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Improving Fertility in Non-Obstructive Azoospermia: Results from An Autologous Bone Marrow-Derived Mesenchymal Stromal/Stem Cell Phase I Clinical Trial

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Abstract

Background: In this phase I clinical trial, our primary objective was to develop an innovative therapeutic approach utilizing autologous bone marrow-derived mesenchymal stromal/stem cells (BM-MSCs) for the treatment of non-obstructive azoospermia (NOA). Additionally, we aimed to assess the feasibility and safety of this approach.

Materials and Methods: We recruited 80 participants in this non-randomized, open-label clinical trial, including patients undergoing NOA treatment using autologous BM-MSCs (n=40) and those receiving hormone therapy as a control group (n=40). Detailed participant characteristics, such as age, baseline hormonal profiles, etiology of NOA, and medical history, were thoroughly documented. Autotransplantation of BM-MSCs into the testicular network was achieved using microsurgical testicular sperm extraction (microTESE). Semen analysis and hormonal assessments were performed both before and six months after treatment. Additionally, we conducted an *in-silico* analysis to explore potential protein-protein interactions between exosomes secreted from BM-MSCs and receptors present in human seminiferous tubule cells.

Results: Our results revealed significant improvements following treatment, including increased testosterone and inhibin B levels, elevated sperm concentration, and reduced levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin. Notably, in nine patients (22.5%) previously diagnosed with secondary infertility and exhibiting azoospermia before treatment, the proposed approach yielded successful outcomes, as indicated by hormonal profile changes over six months. Importantly, these improvements were achieved without complications. Additionally, our *in-silico* analysis identified potential binding interactions between the protein content of BM-MSC-derived exosomes and receptors integral to spermatogenesis.

Conclusion: Autotransplantation of BM-MSCs into the testicular network using microTESE in NOA patients led to the regeneration of seminiferous tubules and the regulation of hormonal profiles governing spermatogenesis. Our findings support the safety and effectiveness of autologous BM-MSCs as a promising treatment modality for NOA, with a particular focus on the achieved outcomes in patients with secondary infertility (registration number: IRCT20190519043634N1).

Keywords: Azoospermia, Clinical Trial, Mesenchymal Stem Cells, Protein-Protein Docking, Reproductive Techniques

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Introduction

Inability to achieve pregnancy despite having regular sexual intercourse for 12 months is defined as "infertility" (1). This pathology affects approximately 8-12% of married couples of reproductive ages worldwide (2). The most severe form of male infertility is non-obstructive azoospermia (NOA), which is the absence of spermatozoa in the ejaculate due to impaired spermatogenesis (3). Therapies for male infertility fall into several categories, including optimization of sperm production, relief of obstruction, and surgical sperm retrieval (4).

Men suffering from NOA have no other treatment options than to attempt testicular sperm retrieval, including testicular sperm extraction (TESA), conventional TESA (cTESE), and microsurgical TESA (microTESE). Among these techniques, the microTESE technique has higher success rates, less damage to the testicular tissue, a larger number of sperm, and a relatively higher chance of sperm cryopreservation than other techniques in NOA cases. Despite the advances in this technique, the clinical pregnancy rate is not favorable (5). Stem cell therapy can give a ray of hope for NOA patients with unsuccessful pregnancies after microTESE surgery (6).

Mesenchymal stromal/stem cells (MSCs) are undifferentiated cells found in the human body. MSCs possess the ability to differentiate into various types of cells, including those in the mesodermal lineage such as osteocytes, adipocytes, and chondrocytes. MSCs can also differentiate into cells in the ectodermal lineage, such as neurocytes, and those in the endodermal lineage, such as hepatocytes. Additionally, MSCs have the capacity for self-renewal (7). MSCs are among the best types of stem cells available for clinical cell therapy. They have several advantages over other stem cells, including multilineage differentiation, secretion of anti-inflammatory cytokines and growth factors, ease of isolation and expansion, and lack of ethical issues. MSCs also exhibit immunosuppressive properties. These cells are derived from various tissues, including bone marrow (BM) and adipose tissue (8). Additionally, in azoospermic rats, MSCs were capable of differentiating into germ cells, spermatids, and spermatocytes in the seminiferous tubules (9).

MSC transplantation is a promising new treatment method proposed for inducing spermatogenesis and treating male infertility (10). MSCs play a crucial role in various processes such as cell survival, proliferation, migration, angiogenesis, and immune modulation (11). Given these benefits, MSCs are considered an ideal material for treating NOA (12). The BM is one of the primary sources of MSCs, and numerous studies have demonstrated that BM-derived MSCs (BM-MSCs) can differentiate into male germ cells *in vitro* (13). In animal models of chemical or surgical NOA, MSCs transplanted into the testes have induced spermatogenesis and/or differentiation of MSCs into germ cells. Additionally, allotransplantation of BM-MSCs has been shown to treat NOA in *in vivo* studies on guinea pigs (14), hamsters (15), mice (16, 17), and rats (18). In summary, stem cell therapy has advanced the treatment of NOA. Several trials (NCT02025270, NCT02641769, and NCT02414295) have been conducted to investigate the effect of BM-MSCs on hormonal levels, testicular size, and sexual potency in NOA cases.

Given that this treatment method has proven satisfactory in animal models but has not yet been tested on human subjects, we conducted a phase I clinical trial experimental study on NOA patients based on the information gathered from the animal model. The aims of this research were to evaluate the safety, feasibility, and efficacy of injecting autologous BM-MSCs into the testicular tissues of NOA patients. Furthermore, several possible mechanisms of spermatogenesis restoration during cell therapy by MSC have been shown (12). Stem cells perform most of these activities through their secreted exosomes, so in this article using the in-silico method and the available information, for the first time, the mechanism of interactions of the proteins in the exosomes of BM-MSCs with the existing receptors of human testis seminiferous duct cells were evaluated.

Materials and Methods

Study design

This experimental study was a non-randomized, open-label, sequential phase I clinical trial that evaluated the use of autologous BM-MSCs for the treatment of NOA. All procedures were conducted in accordance with Protocol No. 8, dated 06/09/2020, which was approved by the Local Ethics Commission of the NJSC Astana Medical University (No. 8, dated 06/09/2020). The trial was registered in the Iranian Registry of Clinical Trials (IRCT) under the code IRCT20190519043634N1. The study was carried out in Astana, Republic of Kazakhstan, from December 2019 to January 2022. Figure S1 (See Supplementary Online Information at www.ijfs.ir) illustrates the schematic process of this intervention. The trial was non-randomized and open-label. The inclusion and exclusion criteria for participants who received NOA treatment using autologous BM-MSCs are listed in Table 1.

