Stem Cell Reviews and Reports (2021) 17:2153–2163
https://doi.org/10.1007/s12015-021-10196-w

Can Autologous Adipose-Derived Mesenchymal Stem Cell Transplantation Improve Sexual Function in People with Sexual Functional Deficiency?

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Accepted: 2 June 2021 / Published online: 15 June 2021

Abstract

Background Sexual functional deficiency occurs at some point in life and becomes a problematic issue in middle-aged adulthood. Regenerative medicine, especially mesenchymal stem cell (MSC) transplantation, has developed extensively, with preclinical and clinical trials emphasizing the benefits of stem cell therapy for restoration of sexual deficiency. This study was designed to develop a new therapeutic stem cell treatment for people with sexual functional deficiency.

Methods Thirty-one patients, including 15 males and 16 females with a medical history of reduced sexual activity, met the inclusion criteria and were enrolled in the study, phase I/IIa clinical trial with a 12-month follow-up. Adipose tissue-derived mesenchymal stem/stromal cells (ADSC) were isolated by type I collagenase digestion and cultured at the Stem Cell Core Facility under ISO 14644-1. Each participant received 1 million cells/kg of body weight via the intravenous route. Safety was evaluated by assessing the occurrence of adverse events or severe adverse events. Efficacy was assessed in males by monitoring testosterone levels and administering the International Index of Erectile Function (IIEF) questionnaire and in females by monitoring anti-Mullerian hormone (AMH), estradiol (E2), and follicle-stimulating hormone (FSH) levels and administering the Female Sexual Functioning Index (FSFI) questionnaire at baseline and 3-, 6-, and 12-months post-transplantation.

Results There was no occurrence of severe adverse events after ADSC administration in our study. Post-transplantation sexual satisfaction was observed in all patients enrolled in this study. Testosterone levels in males increased soon after transplantation and were maintained at high levels for up to 6 months before decreasing again at the 12-month follow-up. No significant changes in AMH, FSH or E2 levels were recorded in female patients.

Conclusions Autologous ADSC infusion is a potential therapeutic option for patients with reduced sexual activity, especially for male patients.

Trial Registration ClinicalTrials.gov. NCT03346967, Registered November 20, 2017.

Keywords Sexual dysfunction · Adipose mesenchymal stem/stromal cell · Reproductive hormones

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Background

Sexual dysfunction in adulthood is a frustrating condition with a high prevalence in the general population worldwide. Several key hormones, including testosterone (T) in males and follicle-stimulating hormone (FSH), estradiol (E2) and anti-Mullerian hormone (AMH) in females, play important roles in the regulation and orchestration of sexual function. The secreted concentrations of these reproductive hormones change throughout the individual's life and are believed to decrease during as the individual ages [1]. In females, this decrease occurs near the time of menopause between the ages of 45 and 55 years [2]. AMH is produced in the ovary by granulosa cells in antral follicles and reaches its highest level at puberty [3] before decreasing to undetectable levels after menopause [4]. Occasionally, AMH begins to decline after 40 years of age with a reduction rate of 0.1 ng/mL/year [5, 6]. Serum E2 level decreases after the age of 50 in both men and women [1]. In contrast, FSH increases with increasing reproductive age and continues to increase after 37 years of age [7]. In males, reproductive hormones decline in parallel with the loss of testicular function [8]. Total serum T level decreases approximately 1-2% per year (equal to 3.2-3.5 ng/dL) beginning in a man's thirties [9, 10]. Thirty percent of men aged 40-79 years suffer from testosterone deficiency, and the decline continues with aging [11]. The decline in reproductive hormones leads to a variety of medical problems, such as reduced sexual function, hot flushes and sweating, depression in women, weak erectile ability, and a reduced amount of ejaculate in men [12]. Hormone replacement therapy (HRT) is one of the main treatments for hormone deficiency in both men and women. However, HRT may have long-term effects on increasing the risk of breast cancer, endometrial cancer, ovarian cancer [13], and uterine cancer in females and the risk of stroke in males [14]. Therefore, there is an urgent need to find an effective method to improve sexual function for both men and women during middle age.

Recently, MSCs have been shown to restore reproductive hormone levels in animal models. Infusion of MSCs increases E2 and AMH levels while decreasing FSH levels in perimenopausal rats [15]. In a rat model of chemotherapy-induced premature ovarian failure (POF), Amin et al. demonstrated that MSCs decreased luteinizing hormone (LH) and FSH levels and increased E2 levels in treated animals compared to the control group [16]. In another study, Li et al. reported the therapeutic efficacy of MSCs for POF. Six weeks after infusion, MSCs restored the levels of serum E2 and FSH in the POF model group to levels similar to the those of the control group [17]. After infusion of MSCs into POF mice, the serum levels of FSH and LH were considerably reduced, whereas the E2 and AMH levels were significantly increased [18]. MSCs can restore the levels of AMH and E2 in mice undergoing natural ovarian aging after 1 week of injection [19]. It has been shown that infusion of human orbital fat-derived stem cells (OFSCs, which are MSCs isolated from human orbital fat tissue) increases testosterone secretion and triggers FSH secretion in male rats that undergo testicular torsion-detorsion surgery [20]. Another study demonstrated that stem cell infusion restores testosterone production in a Leydig cell-disrupted rat model [11].

