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Mesenchymal Stem Cells in Regenerative Medicine, Possible Applications in The Restoration of Spermatogenesis: A Review

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Abstract

Infertility is a common clinical condition and about half of the major causes are due to male-related infertility. Pathogenesis of this abnormality is generally undefined; so establishing a proper treatment option is relatively uncertain. In recent years, several evidences demonstrated that mesenchymal stem cells (MSCs) can be a hope for innovative and efficient treatment of male infertility. This study reviews possible applications of MSCs in the restoration of spermatogenesis in male infertility of both humans and animals to suggest new avenues for future clinical practices. Articles published in "PubMed" and "Google Scholar" from January 1, 2000, to August 1, 2023, were investigated by searching items of "mesenchymal stem cells", "cell therapy", "cell transplantation", and, "regenerative medicine" keywords, in addition to the "urology", "andrology", "reproductive medicine", "male infertility", "azoospermia", and "spermatogenesis". The results obtained from the transplantation of MSCs in the treatment of male infertility seemed encouraging and they revealed the safety and efficacy of these cells to recover spermatogenesis; eventhough further stem cell research is still required before recruiting clinical application of MSCs in the treatment of human male infertility. Undertaking more well-defined, standardized, and reproducible protocols and enrolling larger sample sizes during a longer follow-up period can benefit the relevance of MSC transplantation in the restoration of spermatogenesis and treatment of male infertility. It seems that developing and utilizing stem cell transplantations, exosomes, scaffold delivery systems, and three dimensional (3D) culture methods may open a new window to getting more benefits from cell therapy in the treatment of men infertility.

Keywords: Azoospermia, Male Infertility, Mesenchymal Stem Cell Transplantation, Reproductive Medicine, Spermatogenesis

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Introduction

Infertility is still a common clinical problem influencing both genders, while approximately half of the infertility is male-related. Various terms have been used about male infertility to define sperm abnormalities, including aspermia, oligospermia or oligozoospermia, azoospermia, asthenospermia or asthenozoospermia, teratospermia or teratozoospermia, pyospermia or leukocytospermia and necrospermia (1). Aspermia is described by the complete absence of semen. Oligospermia is defined as the presence of few number of sperms in a semen specimen. Azoospermia is a condition with no sperm in semen. This condition is different from aspermia which has no semen. Asthenospermia is determined as a reduction in the percentage of motile sperm in the sperm specimen. Teratospermia is known as the presence of a large number of spermatozoa with abnormal morphology. Pyospermia is defined as a situation with the abnormal presence of white blood cells and infection in the reproductive tract and semen. Finally, necrospermia is known as a state whereby spermatozoa in the seminal fluid are dead and motionless (2).

Therapeutic strategies in male infertility have mostly focused on the improvement of sperm quality, hormone replacement therapy, assisted reproductive technology (ART), and surgery. Sperm cryopreservation has also been offered as a safe and effective way to preserve fertility in cancer patients before they undergo chemotherapy, radiation, or surgery. These conventional methods in the treatment of male infertility may be difficult to employ from a psychological and clinical point of view (2). To overcome these difficulties, regenerative medicine has opened a new door in the remedy of male infertility by utilizing cell transplantation. In cell transplantation, various stem cells including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), and spermatogonial stem cells (SSCs) have

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been used in the repair or regeneration of reproductive organs (3).

Among the stem cells, long term application of human SSCs is not easily possible (4). Applying iPSCs in the treatment of male infertility is limited due to the high risk of rejection, tumour formation and less potential of mimicking germ cell fate. ESCs are also limited since their current sources, obtained from human embryos and aborted fetuses, bear ethical issues in addition to having high risk of rejection and tumor formation (3). In this relation, MSCs have minimal ethical concerns, while they can be isolated easily from the various tissue resources, and differentiate or trans-differentiate into the multi-lineage cells. They can also secrete paracrine factors and microRNAs (miRNAs) to employ the resident stem cells to play an important role in tissue regeneration, or after cell transplantation to help fusion with the local cells (5). MSCs can create a supportive environment for SSCs to restore endogenous spermatogenesis. *The in vitro* differential potential of various MSC sources in germ cells has been previously described (6). Therefore, this review presented possible regenerative medicine applications of MSCs in the restoration of spermatogenesis.