Bone marrow aspiration, isolation, and characterization

Before BM sampling, all patients underwent an additional examination according to Protocol No. 15 to the clinical protocol for diagnosis and treatment description of surgery and diagnostic intervention dated November 10, 2016, Republic of Kazakhstan. Based on the results of the additional examination, patients provided informed consent (Fig.S2, See Supplementary Online Information at www.ijfs.ir) for BM aspiration. The BM harvesting was performed according to Standard NNMTS ISM PNPK-13.00.06.02 Protocol N10.04.67.02, approved by the Kazakhstan Health Ministry in 2017. The day before surgery, any physical activity, stressful situations, and alcohol intake were excluded.

Table 1: The inclusion and exclusion criteria of participant of NOA treatment by autologous BM-MSCs in clinical trial phase I

The inclusion criteria	The exclusion criteria
20-50 years infertile males	Anatomical abnormalities of the genital tract
Patients with a confirmed diagnosis of NOA based on 2 negative semen analysis with centrifugation (3 months between them)	Patients with grade 2 or 3 varicocele
Patients who have not previously used surgical techniques for sperm retrieval (testicular biopsy)	History of neoplasms and at the moment
	Treated with drug which could improve sperm count and quality one month before observation
	Obstructive azoospermia
	Hypogonadotropic hypogonadism (LH<2 IU / ml and FSH<1 IU/ml)
	Patients with confirmed immunity disorder
	The use of chemotherapy, testosterone, or antiandrogens in the past two years
	Patients who cannot understand the purpose of the study or refuse treatment and follow instructions after treatment
	History of mental disorder
	Participation in another trial

NOA; Non-obstructive azoospermia, BM-MSCs; Bone marrow-derived mesenchymal stromal/stem cells, FSH; Folli-cle-stimulating hormone, and LH; Luteinizing hormone.

Briefly, BM was harvested by percutaneous aspiration from the anterior superior iliac spine (ASIS) on the side convenient for the operator. BM sampling (100-200 mL) was performed through the iliac crest puncture using a special large-diameter needle. The puncture point was 3 cm posterior to the ASIS with the patient supine. Before and after the procedure, the puncture site of the surgical field was treated with an iodine-containing solution, and a sterile bandage was applied to the wound at the end. BM sampling was carried out in the operating room in compliance with the rules of asepsis and antisepsis (19).

The required amount of BM aspirate was 70 mL. The isolation of the BM-MSCs was performed using 0.7 mL Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, USA) supplemented with 0.5 mL streptomycin sulfate (Biochimic, Russian Federation), 0.25 mL penicillin G (sodium salt) (Biochimic, Russian Federation), and 0.5 mL amphotericin B (Biochimic, Russian Federation). Before use, 10 mL of fetal bovine serum (FBS, Biochimic, Russian Federation) was added to the 0.5 mL medium in vials (Biochimic, Russian Federation) to obtain a 10% serum solution, and the medium was warmed up to 37°C. BM cells, consisting of red blood cells (RBCs), white blood cells (WBCs), and MSCs, were separated by passing through syringes (Becton Dickinson, USA) with 16, 18, and 20G needles (Becton Dickinson, USA). Cell pellets were placed in 70% percoll solution (Dickinson, USA) and centrifuged at 460 g for 15 minutes. The adherent cells were localized in the low-density cell fraction. The BM cells were then cultured and adhered to the plastic surface in petri dishes (Becton Dickinson, USA) for 1 to 7 days. The medium was changed every four days until the piles were no longer merged, which usually took from 14 to 21 days. The cultivation was carried out in an incubator (Binder, Germany) at 37°C in a humid environment and at a constant pressure of 5% CO_2. The finished cell suspension was poured into sterile vials, labeled with patient data, names, the number of BM-MSCs, date of collection, and cultivation. Cell biomass was transported in a particular container with a temperature of 37°C (Fig.S3, See Supplementary Online Information at www.ijfs.ir) (20).

Characterization of BM-MSCs was performed through microscopic images of BM-MSCs and flow cytometry technique by 1-laser BD FACS Calibur (20). The data analysis was implemented by the software BD FACSDiva™ (version 6.1.3, Becton Dickinson Labware, USA).

Clinical evaluation

Prior to the start of therapy, serum hormonal profiles [total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and inhibin B], tumor markers (CA 19-9, CYFRA, PSA, AFP, S-100, CEA, SCCA, and CA 72-4), karyotyping analysis, and microdilution of the Y chromosome were measured. LH, FSH, testosterone, and prolactin values were determined using an automatic analyzer Vitros 3600 (Johnson and Johnson, USA) by chemiluminescence, and inhibin B levels by ELISA using Beckman Coulter kits (USA). Tumor markers were determined by ELISA on a biochemical analyzer COBAS INTEGRA 400 Architect 8000 (Abbott). The hormonal profile was determined on the Cobas apparatus, tumor markers on Cobas 401 and Ebot 8000. Semen analysis was determined using the motility mass score (MMS) sperm apparatus twice

before treatment. Ultrasounds of the kidneys, bladder, prostate gland, and scrotum on the Philips clearVue 650 apparatus (according to indications) were performed to exclude inflammatory and oncological changes. In order to exclude genetically determined causes of NOA, a molecular genetic study (i.e., the determination of Y-chromosome microdeletion) and karyotyping were performed at the clinic of ECOMED JSC. Karyotyping was performed according to the standard method on cultured peripheral blood lymphocytes using staining according to the ISCN criteria (International Human Cytogenetics Nomenclature, 2013).

Surgical procedures of BM-MSCs autotransplantation in NOA patients

After BM aspiration and cultivation of autologous BM-MSCs (n=40), they were autotransplanted into the testicular tissue of NOA patients using microTESE. The steps of autotransplantation were as follows. The surgical field was treated three times with an iodine solution. Under epidural anesthesia, a longitudinal incision 3 cm in length was made in the median scrotal region. The albuginea of the testicle was then opened, and the testicular tissue was delicately dislocated outwards. Under an operating microscope (Leica, USA) with 16-40X magnification, promising, large testicular tubules were thoroughly searched for and selected for injection. A solution containing 107 BM-MSCs in 0.3 mL was injected into the convoluted tubules, and another 0.3 mL of a solution containing 107 BM-MSCs was injected into the vas deferens. After completing the procedure on the right testicle, the epididymal membrane wound was sutured layer by layer with 5-0 vicryl suture. A similar procedure was performed on the contralateral left testicle. Additionally, a biopsy of the left testicle was performed using the same procedure for histopathological evaluation. After wound closure, the area was treated with betadine and aseptic bandages. Hemostasis was controlled during all procedures (Fig.S4, See Supplementary Online Information at www.ijfs.ir).