Among human studies, one showed that two of ten idiopathic POF patients who were transplanted with MSCs at Al-Azhar University Hospitals resumed menstruation after 3 months, one of whom became pregnant after 11 months and delivered a healthy full-term baby [21]. The hormone profile of the pregnant case shows the restoration of FSH, LH, E2 and AMH levels to normal levels. Another phase II study demonstrated that allogenic MSC infusion improved immunology-related parameters and the sexual quality of life of females [22]. These studies suggest the potential for MSC infusion in the restoration of reproductive hormone levels.

One of the potential sources of MSCs for clinical use is adipose tissue [23]. Adipose tissue-derived mesenchymal stem cells (ADSCs) are able to differentiate into a variety of different cell lineages, secreting a broad range of cytokines, chemokines and growth factors [24]. Moreover, ADSCs have been shown to have anti-apoptotic, anti-inflammatory, proangiogenic, immunomodulatory and anti-scarring potential [25]. ADSCs have been used in clinical trials to treat many kinds of diseases [26–29].

The aim of this clinical trial was to assess the safety and effectiveness of autologous infusion of ADSCs for aging people with reproductive hormone deficiency.

Materials and Methods

Study Participants and Study Design

This study was an open clinical phase I/IIa trial without a control group. Thirty-one patients, including 15 males and 16 females, were enrolled in this study.

Inclusion Criteria Males (aged 35 to 70 years) with reduced sexual desire and testosterone levels ≤ 12 nMol/dL. Females (aged 40 years to the age of menopause) with reduced sexual activity and AMH ≤ 2 ng/mL or FSH ≥ 10 mIU/mL on the 2^{nd} to 4^{th} day of her menstrual cycle.

Exclusion Criteria Positive laboratory tests for HBV, HCV, HIV, coagulating disorders, cancers, active infection, heart failure, kidney failure, or respiratory failure.

Pre-Intervention Assessment

Prior to intervention, all the participants underwent a thorough clinical examination; serological, biochemical, and hematological tests; heart ultrasound; and chest X-ray. Serum AMH, FSH and E2 levels on the 2nd or 4th day of the menstrual cycle at each interval were examined in females. Serum testosterone levels were assessed in males. The International Index of Erectile Function (IIEF) questionnaire, which includes 15 items divided into the following five domains of sexual function, was used to evaluate male clinical sexual function: erectile function (six items), orgasmic function (two items), sexual desire (two items), intercourse satisfaction (three items), and overall satisfaction (two items) [30]. For females, the Female Sexual Functioning Index (FSFI) questionnaire, which includes 19 items making up the following six domains, was used to assess clinical sexual function: desire, arousal, lubrication, orgasm, satisfaction, and pain [31].

Procedure of Adipose Tissue and the Isolation and Culture of ADSCs

A mass of approximately 1.5 x 1.5 x 1.5 cm of autologous adipose tissue was harvested from each participant's lower abdomen under general anesthesia. This adipose tissue was sent to a laboratory and processed. The tissue was washed 3 times with Hank's balanced salt solution (HBSS; Gibco, USA), cut into small pieces and digested in HBSS with 0.1% (v/v) type I collagenase (Gibco, USA) for 60 minutes at 37°C in AdiPlus Multi-Purpose Medical Centrifuge-VS-6030 (Vision Scientific Co., Ltd., Daejeon, Korea). The digested adipose tissue was washed with HBSS, passed through a strainer with a 70 μm diameter, and centrifuged for 10 minutes at 500 g. The supernatant was discarded, and the pellet was resuspended with PowerStem (PAN-Biotech, Germany) and centrifuged for 5 minutes at 500 g. The cell pellet was resuspended in PowerStem, and cells were counted using a hemocytometer with Turck staining solution (Life Technologies, USA), seeded in culture dishes at a density of 3000-7000 cells/cm^2 and incubated at 37°C under 5% CO_2. ADSCs were cultured in PowerStem with 1% (v/v) penicillin and streptomycin for 5-10 days and then switched to PowerStem medium without antibiotics. When the cell monolayers reached 80-90% confluence, the cells were subcultured after treatment with TrypLE™ select enzyme (Gibco, USA). Both the cell viability and growth rate were evaluated at passages 1-3. All of the culture medium was replaced every third day. The collected medium was tested for bacteria, fungi, endotoxin and mycoplasma.