Sources and selection criteria

Articles published in "PubMed" and "Google Scholar" from January 1st, 2000 to August 1st, 2023, were investigated by searching "mesenchymal stem cells", "cell therapy", "cell transplantation", and "regenerative medicine" terms, in addition to, "urology", "andrology", "reproductive medicine", "male infertility", "azoospermia", and "spermatogenesis".

Regenerative medicine

By utilizing stem cells, scaffolds, and biomaterials, regenerative medicine is considered a modern discipline which can recruit both basic and clinical sciences in the treatment of different diseases. Among different categories of stem cells, MSCs, based on their paracrine effects, have a prominent role in the regeneration of damaged tissues (5). MSC differentiation into germ lines looks a promising therapeutic approach in the treatment of male infertility (7) and in this regard, various MSC sources have successfully been differentiated *in vitro* into germ cells (6, 7).

Characteristics of mesenchymal stem cell

MSCs have the ability of self-renewal, easy expansion and differentiation into cells of different lineages which makes them a proper candidate for cell therapy (8). They are multipotent and they can be derived from various sources, such as bone marrow, adipose tissue, dental pulp and Wharton jelly (5, 8). They are positive for expression of mesenchymal cell surface markers such as CD9, CD13, CD29, CD44, CD47, CD49, CD71, CD54, CD56, CD59, CD73, CD81, CD90, CD98, CD105, CD106, CD120a, CD124, CD140, CD146, CD147, CD151, CD166, CD276 and negative for hematopoietic cell markers such as CD34 and CD45 (5, 8, 9).

MSCs with low immunogenicity can actively participate in the healing of damaged tissue. They secrete immunoregulatory factors that can inhibit the proliferation and function of Thelper 1 (Th1)/Th17 cells, the antigen-presenting function of dendritic cells and macrophages resulting in anti-apoptotic and anti-inflammatory activities. MSCs play anti-inflammatory roles by increasing the secretion of interleukin-4 (IL-4), IL-10, IL-11, IL-13, and transforming growth factor beta (TGF-β), in addition to decreasing the secretion of IL-6, IL-12, IL-21, IL-23 and reduction in the activity of nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) cells (5, 8, 9). Their paracrine properties in the regulation of cell proliferation, survival, and differentiation play an important role in immunomodulation too. They also contribute to angiogenesis and tissue homeostasis which can promote tissue regeneration (5, 8). In a systemic review, it was shown that MSCs from various tissue sources could successfully be differentiated into germ cells or gamete progenitor cells. Successful allotransplantation of these cells or xenotransplantation as well as utilizing their exosomes can be effective in the treatment of non-obstructive azoospermia (NOA) of animal models to restore spermatogenesis and fertility (9). So scientists focused on MSC roles in rehabilitating the microenvironment of spermatogenesis and treatment of male infertility (Fig.1) (7).

Male infertility

Mammalian cells are divided into somatic and germ cells, based on their functions, while germ cells are involved in survival and reproduction. In males, the testis consists of germ cells and other cell types, such as Sertoli and Leydig cells for nourishing and supporting the development of germ cells and spermatogenesis (1). Spermatogenesis is an organized and dynamic cell differentiation started in the early fetus and it is completed after puberty by undergoing different stages of mitosis, meiosis, and finally spermatogenesis (6). For meiosis and the protection of haploid cells from the immune system in spermatogenesis, the blood-testis barrier (BTB) has a crucial role (1, 6).

Several factors can affect spermatogenesis. Advancing age can lead to germ cell apoptosis and abnormal spermatogenesis (1, 10). Since 85% of the testicular parenchyma participates in spermatogenesis, a reduction in the size of the testes can result in less sperm production (6). Additionally, a decrease in DNA integrity can damage the development of germ cells. It can also lead to the formation of abnormal motility, morphology, and function of the sperm, subsequently resulting in male infertility (11). Radiation and chemotherapy are well-known gonadotoxic factors that can negatively impact spermatogenesis, damage the somatic environment within the testis, impede spermatogonial differentiation and lead to male infertility (1, 6, 11).