This phase I clinical trial assessed the efficacy of BM-MSC injection in participants with NAO. After injection of BM-MSCs, patients were evaluated for hormonal levels (FSH, total testosterone, LH, Inhibin B, and Prolactin) and semen analysis after six months.

Histopathologic assessment of testis

The histopathologic assessment of testicular tissue was a critical component of this study, serving as a diagnostic tool to confirm the NOA condition and to evaluate tissue characteristics before and after autologous BM-MSC therapy or hormone therapy. Prior to treatment initiation, all patients underwent a comprehensive evaluation, including two semen analyses, to confirm the presence of NOA. Histopathology slides were utilized to identify eligible patients with NOA for inclusion in the study. During the macroscopic evaluation, small fragments resembling gray flakes were observed in the testicular tissue. These fragments, with an approximate size of 1×1 cm^2, were collected for histopathologic examination.

The collected testicular tissue fragments were subjected to microscopic evaluation. This assessment revealed the presence of numerous seminiferous tubules within the tissue fragments. Microscopic examination was conducted at a magnification of 10x. The observed tubules exhibited characteristics indicative of NOA, including sclerosis and hyalinosis. Only a limited number of tubules (up to 5-7 tubules) contained Sertoli cells, primary spermatocytes, and spermatogonia, and stromal edema was noted in some areas. Importantly, no mature spermatozoa were identified within the tubules, and the basement membrane appeared unchanged.

These findings provided a comprehensive baseline characterization of the testicular tissue before autologous BM-MSC therapy. The histopathologic assessment confirmed the presence of NOA and established the histo-logical features of the testicular tissue, including sclerosis, hyalinosis, and absence of mature spermatozoa. This histopathologic assessment method was crucial in selecting eligible patients for the study and in establishing the baseline tissue characteristics necessary for evaluating the efficacy of autologous BM-MSC therapy.

Hormone therapy rgimen for control group in NOA patients

A total of 40 male patients diagnosed with NOA were enrolled in the control group, which underwent hormone therapy. The hormone therapy regimen consisted of chorionic gonadotropin (hCG) administered at a dosage of 1000 units twice a week and clostilbegit, taken orally at a dosage of 50 mg per tablet once daily. Patients were closely monitored throughout the treatment period for changes in hormonal profiles, including testosterone, FSH, LH, inhibin B, and prolactin, as well as sperm concentration. Data collection involved assessments before the initiation of hormone therapy and at specific intervals during treatment.

Protein structure extraction, protein-protein docking analysis, and data visualization

The protein content of exosomes secreted by BM-MSCs were obtained from previous studies (Table S1, See Supplementary Online Information at www.ijfs.ir). Moreover, the Protein Data Bank (PDB) ID for ligands containing tetraspanin-28 (CD81), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), granulocyte colony-stimulating factor (G-CSF), platelet derived growth factor two A subunits (PDGF-AA), platelet derived growth factor two B subunits (PDGF-BB), transforming growth factor-β (TGFβ), vascular endothelial growth factor A (VEGF-A), ADAM metallopeptidase domain 10 (ADAM10), calcium voltage-gated channel auxiliary subunit alpha2delta 1 (CACNA2D1), notch receptor 2 (NOTCH2), and major histocompatibility complex, class

I, A (HLA-A) were 7mws, 6l4o, 2hgf, 1gnc, 3mjk, 4qci, 1kla, 3qtk, 6be6, 7mix, 2oo4, and 1b0r.

Furthermore, Table S2 (See Supplementary Online Information at www.ijfs.ir) contains the list of the name of receptors that involve in the process of spermatogenesis in human and their role in toll process. Moreover, we exclude proteins that their structure was not exist in PDB. After that, the structure of proteins was achieved from PDB online database. The PDB ID of receptors including androgen receptor (AR), delta-opioid receptor (Delta-O), estrogen receptor (ER), FSH receptor (FSHR), G protein-coupled estrogen receptor 1 (GPER), kapa-opioid receptor (KOR), and mu-opioid receptor (MOR) were 1e3g, 8f7s, 1a52, 1xwd, 5zty, 6vi4, and 8f7r.

The protein-protein docking analysis was conducted by ClusPro server (21). 2D and 3D data visualization was conducted by PDBsum online tool (22) and PyMOL software (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.), respectively. Moreover, network visualization was conducted by Cytoscape software.

Statistical analysis

The results were processed using IBM SPSS Statistics (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The series of hormonal profiles (testosterone, FSH, LH, inhibin B, and prolactin) and sperm concentration were analyzed using paired sample t test before and six months after intervention. Independent sample t tests was performed to evaluate the efficacy of hormone therapy and identify any statistically significant differences in comparison to the cell therapy group, contributing valuable insights into the treatment of NOA. The results were considered significant at $P<0.05$. The outcome data were presented as mean and standard error of mean (SE). Group means and their standard error were reported in the text and graphs (GraphPad Prism version 9 for Windows, GraphPad software, San Diego, CA, USA).

Results

Baseline characteristics of study participants

Initially, 86 patients were screened for eligibility from September 2019 to January 2022, of whom 80 met the eligibility criteria. The flow diagram of the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) describing the number of participants through each step of the study is shown in Figure S5 (See Supplementary Online Information at www.ijfs.ir). Finally, this study recruited 80 male participants with NOA, with ages ranging from 24 to 48 years (mean ± SD age of 31.45 ± 5.18 years in cell therapy group and 31.50 ± 4.10 years in hormone therapy group).

Before treatment, all patients underwent two semen analysis to determine the presence of NOA (Fig.S6, See Supplementary Online Information at www.ijfs.ir). Histopathology slides were used to identify 80 patients who were eligible for autologous BM-MSC therapy due to their NOA condition (Fig.1). During macroscopic evaluation, small fragments in the form of gray flakes, with a total size of 1×1 cm^2 were observed. Microscopic evaluation revealed that the fragments of testicular tissue consisted of numerous seminiferous tubules, with a total count ranging from 30 to 50 in one field of view at a magnification of 10x. These tubules showed sclerosis and hyalinosis, with only a few tubules containing Sertoli cells (up to 5-7 tubules) and primary spermatocytes and spermatogonia with stromal edema. In some areas, the tubular lumens were completely obstructed. No mature spermatozoa were found, and the basement membrane was unchanged. Thus, the testicular tissue exhibited signs of sclerosis and hyalinosis.