Flow cytometry was performed to identify the phenotype of ADSCs at passage 2. Briefly, cells were suspended in PBS (Gibco, USA) with 1% human serum (Pan-Biotech, Germany) and then incubated with anti-CD90-FITC, anti-CD73-APC, anti-CD105-PerCP-Cy5.5, and negative markers, which included anti-CD34-PE, anti-CD11b-PE, anti-CD19-PE, anti-CD45-PE and anti-HLA-DR-PE (all from Becton Dickinson, USA), for 30 minutes at room temperature and in the dark. Cells were washed with PBS (Gibco, USA) and analyzed using BD FACSCanton II system (Becton Dickinson, USA). Data analysis was performed using Navios/Kaluza software (Beckman Coulter, USA). Microbiological tests were performed using the BacT/Alert® 3D microbial detection system (Biomerieux, USA). Cell products were examined for endotoxin levels and mycoplasma using the Endosafe-PTS kit (Charles River) and MycoAlert™ PLUS Mycoplasma detection kit (Lonza), respectively.

ADSC Differentiation Assays

ADSCs were induced into adipogenic, chondrogenic and osteogenic differentiation by using the StemPro® Adipogenesis Differentiation Kit (Gibco, USA). Cells were cultured for 14 days, and the medium was changed every third day. Subsequently, cells were fixed by using 4% PFA (Merck, Germany) for 30 minutes before staining with Oil Red O solution (Merck, Germany) to detect lipid droplets, stained with Alcian Blue solution (Sigma Aldrich) to detect the presence of proteoglycan and stained with Alizarin Red S solution (Merck, Germany) to detect calcium deposition. Stained cells were observed under an inverted microscope containing a camera (Olympus Corporation, Tokyo, Japan).

Cell Infusion

ADSCs were collected after the third passage. On the day of infusion, the ADSCs were harvested, washed twice with NaCl 0.9%, counted and resuspended in NaCl 0.9% in a total volume of 20 ml. Each participant received a dose of $1x10^6$ cells/kg body weight infused through the intravenous route within 30 minutes before being discharged after 24 hours.

Outcome Measures

Monitoring of Procedure-Related Adverse Events and Cellular Infusion -Related Adverse Events

Body temperature, blood pressure, respiratory rate, heart rate, wound and skin manifestations were recorded during adipose tissue harvest, during cell infusion, and at regular intervals during the hospital stay. Telephone interviews were maintained to record any adverse events after discharge.

Monitoring of Effects After Intervention

Participants were followed up and re-examined at 3, 6 and 12 months after cell infusion to evaluate the efficacy of the intervention. Serum AMH, FSH and E2 levels on the 2nd to 4th day of the menstrual cycle at each interval were examined in females. Serum testosterone levels at each follow-up visit were checked in males.

Changes in the sexual ability of males were evaluated using the IIEF questionnaire, and changes in sexual function in females were assessed using the FSFI questionnaire at each follow-up visit.

Statistical Analysis

Data were analyzed using R software version 3.5.2. The total score and each dimension's score of IIEF and FSFI were described by mean ± SD. The preintervention (day 0) and postintervention (3, 6 and 12 months) results were compared using bootstrap method which may be more appropriate for analyzing HRQoL data than conventional statistical methods [32–34]. Parameter changes with a p-value < 0.05 were considered to be significant.

Results

Patient Characteristics

From November 2017 to March 2020, 31 participants were enrolled in the study, including 15 males and 16 females. The average ages of the male and female patients were 48.4 (± 3.1) and 44.6 (± 7.5) years, respectively. The average duration of sexual dysfunction was 1.7 (± 0.9) years for males and 1.3 (± 0.5) for females. The characteristics of the participants are presented in Table 1.

Table 1 Characteristics of the participants at the baseline Anti-Mullerian hormone (AMH), Follicle stimulating hormone (FSH), Estradiol (E2)

Characteristics	Mean ± SD
Female (*N*=16)	
Age (years)	44.6 ± 3.1
Height (cm)	159 ± 3.4
Weight (kg)	55.5 ± 6.8
The duration of sexual dysfunction (years)	1.3 ± 0.5
AMH (ng/mL)	0.7 ± 0.5
FSH (mIU/mL)	10.3 ± 13.9
E2 (pg/mL)	101.2 ± 132.7
Male (N=15)	
Age (years)	48.4 ± 7.5
Height (cm)	166.7 ± 5.9
Weight (kg)	67.6 ± 9
The duration of sexual dysfunction (years)	1.7 ± 0.9
Testosterone (nMol/dL)	9.99 ± 3.1

Autologous Adipose Tissue-Derived Mesenchymal Stem Cells

AD-MSCs from all patients exhibited the typical MSC morphology, including plastic-adherent cells, spindle-shaped cells, and fibroblast-like cells. The doubling times of P0, P1, P2 and P3 MSCs were 54 hours, 28.8 hours, 47.3 hours and 39.5 hours, respectively (supplement material A and B). Analysis of MSC markers by flow cytometry revealed that these cells expressed 99% MSC-positive markers, including CD73, CD90, and CD105 (supplement material C). However, 9 samples (29%) showed negative markers (CD45, CD34, CD19, CD11 and HLA-DR) at a level of 5.69%. There were no significant differences in doubling time or markers between females and males.