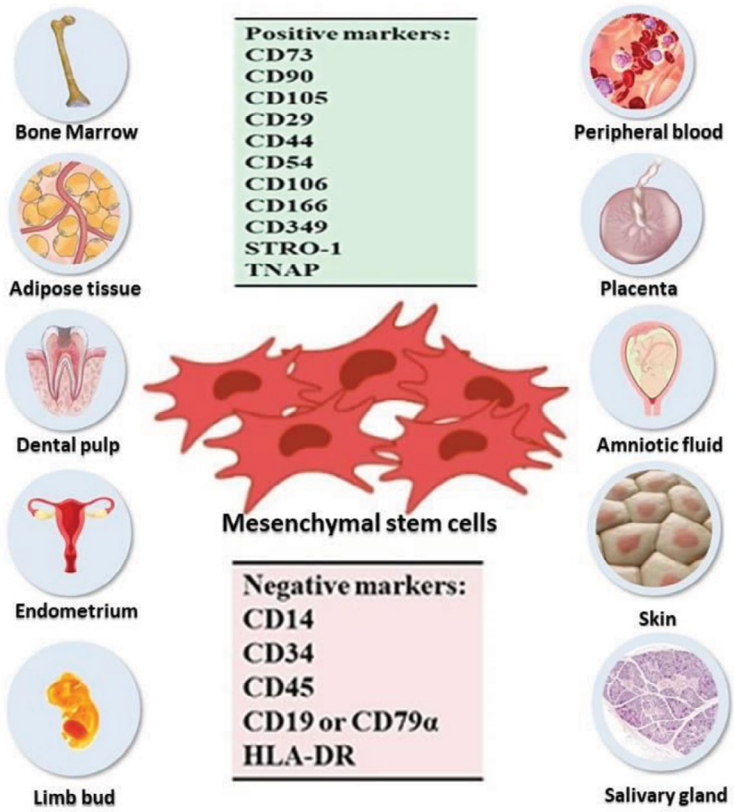

Fig.1: Sources and markers of mesenchymal stem cells.

Globally, 30 million men were reported to suffer from infertility (2). World Health Organization (WHO) report has defined a marriage as infertile when no pregnancy happens within 12 months of unprotected sex (12). Assessment of male infertility consists of a thorough history and physical examination to check abnormalities in the testes, vas deferens, or seminal vesicles, as well as investigation of risk factors for infertility, such as primary or congenital, and acquired or idiopathic causes (Fig.2) (6, 11).

Male infertility is categorized into different types. The absence of sperm in semen and ejaculation is defined as azoospermia. This abnormality is subclassified into two categories, including pretesticular and testicular (NOA), and obstructive azoospermia (OA, post-testicular) (1, 6, 13). In the OA, bilateral distal or proximal obstruction of the reproductive ducts happens, but the spermatogenic process is unaffected. In the NOA, primary or secondary testicular failure occurs and a phenotypic manifestation of three different types of testis histology can be seen, including the sertoli-cell-only syndrome (SCOS), maturation arrest (MA) at different stages of germ cell maturation like, spermatogonial arrest (SGA) and spermatocyte arrest (SCA)- and finally hypospermatogenesis. In azoospermia, almost 40% of the cases are defined as OA and around 60% are determined as NOA. To verify azoospermia, a repeated semen analysis and a hormonal assay would be beneficial. In the NOA, pre-testicular and testicular causes were described (12). In the OA, post-testicular causes were demonstrated, the majority of which were related to obstructions of ducts in the male reproductive system (13).

The major conventional treatment methods for male infertility focus on improving sperm quality which is dependent on etiologic factors (9, 13). SSCs are the basis for spermatogenesis and fertility in males. Damaging these cells may irreversibly alter the spermatogenesis process and lead to NOA. The only conventional solutions to achieve biological fatherhood are assisted reproductive technologies (ARTs), such as testicular biopsy, or testicular sperm extraction (TESE) in combination with intracytoplasmic sperm injections (ICSI), adoption or sperm donation (12).