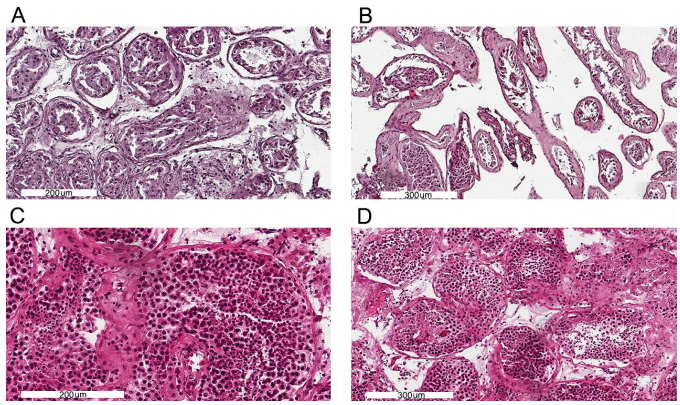

Fig.1: Histopathological evaluation of seminiferous tubules before bone marrow-derived mesenchymal stromal/stem cells (BM-MSCs) therapy in non-obstructive azoospermia (NOA) patients. H&E staining (scale bar: **A, C.** 200 µm and **B, D.** 300 µm).

Table S3 (See Supplementary Online Information at www.ijfs.ir) shows the levels of serum hormonal profiles of total testosterone, LH, FSH, prolactin, and inhibin B before treatment. The patients were followed up for six months after receiving autologous BM-MSCs injection or hormone therapy.

Characterization of BM-MSCs

The BM-MSCs were examined under a light microscope (Leica, USA). BM-MSCs from all participants exhibited fibroblast-like and spindle-shaped cells after attaching to the culture flasks. The results of flow cytometry demonstrated the CD73, CD105, and CD90 MSCs' markers were present and CD34 hematopoietic stem cell marker was absent (Fig.2). The cell viability was at least 94-97%.

Clinical safety and efficacy evaluation

Two weeks after BM aspiration from the anterior superior iliac spine (ASIS), patients showed no complications such as suppuration of the postoperative wound or hematoma in the area. Also, no complications such as un-controlled failure of the needle with the destruction of bone substance in a patient with severe osteoporosis or bleeding due to damage to a blood vessel were observed six months after BM aspiration.

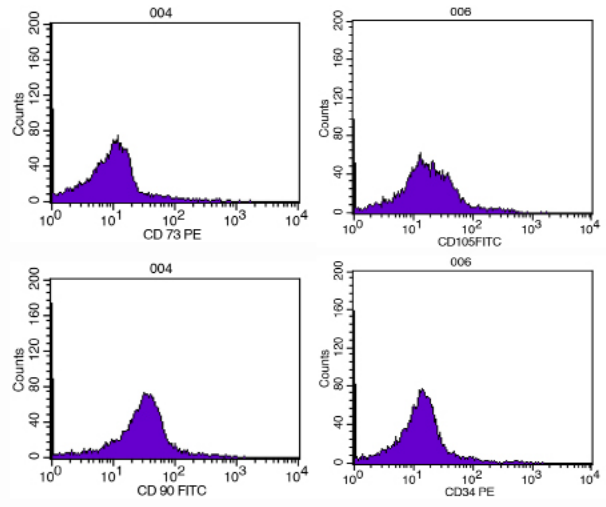

Fig.2: Flow cytometry results confirming markers of bone marrow-derived mesenchymal stromal/stem cells (BM-MSCs) considering the proportion of CD73, CD105, and CD90, MSCs' markers and CD34 hematopoietic stem cell marker.

According to the embryologist, no spermatozoa were found in the material for the *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) and cryopreservation program in biopsy of testicles during surgical procedures. After autotransplantation of BM-MSCs into the testicular networks of patients with NOA, they were followed up for six months. One patient's blood sugar value returned to normal, and patients reported an improvement in general well-being and libido, which was measured using a standardized questionnaire. There were no allergic reactions, wound suppuration, or complications from the cardiovascular, respiratory, nervous, urinary systems, or blood systems in patients. Also, no inflammatory or oncological changes were observed in ultrasound of the kidneys, bladder, prostate gland, and scrotum (Fig.S7, See Supplementary Online Information at www.ijfs.ir). Before starting the intervention, patients underwent tumor marker testing, and their results were within the normal range (Fig.S8, See Supplementary Online Information at www.ijfs.ir). Genetic analysis by karyotype showed that the patients had a normal number of chromosomes (Fig.S9, See Supplementary Online Information at www.ijfs.ir). After six months, the levels of testosterone, LH, FSH, inhibin B, and prolactin were determined (Fig.3). Patients did not donate tumor markers after treatment since we used their own cells for the intervention.

To evaluate the efficacy of the treatment, hormonal profile (Fig.3) and sperm concentration were examined (Figs.3, S10, See Supplementary Online Information at www.ijfs.ir). As observed, testosterone, inhibin B, and sperm concentration increased after treatment, while FSH, LH, and prolactin decreased after treatment. The detailed results of checking the hormonal profile before and six months after treatment are listed in Table S3. The initial FSH value in the patient group was 28.6/21.7 [95% confidence interval (CI): 18.9, 29.8], which decreased by 10.4/8.8 (95% CI: 6.4, 13.6, P<0.001) after treatment while it returned to its normal level in 9 (47.4%) patients. The total testosterone level was 6.8/4.9 (95% CI: 2.8, 9.2) before treatment. After treatment, it rose by 3.2/3.