Further analysis of differentiation potential confirmed that the AD-MSCs were able to differentiate into adipogenic, chondrogenic, and osteogenic cell types, as confirmed by positive staining with Oil O Red, Alcian Blue, and Alizarin Red, respectively (supplement material D).

Adverse Events

Thirty-one patients were followed up for over 1 year after ADSC infusion and showed only mild or nonserious adverse events (AEs). A total of 126 AEs were reported. Notably, one patient had a serious AE (IVF failure), but it was unrelated to MSC-based therapy. Two events (1.6%) were related to the intervention: surgical site infection and high liver enzymes. Thirty-seven events (29%) were considered to be related to the procedure, with the most common being uterine fibroids (Table 2). The most common unrelated AEs were cough, dry eyes, and dyslipidemia, which were reported between 1 month and 12 months post-infusion.

The Improvement in Sexual Quality of Life Among Males

After ADSC infusion at 3 months, 6 months, and 12 months, there were significant changes in three domains compared to baseline: erectile function, intercourse satisfaction and overall satisfaction (p – value <0.05). The other domain including orgasmic function and sexual desire has increased without statistical significance (Table 3).

Table 2 Summary of the safety during the study None were related to the ADSC transplantation. Adverse event (*AE*), Serious Adverse Event (SAE)

AE/SAE	Number	Classifications of AE/SAE	Note
IVF failure	1	SAE	Unrelated to the intervention
AE/SAE was not associated with the intervention	47 (37%)	AE	
AE/SAE was less associated with the intervention	40 (32%)	AE	
AE/SAE may be related to the intervention	37 (29%)	AE	
AE/SAE related to the intervention	2 (1.6%)	AE	
Total	126 AE + 1 SAE		

Testosterone Levels in Males

The testosterone levels significantly increased at 3 months (P<0.05) and then decreased gradually at 6 months and 12 months; however, they were still significantly higher than those at baseline P<0.05) (Table 4). One male did not check in at 6 months.

The Improvement in Sexual Quality of Life Among Females

The quality of sexual life among females also significantly improved in all six domains after ADSC infusion. In general, the sexual quality of life was significantly increased (Table 5). One female who had IVF failure did not complete the 6-month assessment.

Reproductive Hormone Levels in Females

The results of AMH, FSH and E2 are presented in Table 6. There were no significant changes in AMH, FSH, or E2 levels at 3 months, 6 months and 12 months after ADSC infusion compared to baseline. One female was lost to follow-up at 6 months.

We divided patients into two groups, under 45 years old and over 45 years old, to determine whether age affects the results of ADSC infusion on reproductive hormones in females. We found that E2 tended to increase at 3 months after ADSC infusion and decreased at 6 and 12 months in the under 45-year-old group, whereas E2 declined at all time points in the over 45-year-old group compared to baseline (Fig. 1, upper panel). In the under 45-year-old group, FSH had a mild elevation at 3 months, although no changes were observed at 6 and 12 months after MSC therapy compared to baseline, while FSH levels were not different in the over 45-year-old group (Fig. 1, middle panel). There were no changes in AMH levels in either group (Fig. 1, lower panel).

Discussion

To our knowledge, this is the first report using ADSC therapy to treat reproductive hormone deficiency in humans. Autologous ADSCs were isolated from the patient's adipose tissue through enzyme digestion. Our isolated and expanded

Table 3 The sexual life quality in males participated in the study using IIEF questionnaire International Index of Erectile Function (IIEF)

Domain	Baseline (*N*=15)	After 3 months (*N*=15)	Change in QOL score (Mean difference)	After 6 months (*N*=14)	Change in QOL score (Mean difference)	After 12 months (*N*=15)	Change in QOL score (Mean difference)
	Mean (SD) Median [Min, Max]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]
Erectile Function	19.0 (7.82) 23.0 [1.00, 28.0]	23.4 (3.58) 23.0 [17.0, 29.0]	4.27* [0.80; 8.13]	21.9 (5.57) 24.0 [11.0, 28.0]	2.73* [0.40; 6.47]	23.7 (5.16) 25.0 [8.00, 28.0]	4.53* [1.33; 8.33]
Orgasmic Function	7.47 (2.26) 8.00 [1.00, 10.0]	8.13 (1.36) 9.00 [6.00, 10.0]	0.67 [-0.47; 1.87]	7.93 (1.71) 8.00 [3.00, 10.0]	0.47 [-0.53; 1.60]	7.73 (2.12) 8.00 [1.00, 10.0]	0.27 [-1.07; 1.73]
Sexual Desire	6.07 (2.05) 6.00 [2.00, 10.0]	6.73 (1.44) 6.00 [4.00, 9.00]	0.67 [-0.20; 1.47]	6.40 (1.84) 6.00 [3.00, 10.0]	0.33 [-0.73; 1.33]	6.60 (1.64) 7.00 [2.00, 9.00]	0.53 [-0.27; 1.53]
Intercourse Satisfaction	7.33 (3.58 9.00 [0, 12.0]	9.13 (2.47) 9.00 [5.00, 14.0]	1.73* [0.60; 3.20]	8.67 (2.79) 9.00 [3.00, 12.0]	1.27* [0.27; 2.60]	9.33 (2.41) 9.00 [3.00, 12.0]	2.00* [0.67; 3.53]
Overall Satisfaction	5.53 (1.36) 5.00 [4.00, 8.00]	6.60 (1.24) 6.00 [4.00, 9.00]	1.07* [0.20; 1.93]	6.40 (1.72) 6.00 [3.00, 9.00]	0.87* [0.33; 1.47]	7.07 (1.33) 7.00 [4.00, 9.00]	1.53* [0.87; 2.20]

