for the treatment of a patient diagnosed with DOR.

4. CasePresentation

A female who has infertility due to DOR was referred to Imam Khomeini Hospital in Sari for treatment. She had 3 follicles and 0.7 ng/ml level anti mullerian hormone (AMH). The patient underwent routine medical treatments as well as assisted reproduction, but unfortunately, the desired results were not achieved. Therefore, the patient was enrolled in our study's phase 1 clinical trial, with an ethics code IR.MAZUMS.IMAMHOSPITAL.REC.1400.041 and IRCT code IRCT20210228050535N1. The patient was thoroughly consulted with her primary doctor, who provided all the necessary study information. After considering all the details, the patient freely agreed to participate by signing the declared consent form outlining the study's objectives, procedures, potential risks, and benefits. The patient's decision to participate was entirely voluntary, and she was assured that she could withdraw from the study at any time without any negative consequences. Upon obtaining informed consent, the patient was enrolled in this study. A sample of menstrual blood was collected from the patient. Menstrual blood was collected from the patient using a menstrual cup on the second day of menstruation to isolate exosomes. Then, autologous menstrual blood mesenchymal stem cells (Mens-MSCs) were isolated, cultured, and characterized. The cultured cells exhibited a spindle-shaped, fibroblast-like morphology and adhered to the flask. During the third passage, isolated cells were analyzed using flow cytometry to determine their characteristics. The analysis showed positive expression of CD73 and CD105 as mesenchymal markers but negative expression of hematopoietic markers. The exosomes were isolated and purified from the culture medium using size exclusion chromatography (SEC). The characteristics of exosomes were determined, and their sterility was checked. Exosomes were obtained from the culture medium of the characterized cells. These exosomes positively expressed CD9, CD63, and CD81 markers in flow cytometry studies. Moreover, the transmission electron microscopic observation revealed that the isolated exosomes had a cup-shaped morphology. Additionally, the size of the isolated exosomes, evaluated by dynamic light scattering, ranged from 30 to 150 nm. Furthermore, no evidence of contamination was detected in the isolated exosomes.

The patient underwent an ultrasound to check for any cysts or malignancies in her ovaries 2 weeks before the injection. Since no abnormalities were detected, the patient was permitted to receive EXO-MensMSCs. During the procedure, the patient was under conscious analgesia. Ultrasound guidance was used to perform exosome injection. In the follicular phase, 2 ccs of exosome with a total protein amount of 90 µg/ml were injected into each ovary in 7 to 10 areas, from the cortex to the medulla. After the injection, the treatment's effectiveness was evaluated three months later, and safety was assessed until now. Clinical symptoms such as fever, pain, infection, bleeding, and allergic reactions were monitored to evaluate safety. The effectiveness of exosome therapy was determined based on the incidence of natural pregnancy in the patient within three months after the injection. On the day of the injection, the patient experienced no nausea, infection, bleeding, or fever. Consequently, she was discharged from the hospital after 4 hours. The participant was monitored daily and showed no severe clinical signs such as swelling, fluid collection, or fever. The patient experienced some pain around the injection area that persisted for up to 24 hours but eventually subsided. An experienced doctor conducted a physical examination and ultrasound during the follow-up, but no suspicious symptoms were observed. The serological assessment showed no level of AMH improvement at follow-ups. Nevertheless, follicle improvement was observed, and 7 follicles were shown in the ultrasound assessment. Almost two months after the injection, the patient encountered menstruation arrest. The possibility of pregnancy was raised. An ultrasound was conducted, which revealed the presence of an embryonic sac ten weeks after the injection (Figure 1).

Figure 1:

Figure1: Ultrasound result: The participant was monitored daily after injection and showed no severe clinical signs. Almost two months later, menstruation arrest occurred, and the possibility of pregnancy was raised. An ultrasound confirmed pregnancy two months after injection.

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5. Discussion

DOR refers to a medical condition characterized by a reduction in both the quantity and quality of ovum present in the ovary. This decline is accompanied by a decrease in the levels of AMH, a decrease in the number of antral follicles, and an elevation in the levels of follicle-stimulating hormone (FSH). This phenomenon results in impaired fertility and below-average outcomes, even in cases when assisted reproductive techniques (ARTs) are being performed. Regarding current developments in IVF treatment approaches, the management of patients diagnosed with DOR remains a significant hurdle in the field of clinical practice. In order to enhance outcomes, researchers have directed their efforts towards the identification of targeted pharmaceutical interventions aimed at enhancing ovarian functioning in individuals diagnosed with DOR [6]. The ovarian reserve is influenced by various factors, including hormones, metabolites, environmental factors, diseases, treatments, the initially formed ovarian reserve, and unknown reasons. In recent years, many women have opted to delay pregnancy for personal or professional reasons. The previously mentioned trend has resulted in a rise in incidences of DOR within the field of clinical reproductive medicine. Unfortunately, the causes of DOR are poorly understood, and it presents with complex clinical features, making targeted treatment difficult for physicians [13]. In this study, we focused on a woman who was struggling with infertility due to DOR. No specific reason for the decrease in ovarian reserves was discovered in this patient. She initially underwent routine treatments, which included medication and assisted reproduction. However, despite undergoing these treatments for a certain period of time, there was no significant improvement in her condition. Following disappointment with previous treatments, the patient decided to undergo an intraovarian injection of EXO-MensMSCs as a volunteer.

The potential implications of DOR are significant, involving not only effects on conception but also on general well-being attributable to the occurrence of early menopause [14]. There are several routine treatments available for infertility, including IVF, ICSI, IUI, hormone therapy, and egg donation. Women who undergo high-tech infertility treatments and become pregnant are at a higher risk of undergoing pregnancy difficulties. Therefore, all infertility patients must be informed of the increased risks.In order to minimize the potential complications associated with pregnancy in women undergoing infertility treatments, it is essential to focus on optimizing the treatment. This can be achieved through the adoption of less intense stimulation protocols, which can help prevent the ovarian hyperstimulation syndrome (OHSS) [4]. Our study revealed that the patient who received EXO-MensMSCs injection was discharged from the hospital without experiencing any acute side effects such as bleeding, fever, nausea, or infection. The patient was closely monitored through monthly follow-up sessions and examinations, and no signs of severe side effects were reported. It is noteworthy that two months after the injection, the patient was able to conceive naturally, indicating the safety and feasibility of the treatment.

6. Conclusion

According to result, we could say that autologous exosomes derived from Mens-MSCs could be a suitable therapeutic option to restore the ovarian function of DOR patients and increase the chance of normal pregnancy. Exosome injection can increase ovarian function and fertility in humans without any critical side effects. Overall, these results give new hope to those who have longed for parenthood. Considering the single-dose injection in this study, the efficiency of exosomes can be enhanced by increasing the dose or using repeated injections in future studies. In conclusion, our data suggest that treating DOR using autologous EXO-MensMSCs is safe and promising.

6.1 Author contributions:

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6.2 Conflicts of interest: There is no conflict of interest between the authors.

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