Transplantation of own cryostored SSCs has been mentioned as a promising technique for fertility restoration when the SSC pool has been depleted (12). However, SSC transplantation in the treatment of male infertility has also several obstacles and challenges, including the efficiency of cryopreservation, experience and knowledge regarding cryopreservation of SSCs, exclusion of malignant cell contamination in cancer patients, and socio-cultural attitudes (11). As TESE-ICSI has shown limited success in NOA and there are many shortcomings with the transplantation of SCCs, there is a need to overcome these challenges (12). Transplantation of MSCs via their self-renewal properties and their ability to differentiate into multiple lineages may be a treatment of choice in male infertility, especially in patients with NOA (1, 6, 9). So based on these properties, MSC transplantations in male infertility can promote spermatogenesis due to their paracrine activities. This can affect Leydig cells by secretion of testosterone for sperm production (6, 9).

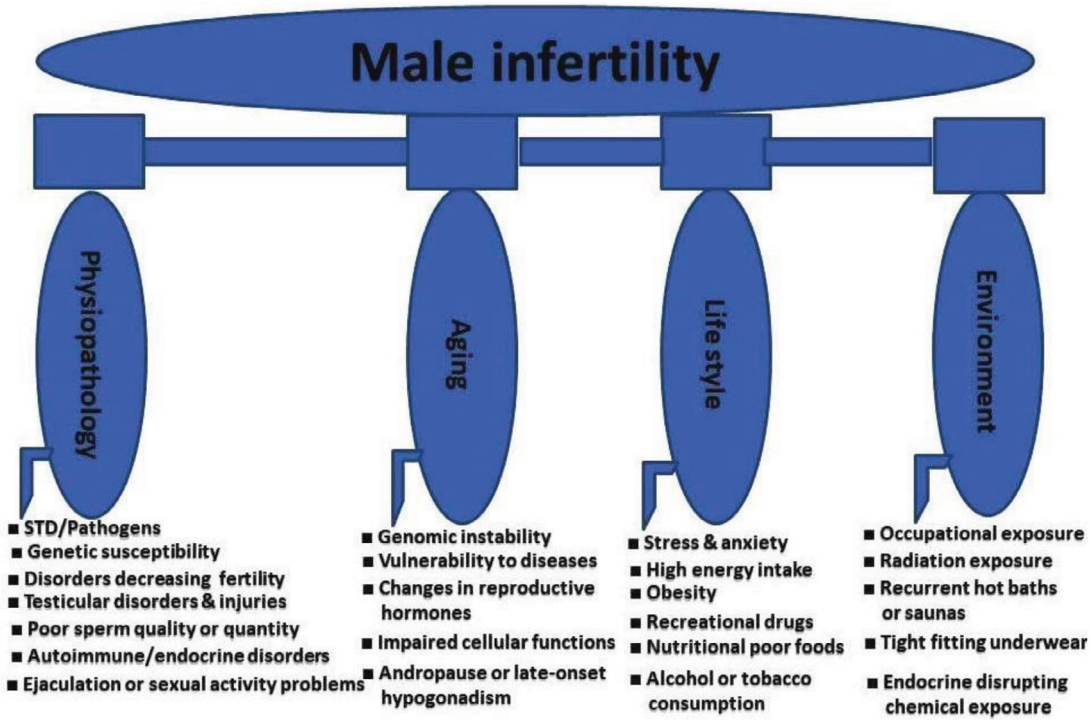

Fig.2: Multifactorial etiology of male infertility.

mesenchymal stem cell transplantation in male infertility

The final goal of fertility treatment is to provide pregnancy and to reach a successful outcome. In animal and human studies, it seems that regenerative medicine by applying cell transplantation, tissue engineering and growth factors has overcome the therapeutic drawbacks and it has opened a new window to improve the tissue or organ normal function and to enhance the fertilization capacity. Cell transplantation can utilize various stem cell sources in the treatment of infertility. In fertility treatment, animal and human studies have been divided into two major categories: i. *In vitro* differentiation into germ cells or gametes and ii. *In vivo* stem cell transplantation into reproductive organs. In the case of specifically transplanting into the testis, MSC can promote the local microenvironment of spermatogenesis to restore the spermatogenic process via secretion of nutritional factors after transplantation (14-21).