*Bootstrap P – value < 0.05

Table 4 Serum testosterone levels at baseline and follow-up the serum testosterone levels were measured at baseline, 3 months, 6 months and 12 months after ADSC transplantation

Indicator	Baseline (*N*=15)	After 3 months (*N*=15)	Compared to baseline (Mean difference)	After 6 months (*N*=14)	Compared to baseline (Mean difference)	After 12 months (*N*=15)	Compared to baseline (Mean difference)
	Mean (SD) Median [Min, Max]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]
Testosterone (nMol/dL)	9.99 (3.13) 9.49 [5.84, 19.9]	12.4 (3.49) 13.1 [5.73, 17.1]	2.30* [0.94; 3.67]	12.6 (3.82) 12.0 [6.31, 18.7]	2.57* [0.90; 4.49]	11.8 (4.27) 10.8 [6.01, 19.2]	1.77* [0.26; 3.48]

*Bootstrap P – value < 0.05

ADSCs were evaluated for the expression of the MSC surface markers CD73, CD90 and CD105, as in other studies [35–37]. The negative surface markers in some samples of our study are probably due to CD34 or HLA-DR expression. CD34 was demonstrated to be expressed in MSCs from adipose tissue at passages 2 to 4 [38]. Other studies have shown an increase in HLA-DR expression, especially in culture media containing IFN-γ [39]. Therefore, further investigation is needed to determine exactly which markers can be expected to be present.

Our results indicated that ADSC infusion was safe for both males and females with sexual deficiency. All related events were resolved without complications. There were no severe AEs that were related to MSC therapy during either the treatment or 12-month follow-up period after the infusion.

Sexual function was improved in men, based on both clinical and laboratory manifestations. Testosterone increased significantly after ADSC infusion. However, it decreased gradually after 6 months and then after 12 months. Clinically, overall satisfaction was significantly improved, while each of the other domains, namely, erectile function, organism, sexual desire, and intercourse satisfaction, increased, but not significantly. These results suggest

Table 5 The sexual life quality in female patients participated in the study using FSFI questionnaire Female sexual functioning index (FSFI)

Domain	Baseline (*N*=16)	After 3 months (*N*=16)	Change in QOL score (Mean difference)	After 6 months (*N*=15)	Change in QOL score (Mean difference)	After 12 months (*N*=16)	Change in QOL score (Mean difference)
	Mean (SD) Median [Min, Max]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]
Desire	2.81 (0.811) 3.00 [1.20, 4.20]	3.38 (0.615) 3.60 [2.40, 4.80]	0.56* [0.22; 0.98]	3.32 (0.594) 3.60 [2.40, 4.80]	0.50* [0.19; 0.85]	3.90 (0.727) 3.60 [2.40, 4.80]	1.09* [0.68; 1.50]
Arousal	3.08 (1.25) 3.00 [0, 5.70]	3.38 (1.28) 3.30 [0, 5.70]	0.30* [0.13; 0.49]	3.46 (1.28) 3.30 [0, 5.70]	0.38* [0.17; 0.64]	4.05 (0.910) 3.75 [3.00, 5.70]	0.98* [0.51; 1.50]
Lubrication	3.92 (1.43) 3.75 [0, 6.00]	4.05 (1.43) 4.05 [0, 6.00]	0.13 [-0.02; 0.32]	4.26 (1.44) 4.80 [0, 6.00]	0.34* [0.08; 0.63]	4.97 (0.790) 5.10 [3.60, 6.00]	1.03* [0.60; 1.56]
Orgasm	3.60 (1.35) 3.60 [0, 6.00]	3.68 (1.32) 3.80 [0, 6.00]	0.08 [-0.10; 0.28]	3.73 (1.24) 4.00 [0, 5.20]	0.13 [-0.08; 0.33]	4.60 (0.800) 4.60 [3.20, 6.00]	0.98* [0.50; 1.58]
Satisfaction	3.55 (0.837) 3.60 [2.00, 5.20]	3.55 (0.837) 3.60 [2.00, 5.20]	0.25 [-0.03; 0.58]	3.87 (0.823) 4.00 [2.00, 5.20]	0.31* [0.10; 0.55]	4.55 (0.728) 4.80 [3.60, 6.00]	1.33* [0.68; 2.13]
Reduce Pain	3.70 (1.98) 3.80 [0, 6.00]	3.95 (1.59) 3.80 [0, 6.00]	0.23 [-0.05; 0.65]	4.13 (1.54) 3.60 [0, 6.00]	0.42 [-0.15; 1.12]	5.05 (0.850) 5.00 [3.60, 6.00]	1.00* [0.68; 1.33]
All items	20.7 (6.99) 21.1 [3.20, 31.9]	22.2 (6.63) 21.6 [5.00, 32.5]	1.58* [0.56; 2.77]	22.8 (6.28) 23.7 [5.00, 32.9]	2.09* [1.03; 3.34]	27.1 (3.92) 28.2 [21.0, 33.7]	6.44* [4.03; 9.15]