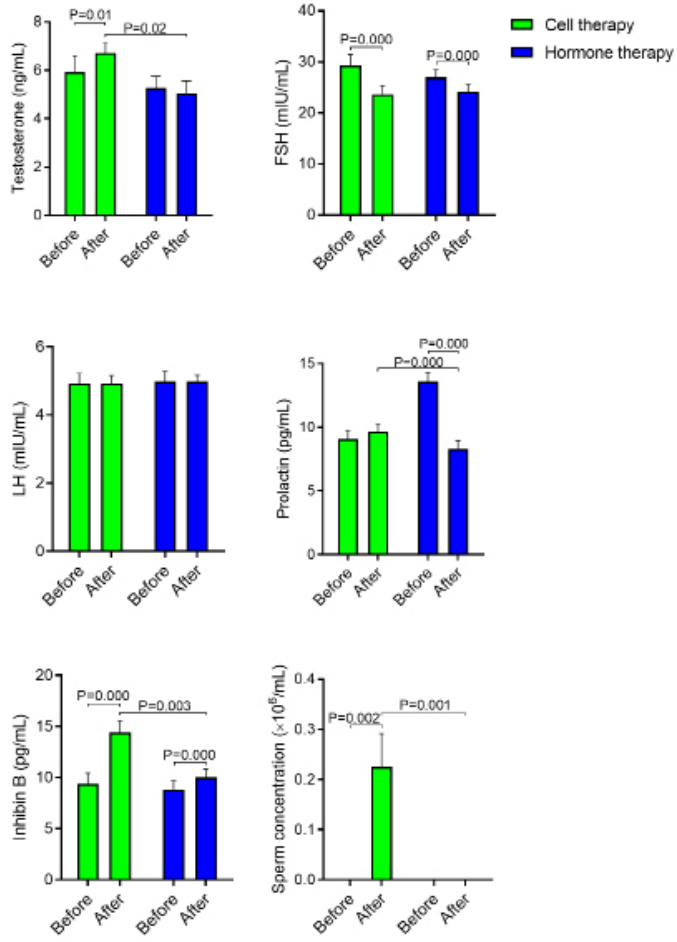

Fig.3: The mean values and standard errors (SE) associated with hormonal profiles, including testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B, and prolactin, along with sperm concentration data, both prior to and six months after the treatment of non-obstructive azoospermia (NOA) using autologous bone marrow-derived mesenchymal stromal/stem cells (BM-MSCs) in a Phase I clinical trial. The study encompassed a cell therapy group (n=40) and a control hormone therapy group (n=40). Significance levels are represented above the columns. Data analysis was conducted using independent sample t tests and paired-sample t tests for comparison.

Adverse effects and safety profile

In this study, we rigorously monitored and documented adverse effects and safety concerns associated with the autologous BM-MSC treatment approach for NOA. Our paramount concern was the well-being of the participants, and we implemented comprehensive measures to ensure their safety.

During the course of this study, we observed and thoroughly documented any adverse events that occurred following BM-MSC transplantation into the testicular tissue using microTESE. Adverse events were categorized by severity, and their occurrence was recorded. Any complications, discomfort, or unintended effects were noted.

In the event of adverse events, participants received prompt and appropriate medical care. Our study adhered to established protocols for managing adverse events, and we took all necessary steps to minimize any potential harm to participants.

We are pleased to report that the overall safety profile of the autologous BM-MSC treatment approach was favorable. No severe or life-threatening adverse events were observed. Most adverse events were of mild to moderate severity and resolved with appropriate management.

Our study was conducted in strict compliance with regulatory guidelines and ethical standards for monitoring and reporting adverse events in clinical trials. We ensured transparency and accountability in safety reporting to protect the well-being of our participants.

CD81, PDGF-AA, GCSF and HLA-A had the most binding affinity to receptors that involve in the process of spermatogenesis

According to our *in-silico* analysis, CD81, PDGF-AA, GCSF and HLA-A demonstrated the most binding affinity to the receptors that participate in spermatogenesis (Table 2). Furthermore, detailed data about binding site of mentioned ligands with receptors that play an important part in the process of spermatogenesis are shown in Figure 4.

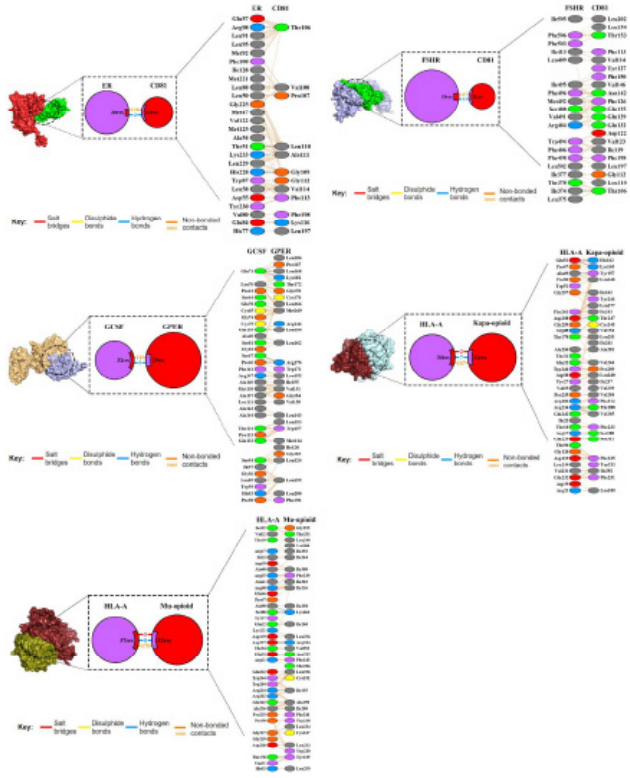

Fig.4: Detailed information about interactions between CD81 and Transforming growth factor beta (TGFβ) had the most binding affinity to receptors that involve in the process of spermatogenesis. Blue, red, green, gray, purple, orange and yellow olives represent Positive; negative, neutral, aliphatic, aromatic, Pro-Gly, and cysteine amino acids, respectively.

Table 2: Binding affinities of the protein content of exosomes are secreted by mesenchymal stromal/stem cells and receptors that involve in the process of spermatogenesis (Kcal/mole)

Proteins	Receptors						
	AR	Delta-O	ER	FSHR	GPER	KOR	MOR
CD81	-1071.7	-987.5	-1199.1	-1528.3	-1406.7	-1413.1	-1514.5
FGF2	-714.7	-705	-732.7	-1060.4	-917.5	-908.6	-944.4
HGF	-607.7	-566.4	-750.3	-630.3	-860.2	-940.2	-918.4
PDGF-BB	-823.7	-902.4	-1045.9	-1322.8	-1217	-1115.6	-1210.7
PDGF-AA	-1072.1	-957.5	-1000.8	-1380.7	-1315.6	-1460.4	-1356.7
TGFβ	-936.6	-655.7	-1074.2	-784.4	-1250.2	-1469	-1470.8
VEGF-A	-883.9	-851.5	-957.4	-1411.2	-1187.2	-1202.9	-1380.4
GCSF	-859.4	-953.6	-1113.9	-831.8	-1607.7	-1340.3	-1479.5
ADAM10	-873.4	-758	-814.2	-1266.5	-1318.3	-1353	-1268.4
CACNA2D1	-822.9	-962.4	-943	-1267.4	-1267.8	-1215.8	-1190.4
NOTCH2	-836.7	-554.5	-865.6	-1396.1	-1348.6	-1427	-1397.4
HLA-A	-903.2	-755.2	-973.4	-746.9	-1308.9	-1677.5	-1585.6

Discussion

This groundbreaking non-randomized, openlabel phase I clinical trial represents significant advancement in the realm of NOA treatment. Autologous BM-MSCs, harvested from the patient's own BM, offer a promising avenue for addressing NOA, a condition with diverse etiologies, including genetic anomalies, gonadotoxin exposure, infections, varicocele, trauma, and chemotherapy-induced damage (3). Moreover, in some cases, NOA may remain idiopathic (23). The conventional treatments, such as hormonal therapies and surgical interventions, have demonstrated limited efficacy, underscoring the urgency for alternative therapeutic strategies (24). The transplantation of autologous BM-MSCs has emerged as a safe and potentially transformative approach, demonstrating the ability to trigger spermatogenesis in patients with a history of children. This innovative technique focuses on regenerating the seminiferous tubular microenvironment, promoting the proliferation and differentiation of spermatogonia, thus rekindling the cycle of spermatogenesis (15). The presence of sperm in post-treatment semen analysis provides compelling evidence for the viability of BM-MSCs as a novel NOA treatment method, offering renewed hope for affected individuals.