***In vitro* utilization of mesenchymal stem cells for the generation of male germ cells**

MSCs from different sources have been used *in vitro* for the generation of male germ cells, such as umbilical cord stem cells (UCSCs) (14-16), adipose tissue derived stem cells (AdSCs) (16-18), bone marrow derived stem cells (BMSCs) (17, 19), and Wharton's jelly stem cells (WJSCs) (20). Table 1 shows *in vitro* studies of MSCs sorted from different sources to differentiate into germ cells. By incubating human UCSCs with retinoic acid (RA), testosterone, and conditioned medium of testicular cell cultures showed high expression levels of male germ cell markers as well as proteins (14, 15). Human umbilical cord perivascular cells (UCPVCs) as a promising cell source, were utilized in transdifferentiation of germ cells and preservation of male fertility (16). For AdSCs, *in vitro*, transdifferentiating of human AdSCs into male germlike cells was demonstrated by rabbit Sertoli cells, while they expressed germ cell-specific markers (17, 21). ADSCs have been reprogrammed into primordial germ cell-like cells, and they had lower efficiency in comparison with AdSC-derived iPSCs (22). Comparing AdSCs with BMSCs, by adding BMP-4 to the culture medium, it was shown that the differentiation potential of BMSCs into primordial germ cells was better than AdSCs (23). AdSCs and BMSCs in the presence of RA were reported to have differentiation potential into male germ cells and expressed germ cell markers, like Mvh, Dazl, Stra8, and Scp3 (17). Kumar et al. (24) illustrated that adding RA to the culture medium of BMSCs could induce differentiation into germ cell-like cells. RA and titanium nanotubes coated with fibrin were demonstrated to offer a proper two-dimensional (2D) scaffold for BMSCs to differentiate into germ-like cells using *in vitro* maturation (IVM) and ART (25). Moreover, the efficacy of BMSCs on survival and expansion of the mice spermatogonial stem/progenitor cells (SSPC) and their differentiation to round spermatids was illustrated (26). BMSCs in the presence of testicular cells were shown to increase the expression of the male germ cell-specific genes (17, 19, 20). Ge et al. (27) showed that murine skin-derived multipotent papillary dermal fibroblast progenitors had *the* potential to generate male germ line cells. Table 1 presents *in vitro* studies of MSCs from different sources to differentiate into germ cells.

Table 1: *In vitro* studies of MSCs from different sources to transdifferentiate into germ-like cells

Type of stem cell	Other treatments	Outcome	Reference
UCSCs	RA, MPLA	Induction of migration and differentiation into germ-like progenitor cells, expression of male germ cell markers as well as proteins	(14)
UCSCs	RA, testosterone, conditioned medium of testicular cell cultures	High expression levels of C-KIT, DAZL, PIWIL2, and DDX4 in mRNA and protein levels, differentiation potential into germ-like progenitors	(15)
UCPVCs	NA	Being promising cell sources to be utilized in the preservation of male fertility strategies	(16)
AdSCs, BMSCs	RA	Remarkable expression of germ cells characteristic markers (Mvh, Dazl, Stra8, and Scp3)	(17)
AdSCs	LIF, GDNF, EGF, RA	Increase in expression of male germ cell markers (c-Kit and Mvh), differentiation into germ-like progenitors	(18)
BMSCs	RA, testicular cells	Increased expression of germ cell-specific markers (Dazl, Piwil2 and Stra8), differentiation potential into germ-like progenitors	(19)
WJSCs	RA and Sertoli cell-conditioned medium	Transdifferentiation into advanced stages of germ cells with an increase in expression of male germ cell-specific genes	(20)
AdSCs	Rabbit Sertoli cells	Transdifferentiating human AdSCs into male germ-like cells, expressing germ cell-specific markers	(21)
AdSCs	Reprogramming	Differentiation into primordial germ cell-like cells, lower efficiency in comparison with AdSC-derived iPSCs	(22)
AdSCs, BMSCs	BMP-4	Differentiation into primordial germ cells, BMCs were better capable of differentiation into primordial germ cells	(23)
BMSCs	Retinoic acid	Differentiation into germ-like cells	(24)
BMSCs	Retinoic acid, titanium nanotubes-coated fibrin formation, ultraviolet radiation	Offering a proper 2D scaffold for transdifferentiated germ-like cells, IVM for ART	(25)
BMSCs	SSPC	Differentiation into round spermatids	(26)
Skin-derived multipotent papillary dermal fibroblast cells	NA	Generating cells of the male germline progenitors	(27)