*Bootstrap P – value < 0.05

Table 6 Serum AMH, FSH, E2 levels at baseline and follow-up the serum levels of AMH, FSH and E2 were measured at baseline, 3 months, 6 months and 12 months after ADSC transplantation. Anti-Mullerian hormone (AMH), Follicle stimulating hormone (FSH), Estradiol (E2)

Indicator	Baseline (*N*=16)	After 3 months (*N*=16)	Compared to baseline (Mean difference)	After 6 months (N=15)	Compared to baseline (Mean difference)	After 12 months (*N*=16)	Compared to baseline (Mean difference)
	Mean (SD) Median [Min, Max]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]	Mean (SD) Median [Min, Max]	Mean [95% CI]
FSH (mIU/mL)	10.3 (14.0) 4.83 [1.33, 49.7]	10.7 (9.03) 8.03 [2.69, 40.1]	0.81 [–4.14; 3.69]	12.8 (11.2) 8.14 [2.74, 41.2]	2.56 [–3.38; 8.04]	13.7 (9.91) 13.2 [1.96, 34.8]	3.26 [–1.07; 7.63]
Estradiol (pg/mL)	101 (133) 68.2 [4.00, 551]	67.1 (84.6) 38.6 [4.00, 347]	–31.77 [–124.37; 36.94]	74.3 (131) 22.9 [4.00, 490]	–31.96 [–130.85; 66.02]	74.5 (140) 29.6 [4.00, 571]	–23.95 [–126.36; 72.95]
AMH (ng/mL)	0.714 (0.508) 0.573 [0.01, 1.85]	0.563 (0.473) 0.463 [0.0100, 1.59]	–0.15* [–0.28; -0.02]	0.690 (0.701) 0.397 [0.01, 2.36]	–0.06 [–0.29; 0.26]	0.429 (0.403) 0.228 [0.0100, 1.52]	–0.29 [–0.42; -0.16]

*Bootstrap P – value < 0.05

that stem cell infusion may have to be repeated every 12 months to maintain the quality of sexual life and reproductive hormones in males.

In females, sexual function was remarkably improved after ADSC infusion, as evaluated through the FSFI, including the six domains sexual desire, arousal, lubrication, orgasm, satisfaction, and reduce pain.

The female patients in this study were at the menopausal transition stage. This stage starts at approximately 35 years old [40]. During this time, the levels of FSH increase while the levels of E2 decrease, and AMH is undetectable with increasing age [41, 42]. Interestingly, we observed an increase in E2 levels in women under 45 years old at 3 months after ADSC infusion but not in women over 45 years old. This result suggests that MSC infusion may have an effect in younger females and should be performed early in women under 45 years of age to improve or maintain sexual hormone levels. This idea was supported by the study of Edessy et al. in which autologous MSC were injected into the ovaries of women younger than 40 years old suffering from POF and showed that FSH, LH, E2 and AMH levels were improved after the injection [21]. Moreover, there are significant differences in clinical application [43–45], as well as in cytokine and chemokine secretion profiles [46, 47], between ADSCs and bone marrow stem cells. Therefore, differences in the results obtained in these two studies are probably due to differences in cell source, routes of application for MSC transplantation and the age of patients.