Allogeneic BM-MSCs differentiated into spermatogenic-like cells and injected into the recipient seminiferous tubules induced by busulfan enhanced the restoration of endogenous fertility in rats (18). Busulfan, a chemotherapeutic agent, typically used in low-dose long-term protocols for treating chronic myeloid leukemia or as a myeloablative agent before allogeneic hematopoietic cell transplantation, has a significant impact on germ cells, making them more susceptible to its side effects (25, 26). These findings in rodent models demonstrate the potential of allogeneic BM-MSCs in facilitating spermatogenesis recovery in azoospermic individuals, particularly those affected by busulfan-induced NOA (14, 18). While these preclinical results are promising, further investigations and clinical trials are essential to validate the efficacy and safety of this approach in humans, bridging the gap between bench and bedside.

Exploring the effects of other MSC types on NOA has expanded our understanding of potential therapeutic avenues. For instance, adipose tissue-derived MSCs (AT-MSCs) have shown promise in azoospermic models, inducing spermatogenesis in both rat and hamster testes affected by busulfan-induced NOA (9, 15). The ability of AT-MSCs to stimulate spermatogenesis highlights the potential of alternative MSC sources for NOA treatment (27). However, further research is needed to compare the efficacy of BM-MSCs versus AT-MSCs and assess their long-term effects on fertility in clinical settings.

In the current study, autologous BM-MSCs from ASIS were employed to minimize the risk of graft rejection associated with allogeneic transplantation. The successful isolation and characterization of BM-MSCs, with their remarkable multipotent capabilities and tissue-regenerative potential, underscore the therapeutic promise of these cells (28). Despite the invasive nature of BM-MSCs isolation, the abundance and well-established history of BM-MSCs as a source of stem cells for clinical applications make them a viable choice for cell-based therapies (29). Nevertheless, as the field of regenerative medicine continues to evolve, other sources and isolation methods for MSCs warrant exploration to optimize treatment outcomes while minimizing patient discomfort (30-32).

In this study, the injection of autologous BM-MSCs into the testicular networks of NOA patients had a significant impact on their hormonal profiles. This represents a pivotal aspect of the therapeutic mechanism, shedding light on the role of BM-MSCs in regulating hormonal imbalances associated with NOA. The restoration of testosterone and inhibin B levels through the modulation of FSH and LH underscores the potential of BM-MSCs to rejuvenate spermatogenesis by fine-tuning the hormonal milieu in the testicular microenvironment (33). This hormonal rebalancing offers further insights into the multifaceted mechanisms through which BM-MSCs contribute to spermatogenesis recovery, providing a promising avenue for future research and therapeutic interventions.

While the results of this non-randomized study are encouraging, there remain several areas for improvement and further exploration. The injection technique for precise delivery of MSCs into the reticulum needs refinement to enhance treatment efficacy. Additionally, the administration of antiestrogens and hCG post-surgery introduces confounding variables that should be carefully controlled in future trials. Collaborative efforts among fertility treatment centers can help replicate these results and expand the scope of this promising cell-based therapy for NOA, offering renewed hope to individuals grappling with this challenging condition.

The significance of AR in spermatogenesis cannot be overstated, as it plays a pivotal role in both classical and non-classical testosterone-based pathways within Sertoli cells (34). This study has elucidated a noteworthy finding: PDGF-AA, a factor contained within exosomes, exhibits the highest binding affinity for AR. This finding aligns with prior research, highlighting the compelling potential of BM-MSC-derived exosomes to influence spermatogenesis through their interaction with AR. These exosomes, through PDGF-AA and other components, may orchestrate a complex regulatory network within the testicular microenvironment, promoting the intricate process of spermatogenesis.

Delta opioid receptors, known to impact sperm motility and human spermatozoa, are integral to male fertility (35). CD81, a key protein within BM-MSC exosomes, exhibits the highest binding affinity to these receptors. This *in-silico* insight suggests that exosomes may exert their modulatory effect on spermatogenesis by virtue of CD81's influence on delta opioid receptors. This interaction highlights a potential mechanism through

which exosomes enhance male fertility, emphasizing the intricate interplay between exosomal components and essential receptors in sperm function and production.

Indepth examination reveals CD81's propensity to bind to estrogen receptors, whose activation can exert varying effects on spermatogenesis. This receptor family assumes diverse roles across species, particularly stimulating spermatogenesis in humans (36). CD81's affinity for estrogen receptors implies its potential role in amplifying their effects, accentuating the impact of exosomes in spermatogenesis regulation. This finding underscores the multifaceted nature of BM-MSC-derived exosomes in orchestrating the complex process of spermatogenesis.

FSHR stands as a cornerstone in spermatogenesis, influencing diverse events crucial for male reproductive health (37). CD81's pronounced affinity for FSHR suggests a potential pathway through which exosomes may activate and support spermatogenesis. This interaction accentuates the versatility of exosomal components in regulating essential receptors and contributing to the intricate orchestration of spermatogenesis. Further research will be pivotal in unraveling the precise mechanisms underlying this interaction and its therapeutic implications.

Emerging evidence linking GPER levels to sperm parameters underscores the receptor's critical role in spermatogenesis (38). The affinity of G-CSF for GPER suggests a potential mechanism by which BM-MSC-derived exosomes positively impact spermatogenesis through the influence of G-CSF on GPER. This finding strengthens the notion that exosomal components can modulate essential receptors, fostering an environment conducive to male reproductive health.

Our study delved into the intricate interactions between HLA-A, a constituent of BM-MSC-derived exosomes, and the MOR and KOR, both of which are present in spermatozoa. While prior research has elucidated MOR critical role in sperm motility (39), KOR precise function in spermatogenesis remained uncertain (35). However, our *in-silico* investigation shed light on KOR's involvement in the spermatogenesis process, marking a significant contribution to the understanding of male fertility mechanisms. Importantly, our findings demonstrated that HLA-A displayed the highest binding affinity to both of these receptors, suggesting that the proteins within BM-MSC exosomes, particularly HLA-A, might amplify spermatogenesis processes through their influence on these receptors (40). This *in-silico* discovery unveils a potential regulatory mechanism by which BM-MSC exosomes contribute to the complex orchestration of spermatogenesis, underscoring the promise of these exosomes as therapeutic agents.