AdSCs; Adipose tissue-derived stem cells, ART; Assisted reproductive technology, BMSCs; Bone marrow-derived stem cells, BMP-4; Bone morphogenic protein-4, EGF; Epidermal growth factor, GDNF; Glial cell line-derived neurotrophic factor, IVM; *In vitro* maturation, LIF; Leukemia inhibitory factor, MPLA; Monophosphoryl lipid A, NA; Not available, RA; Retinoic acid, SSPC; Spermatogonial stem/progenitor cells, UCPVCs; Umbilical cord perivascular cells, UCSCs; Umbilical cord stem cells, and WJSCs; Wharton's jelly stem cells.

Potential mechanisms in the treatment of male infertility by mesenchymal stem cells

At least, three potential mechanisms were described by which MSCs may improve recovery of spermatogenesis, mostly in rodent models of azoospermia, including: i. Their direct differentiation potential into gametes, Sertoli or Leydig cells (9, 17, 25, 26), ii. Their paracrine secretions and mechanisms can protect and promote regeneration of the testicular niche by directly regulating different stem cells and progenitors, and iii. Indirect effects via immunomodulation of both paracrine and non-paracrine mechanisms make them a therapeutic potential to regulate the survival of testicular niche cells or SSCs (9, 17, 25, 26). Various factors including fibroblast growth factor-2 (FGF2), stem cell factor (SCF), leukemia inhibitory factor (LIF), epidermal growth factor (EGF), bone morphogenetic protein-4 (BMP4), BMP6, FGF5, serpine, angiopoeitin pro-inflammatory M1 and anti-inflammatory M2 cells are involved in regulation of testicular niche and angiogenesis (9, 17, 25). These mechanisms can favor a regenerative environment for the resumption of spermatogenesis by SSCs after testicular injury (9, 17, 25). The effect of treatment with MSCs may be due to the presence of secretomes, like vascular endothelial

growth factor (VEGF), insulin-like growth factor (IGF), miRNA, and having exosomes that can regulate their differentiation and regeneration characteristics in injured tissues (9, 17, 25).

In vivo studies of mesenchymal stem cells in the treatment of male infertility

In the treatment of infertility by MSCs, various animal models were utilized including mouse (28), rat (29), hamster (30), Guinea pig (31), etc. In addition, different methods were applied to induce male infertility, such as the utilization of genotoxic chemicals -like busulfan and physical- and surgical methods (28). Other genotoxic chemicals, except busulfan (30) have been mentioned in the literature, including cyclophosphamide (32), cisplatin (33), cadmium chloride ($CdCl_2$) (34), calcium chloride ($CaCl_2$) (35, 36), doxorubicin hydrochloride (DOX) (30), ethane dimethanesulfonate (EDS) (37), lead (LN) (38), etc. Physical and surgical methods, such as electron beam irradiation (38) and testicular torsion (39), have respectively been used for inducing male infertility. In treatment of male infertility, various MSC sources have been transplanted in animal models, such as BMSCs (38, 40), AdSCs (10, 28, 33, 35), amniotic fluid-derived stem cells (AFSCs) (41), placental mesenchymal stem cells (PSCs) (37), UCPVCs (42), urine-derived stem cells (USCs) (43) and amnion (44).