In Table 7, the efficacy of MSCs in improving sexual hormone levels and function in animal models, as well as both men and women, is summarized; however, the mechanism underlying MSC-based therapy is still not well understood. In females, E2 is produced by the ovary by stimulating the synthesis of steroidogenic enzymes such as P450arom. MSCs express P450arom mRNA and protein [48] suggesting that MSCs have the ability to synthesize and release E2. Human umbilical cord MSCs secrete cytokines such as hepatocyte growth factor (HGF), vascular endothelial cell growth factor (VEGF) and insulin-like growth factor-1 (IGF-1), which improve ovarian reserve function in aging rats [15]. In a study relating to male patients, MSCs were demonstrated to differentiate into Leydig cells in vivo [49]. However, some studies demonstrated very low rates of in vivo engraftment and differentiation of transplanted MSCs into target cells [50, 51] This suggests that MSC differentiation might not be an essential mechanism for the beneficial effects of MSCs. Recent studies suggested that the therapeutic mechanisms of MSC transplantation may be through secretion of growth factors and extracellular vesicles that stimulate the survival and recovery of damaged tissue [50–52]. Further studies are necessary to investigate the exact mechanism of MSCs in recovering reproductive hormones in patients with sexual deficiency.

We acknowledge some limitations in our study, including its relatively small cohort size and lack of a control group. Larger randomized controlled trials are necessary to better assess the potential benefit of ADSCs in the improvement of sexual deficiency.

In conclusion, autologous ADSC transplantation is safe and may improve sexual quality of life in patients of both sexes.

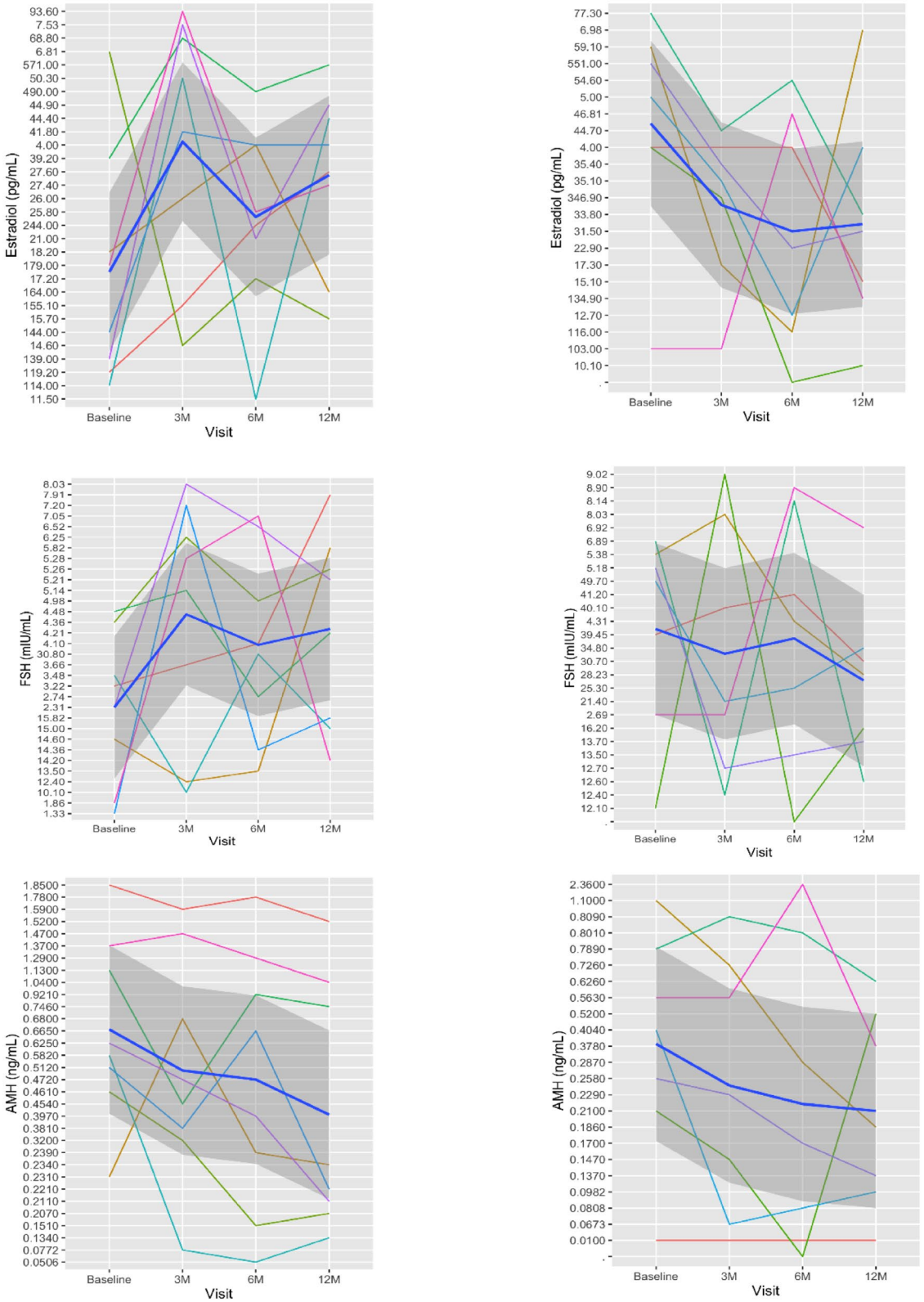

Fig. 1 The changes of AMH, FSH, E2 between participants under 45 years old versus participants over 45 years old. The serum levels of E2 (Estradiol, upper panel), FSH (Follicle stimulating hormone, middle panel) and AMH (Anti-Mullerian hormone, lower panel) were measured at baseline, 3 months, 6 months and 12 months after ADSC transplantation