Figure S11 (See Supplementary Online Information at www.ijfs.ir) provides a visual representation of the effects of proteins derived from exosomes originating from BM-MSCs on receptors central to human spermatogenesis. Our results corroborate earlier studies and fortify the findings of our current research. Nevertheless, it is crucial to emphasize that further comprehensive investigations are imperative to validate the insights gained through this analysis. Future research endeavors should focus on elucidating the intricate molecular mechanisms and signaling pathways that underlie the actions of BM-MSC exosome proteins on spermatogenesis-related receptors, thereby advancing our comprehension of these complex processes.

While our study offers valuable insights into the potential benefits of autologous BM-MSC therapy for NOA, we acknowledge several limitations that warrant consideration:

Sample size: Although we increased the sample size to enhance the robustness of our findings, our study still included a limited number of participants. Further research with larger cohorts is needed to confirm and generalize our results.

Follow-up duration: Our study had a follow-up period of six months after autologous BM-MSC therapy. Longer-term follow-up is essential to assess the durability and sustainability of the observed improvements in spermatogenesis and fertility outcomes.

Heterogeneity of NOA etiology: The etiology of NOA can vary among individuals, encompassing congenital, genetic, environmental, and idiopathic factors. Our study included patients with diverse etiologies, which may introduce variability in treatment responses. Future research may benefit from stratified analyses based on etiology.

Adverse events reporting: While we diligently monitored and reported adverse events, longer-term studies with extended follow-up periods are necessary to comprehensively assess the safety profile of autologous BM-MSC therapy.

Control group: Although we included a control group receiving hormone therapy, a placebo-controlled group would have provided a more robust basis for evaluating the specific effects of BM-MSC therapy. Future studies with placebo controls could offer additional insights.

Generalizability: Our study focused on a specific patient population with NOA. The generalizability of our findings to other patient groups or populations with different characteristics should be explored in future investigations.

In-silico analysis: While our *in-silico* analysis identified potential protein-protein interactions, these findings should be validated through *in vitro* and *in vivo* experiments to establish their clinical significance.

Despite these limitations, our study represents a significant step toward understanding the potential benefits of autologous BM-MSC therapy in NOA treatment. Further research addressing these limitations will contribute to a more comprehensive understanding of this promising therapeutic approach.

Conclusion

Our study introduces a novel therapeutic approach for the treatment of patients with NOA, combining autologous BM-MSCs with microTESE. The detection of sperm in semen analysis data provides compelling evidence of the efficacy of this innovative treatment method, not only for NOA patients but also for those with secondary infertility who have a history of children. In this treatment paradigm, autologous BM-MSCs, with their diverse properties, including self-renewal capabilities, foster the regeneration of seminiferous tubules. Additionally, our hormonal analysis highlights the role of autologous BM-MSCs in hormone production, crucial for regulating the spermatogenesis cycle. Consequently, the combination of autologous BM-MSCs with this approach holds promise in stimulating sperm production and addressing the needs of NOA patients. Moreover, our *in-silico* analysis, exploring the binding affinities between the protein content of BM-MSC-derived exosomes and receptors integral to spermatogenesis, underscores the multifaceted mechanisms through which these exosomes may contribute to male fertility. These findings collectively signify the potential of BM-MSC-based therapies in advancing the treatment landscape for NOA and warrant further investigations to uncover the full spectrum of their therapeutic capabilities.

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Authors' Contributions

U.Z., N.T., R.Sh., M.M., A.T.; Conceptualization. A.T.; Formal analysis and Project administration. U.Z., A.T.; Funding acquisition. R.Z., U.Z., M.A., A.Z., N.J., D.S., R.Sh. D.A., A.H., N.T., B.A., H.B., R.Sh., F.R., A.T.; Investigation. R.Z., U.Z., M.A., A.Z., N.J., D.S., R.Sh., D.A., M.F., N.T., H.B., M.M., Sh.B., F.R., A.T.; Methodology. M.A., A.H., M.F., N.M.M., A.A.K., Y.S., A.T.; Resources. A.Z.; Software. U.Z., B.A., N.M.M., A.T.; Supervision. R.Z., A.Z., N.J., D.A.; Writing-original draft. U.Z., M.F., N.T., B.A., N.M.M., A.A.K., Y.S., R.Sh., M.M., Sh.B., F.R., A.T.; Writing-review & editing. All authors read and approved the final manuscript.