Among various methods to induce male infertility such as gonadotoxic chemicals, busulfan as a chemotherapeutic agent is conventionally applied in the treatment of leukemia. It has also been utilized for induction of male infertility since busulfan causes ROS-mediated apoptosis, reduction of SSCs and subsequent fertility loss (28). In busulfan-induced male infertility, transplantation of MSCs was demonstrated as a therapeutic approach to restore fertility (45). Cyclophosphamide is a chemotherapeutic agent which has also been efficiently used as a gonadotoxic chemical to induce male infertility at one dose of 150 mg/kg intraperitoneally followed by daily injections of 8 mg/kg for seven days (46). Cisplatin or cisplatinum, also called cis–diamminedichloroplatinum II, as an antineoplastic drug against cancers, has been efficiently utilized for induction of male infertility by injection of a single dose of 7 mg/kg intraperitoneally (34). $CdCl_2$, as a highly toxic gonadotoxic metal, has also been employed to induce male infertility with intraperitoneal injection of 0.4 mg/kg for five weeks or at a single dose of 2.5 mg/testis/100 g body weight dissolved in 0.1 ml of normal saline (37, 47). DOX from the family of anthracycline antibiotics and as an anticancer drug has been effectively used as a gonadotoxic chemical for induction of male infertility by intraperitonial injection of three doses of 3 mg/kg every two days (29). Endocrine-disrupting substances (EDS) are the other gonadotoxic chemicals that have been utilized for the induction of male infertility at a single dose of 50 mg/kg (37). LN is another gonadotoxic chemical inducing male infertility when it is injected intravenously at a single dose of 23.3 mg/kg (38).

Among the physical methods to induce male infertility, exposure to electron beam irradiation, at doses 6, 8, 10, or 12 Gray (Gy) from a linear accelerator to the scrotum, has been introduced in the literature. Hyperthermia by long-term exposure to a temperature of 43°C is also a physical method of male infertility induction (38). Considering surgical methods to induce male infertility, testicular torsion for more than 360° and longer than 24 hours (such as 720° testicular torsion for 3-4 hours) has been in favor of infertility (39). Table 2 just demonstrates *in vivo* studies of busulfan, as a gonadotoxic chemical, to induce male infertility in different animal models, while treatment with the various MSC sources has also been described. Table 3 illustrates genotoxic chemicals, except for busulfan, together with physical methods and surgical procedures in different animal models to induce male infertility, while treatment with different MSC sources has been mentioned too.

In vivo studies of busulfan-induced male infertility treated by mesenchymal stem cell transplantation

As presented in Table 2, AdSCs, AdSCs-conditioned medium (AdSC-CM), AFSCs, BMSCs-CM, PSCs, UCPVCs, USCs and USC-exos (exosomes) have been used in the treatment of male infertility induced by busulfan in different animal models i.e. Guinea pig, hamster, mouse and rat). Several studies have successfully used AdSCs in the treatment of busulfan-induced male infertility. They showed an increase in sperm count and motility with normal morphology (28, 31), a repairing effect in the injured epithelial tissue of seminiferous tubules (30, 45), an upregulation in the expression of male germ cell-related markers of Oct4, Stella, Ddx4, Dazl, PGP9.5, Stra8, and ITGα6 as well as proteins of TGFβ-SMAD2/3, JAK2-STAT3, and AKT (48), and an increase in the total diameter, cellular diameter, cellular area, cross-sectional area and spermatogenesis index of the seminiferous tubules (49). Transdifferentiation of AdSCs into spermatogenic cells and recovery of spermatogenesis were also visible (50). When AdSCs, BMSCs and BMSCs conditioned media (BMSC-CM) were compared in the treatment of male infertility of busulfan-induced azoospermia, AdSCS were less favourable than BMSCs to recover spermatogenesis (51).

Many researchers have efficiently utilized BMSCs in the treatment of busulfan-induced male infertility and reported BMSCs to survive and reside in recipient seminiferous tubules, while expressing SSC marlers like Vasa, Stella, SMAD1, Dazl, GCNF, HSP90α, integrinβ1, and c-kit (52). Transplantation of BMSCs increased the overall tubular fertility index (TFI) and decreased the expression of DNMT3A and H4K5ac in germ cells (34). They also elevated expression of the meiosis-associated genes of *Miwi*, *Stra8*, *CyclinA1*, *Pgk2* and *Scp3* and Sertoli cell barrier functional factors, i.e. ICAM-1 and N-cadherin (40). An enhanced testosterone and estradiol serum levels, upregulated expression of germ cell-specific genes miRNA-21, miRNA-34b, miRNA-34c, miRNA-122, miRNA-449a, miRNA-449b, and miRNA-449c, downregulation expression of miRNA-19b, miRNA-100, miRNA-141, miRNA-146a, miRNA-429, and let-7a. Restoration of the disrupted expression of Ccnd1, E2F1, Myc, PLCXD3, and ERα were also found, following