Table 7 The effect of MSCs in pre- and clinical studies Mesenchymal stem cell (MSCs), Proliferating cell nuclear antigen (PCNA), Human amniotic mesenchymal stem cells (hAMSCs), MSCs from human orbital fat tissues (OFSCs), Human umbilical cord mesenchymal stem cells (UCMSCs), MSCs derived from the chorionic plate (CP-MSCs), Bone marrow stem cells (BMSCs), Human placenta mesenchymal stem cells (hPMSCs), Estradiol (E2), Follicle stimulating hormone (FSH), Anti-Mullerian hormone (AMH), hepatocyte growth factor (HGF), vascular endothelial cell growth factor (VEGF), insulin-like growth factor-1 (IGF-1), treatment-emergent serious adverse events (TE-SAEs), tumor necrosis factor alpha (TNF-α), anti-zona pellucida antibody (AzpAb), FSH receptor (FSHR)

Model	MSC source	Results	Reference
Rat	MSC	• Primordial follicles were found • PCNA (proliferating cell nuclear antigen) immunoreactivity was detected • Collagen fiber was decreased	Amin et al., 2013 [16]
Mouse	hAMSCs	• Follicle numbers were improved. • The proliferation rate and marker expression level of ovarian granular cells were promoted.	Ding et al., 2018 [19]
Human	MSCs	• Two cases resumed menstruation after three months. • One case got pregnant after 11 months and delivered a healthy full-term baby.	Edessy et al., 2016 [21]
Rat	OFSCs	• Prevented torsion-induced infertility judging from Johnsen's score • Serum testosterone was increased. • FSH level was balanced. • FSCs also produced stem cell factors in the damaged testis.	Hsiao et al., 2015 [20]
Rat	UCMSCs	• E2 and AMH were increased, FSH was decreased • Follicle number was increased • Ovarian expressions of HGF, VEGF, and IGF-1 protein were elevated	Li et al., 2017 [15]
Mouse	CP-MSCs	• The serum hormone level and ovarian function were restored • The levels of serum E2 and FSH were similar to the values in the wild-type group. • Ovarian function was significantly improved	Li et al., 2018 [17]
Human	MSCs	• No therapy-related TE-SAEs occurred at one month. • The female sexual quality of life questionnaire improved. • Serum TNF-α levels decreased, B cell intracellular TNF-α improved • Early and late activated T-cells were also reduced	Tompkins et al., 2017 [22]
Rat	MSCs	• Differentiated into Leydig cells or adrenocortical cells	Yazawa et al., 2006 [49]
Rat	BMSCs	• BMSCs can synthesize and release E2	Zhang et al., 2012 [48]
Mice	hPMSC	• Recover the estrus cycle • The basal follicles and sinus follicles were higher, and the follicle number was significantly decreased with atresia. • The serum levels of FSH, LH, and AzpAb were reduced, the E2 and AMH levels were significantly increased. • The AMH and FSHR expression were significantly higher • Granulosa cell apoptosis was decreased.	Zhang et al., 2018 [18]

Abbreviation MSC: mesenchymal stem cell; ADSC: adipose tissue-derived mesenchymal stem/stromal cell; AMH:: Anti-Mullerian hormone; FSH: Follicle stimulating hormone; E2: Estradiol; AE: Adverse event; SAE: Serious adverse event; IIEF: International Index of Erectile Function; FSFI: Female Sexual Functioning Index

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12015-021-10196-w.

Acknowledgments We thank the patients for their trust and willingness to participate in this clinical trial. We acknowledge the support of Vinmec International Hospital.

Authors' Contributions L.N.T. and P.T.M.D. performed the literature research, designed the study and conducted ADSC transplantation. N.T.T.S., T.M.H., and N.B.H. recruited the patients and performed the clinical examinations and follow-up studies. P.T.M.D. and H.M.D. performed the isolation and culture of ADSCs and quality control tests. N.H.P. collected and analyzed the data. L.N.T. and P.T.M.D. wrote the article, and it was reviewed by all authors.

Funding This work was supported by Vingroup Joint Stock Company.

Availability of Data and Materials The data support the findings of this study are included in this article. Unpublished data are available from the corresponding author upon reasonable request and with permission from the Ethics Committee of Vinmec International Hospital.

Declarations

Ethics Approval This study was approved by The Clinical Research Ethics Committee of Vietnam Ministry of Health (November 30th, 2017). Written informed consent was obtained from each participant. This trial was registered witNoth ClinicalTrials.gov (NCT03346967). Participants waived any hospital fees for screening and treatment-related interventions.

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