References

1. Khizroeva J, Nalli C, Bitsadze V, Lojacono A, Zatti S, Andreoli L, et al. Infertility in women with systemic autoimmune diseases. Best Pract Res Clin Endocrinol Metab. 2019; 33(6): 101369.
2. Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum Reprod Update. 2015; 21(4):411-426.
3. Chiba K, Enatsu N, Fujisawa M. Management of non-obstructive azoospermia. Reprod Med Biol. 2016; 15(3): 165-173.
4. Minhas S, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al. European association of urology guidelines on male sexual and reproductive health: 2021 update on male infertility. Eur Urol. 2021; 80(5): 603-620.
5. Kang C, Punjani N, Schlegel PN. Reproductive chances of men with azoospermia due to spermatogenic dysfunction. J Clin Med. 2021; 10(7): 1400.
6. Deng CC, Liu GH. Stem cell therapy for non-obstructive azoospermia. Zhonghua Nan Ke Xue. 2020; 26(4): 351-356.
7. Zakrzewski W, Dobrzynski M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019; 10(1): 68.
8. Kim HJ, Park JS. Usage of human mesenchymal stem cells in cell-based therapy: advantages and disadvantages. Dev Reprod. 2017; 21(1): 1-10.
9. Mehrabani D, Hassanshahi MA, Tamadon A, Zare S, Keshavarz S, Rahmanifar F, et al. Adipose tissue-derived mesenchymal stem cells repair germinal cells of seminiferous tubules of busulfan-induced azoospermic rats. J Hum Reprod Sci. 2015; 8(2): 103-110.
10. Chang Z, Zhu H, Zhou X, Zhang Y, Jiang B, Li S, et al. Mesenchymal stem cells in preclinical infertility cytotherapy: a retrospective review. Stem Cells Int. 2021; 2021: 8882368.
11. Song N, Scholtemeijer M, Shah K. Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential. Trends Pharmacol Sci. 2020; 41(9): 653-664.
12. Zhankina R, Baghban N, Askarov M, Saipiyeva D, Ibragimov A, Kadirova B, et al. Mesenchymal stromal/stem cells and their exosomes for restoration of spermatogenesis in non-obstructive azoospermia: a systemic review. Stem Cell Res Ther. 2021; 12(1): 229.
13. Yan G, Fan Y, Li P, Zhang Y, Wang F. Ectopic expression of DAZL gene in goat bone marrow-derived mesenchymal stem cells enhances the trans-differentiation to putative germ cells compared to the exogenous treatment of retinoic acid or bone morphogenetic protein 4 signalling molecules. Cell Biol Int. 2015; 39(1): 74-83.
14. Hajihoseini M, Vahdati A, Hosseini SM, Mehrabani D, Tamadon A. Induction of spermatogenesis after stem cell therapy of azoospermic guinea pigs. Vet Arh. 2017; 87(3): 333-350.
15. Tamadon A, Mehrabani D, Rahmanifar F, Jahromi AR, Panahi M, Zare S, et al. Induction of spermatogenesis by bone marrow-derived mesenchymal stem cells in busulfan-induced azoospermia in hamster. Int J Stem Cells. 2015; 8(2): 134-145.
16. Kadam P, Ntemou E, Baert Y, Van Laere S, Van Saen D, Goossens E. Co-transplantation of mesenchymal stem cells improves spermatogonial stem cell transplantation efficiency in mice. Stem Cell Res Ther. 2018; 9(1): 317.
17. Sherif IO, Sabry D, Abdel-Aziz A, Sarhan OM. The role of mesenchymal stem cells in chemotherapy-induced gonadotoxicity. Stem Cell Res Ther. 2018; 9(1): 196.
18. Rahmanifar F, Tamadon A, Mehrabani D, Zare S, Abasi S, Keshavarz S, et al. Histomorphometric evaluation of treatment of rat azoosper-mic seminiferous tubules by allotransplantation of bone marrow-derived mesenchymal stem cells. Iran J Basic Med Sci. 2016; 19(6): 653-661.
19. Chahla J, Mannava S, Cinque ME, Geeslin AG, Codina D, LaPrade RF. Bone marrow aspirate concentrate harvesting and processing technique. Arthrosc Tech. 2017; 6(2): e441-e445.
20. Baghaei K, Hashemi SM, Tokhanbigli S, Asadi Rad A, Assadzadeh-Aghdaei H, Sharifian A, et al. Isolation, differentiation, and characterization of mesenchymal stem cells from human bone marrow. Gastroenterol Hepatol Bed Bench. 2017; 10(3): 208-213.
21. Desta IT, Porter KA, Xia B, Kozakov D, Vajda S. Performance and its limits in rigid body protein-protein docking. Structure. 2020; 28(9): 1071-1081. e3.
22. Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM. PDBsum: Structural summaries of PDB entries. Protein Sci. 2018; 27(1): 129-134.
23. Kasak L, Laan M. Monogenic causes of non-obstructive azoospermia: challenges, established knowledge, limitations and perspectives. Hum Genet. 2021; 140(1): 135-154.
24. Shiraishi K. Hormonal therapy for non-obstructive azoospermia: basic and clinical perspectives. Reprod Med Biol. 2015; 14(2): 65-72.
25. Garcia-Perez L, van Roon L, Schilham MW, Lankester AC, Pike-Overzet K, Staal FJT. Combining mobilizing agents with busulfan to reduce chemotherapy-based conditioning for hematopoietic stem cell transplantation. Cells. 2021; 10(5): 1077.

26. Panahi M, Keshavarz S, Rahmanifar F, Tamadon A, Mehrabani D, Karimaghai N, et al. Busulfan induced azoospermia: Stereological evaluation of testes in rat. Vet Res Forum. 2015; 6(4): 273-278.
27. Karimaghai N, Tamadon A, Rahmanifar F, Mehrabani D, Raayat Jahromi A, Zare S, et al. Spermatogenesis after transplantation of adipose tissue-derived mesenchymal stem cells in busulfan-induced azoospermic hamster. Iran J Basic Med Sci. 2018; 21(7): 660-667.
28. Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: an updated systematic review and meta-analysis. EClinicalMedicine. 2020; 19: 100249.
29. Andrzejewska A, Lukomska B, Janowski M. Mesenchymal stem cells: from roots to boost. Stem Cells. 2019; 37(7): 855-864.
30. Kingery MT, Manjunath AK, Anil U, Strauss EJ. Bone marrow mesenchymal stem cell therapy and related bone marrow-derived orthobiologic therapeutics. Curr Rev Musculoskelet Med. 2019; 12(4): 451-459.
31. Kouchakian MR, Baghban N, Moniri SF, Baghban M, Bakhshalizadeh S, Najafzadeh V, et al. The clinical trials of mesenchymal stromal cells therapy. Stem Cells Int. 2021; 2021: 1634782.
32. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. Signal Transduct Target Ther. 2022; 7(1): 92.
33. Walker WH. Androgen actions in the testis and the regulation of spermatogenesis. Adv Exp Med Biol. 2021; 1288: 175-203.
34. Wang JM, Li ZF, Yang WX. What does androgen receptor signaling pathway in sertoli cells during normal spermatogenesis tell us? Front Endocrinol (Lausanne). 2022; 13: 838858.
35. Olabarrieta E, Totorikaguena L, Romero-Aguirregomezcorta J, Agirregoitia N, Agirregoitia E. Delta and kappa opioid receptors on mouse sperm cells: Expression, localization and involvement on in vitro fertilization. Reprod Toxicol. 2020; 93: 211-218.
36. Walczak-Jędrzejowska R, Forma E, Oszukowska E, Bryś M, Marchlewska K, Kula K, et al. Expression of G-protein-coupled estrogen receptor (GPER) in whole testicular tissue and laser-capture microdissected testicular compartments of men with normal and aberrant spermatogenesis. Biology. 2022; 11(3): 373.
37. Santi D, Crépieux P, Reiter E, Spaggiari G, Brigante G, Casarini L, et al. Follicle-stimulating hormone (FSH) action on spermatogenesis: a focus on physiological and therapeutic roles. J Clin Med. 2020; 9(4): 1014.
38. Barut O, Seyithanoglu M, Kucukdurmaz F, Demir BT, Olmez C, Dogan NT, et al. Relationship between the G protein–coupled oestrogen receptor and spermatogenesis, and its correlation with male infertility. Andrologia. 2020; 52(10): e13779.
39. Olabarrieta E, Totorikaguena L, Romero-Aguirregomezcorta J, Agirregoitia N, Agirregoitia E. Mu opioid receptor expression and localisation in murine spermatozoa and its role in IVF. Reprod Fertil Dev. 2020; 32(4): 349-354.
40. Wang ZG, He ZY, Liang S, Yang Q, Cheng P, Chen AM. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res Ther. 2020; 11(1): 511.