the transplantation of BMSCs (53). There was an increase in the cellular and total diameters, as well as cellular and cross-sectional areas in the basement membrane of seminiferous and epididymis tubules together with an increase in the survival, migration, homing and differentiation of these cells (54, 55). This resulted in the restoration of spermatogonial structures, such as spermatogonia, primary spermatocytes, spermatids and sperms in seminiferous tubules and the Sertoli cells expressing FSH receptor, after BMSCs transplantation, suggesting efficacy of these cells in the treatment of male infertility (10, 34, 40, 54-66).

Researchers have also used PSCs in treatment of busulfan-induced male infertility and reported restoration of the disrupted spermatogenesis, improvement in semen parameters, an increase in testosterone level and testis size based on the promoted autophagy, protection against oxidative stress, decrease in testicular oxidative damage, increase in the expression of proliferation genes (*PCNA* and *KI67*), and finally decrease in apoptotic genes (*γ-H2AX*, *BRCA1*, and *PARP1*) (42). AFSCs are the other choice among the MSCS which have been successfully utilized in the treatment of busulfan-induced male infertility. This caused the resumption of spermatogenesis, due to attenuation of degenerative and oxidative changes in the testis (41). HUCPVCs were another alternative, among MSCS, applied to treat busulfan-induced male infertility and recover spermatogenesis (67). Transplantation of USCs and their exosomes (USC-exos) were also illustrated to be beneficial in the treatment of busulfan-induced male infertility to restore spermatogenesis, based on the increase in expression levels of spermatogenic genes (*Pou5f1*, *Prm1*, *SYCP3*, and *DAZL*) and proteins, such as UCHL1 (43). USCs were shown to affect the proliferation of spermatogonia verified by expression of the germ cell markers, like octamer-binding transcription factor 4 (OCT4), α6 integrin, c-Kit and VASA (68). UCSCs among MSCs were the other group of stem cells utilized to treat busulfan-induced male infertility and to improve spermatogenesis through an increase in mRNA of the genes related to meiosis (like *Vasa*, *SCP3*, and *PgK2*) together with a decrease in FSH and LH hormonal levels (69). UCSCs can lead to a rise in germ cell-specific genes of miwi and synaptonemal complex protein (Scp3) too (70). SSCs in the presence of MSCs were another therapeutic alternative to restore spermatogenesis in busulfan-induced male infertility by increasing TFI, and recovery of the endogenous SSCs (71). By investigating the fertility protective effects of human amnion MSCs (AMSCs) against busulfan-induced testis toxicity in mouse, it was shown that hAMSC could restore spermatogenesis and positively impact testosterone, testicular tissue, cell proliferation, cell apoptosis, oxidative damage and defense, and expression of GCS and meiosis genes (44). Table 2 reveals the results of *in vivo* studies using the various MSC sources in different animal models to treat busulfan-induced male infertility.

In vivo studies of MSC transplantation in male infertility induced by different methods except busulfan

When BMSCs were utilized in the treatment of male infertility induced by cyclophosphamide, restoration of testicular function and enhanced spermatogenesis happened through reducing apoptosis and phosphorylated levels of ERK, AKT, and p38MAPK proteins (32); treatment with this cell type also led to an improvement in androgen hormonal profile (46). When cisplatin was used to induce testicular damage, administration of AdSCs (34) or BMSCs (72) improved spermatogenesis based on the improvement of testicular architecture, and the increase in testosterone level (33). These MSCs could affect malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and gene expressions of Bax, inducible nitric oxide synthase (iNOS), caspase-3, and p38-MAPK (72).

In male infertility induced by intraperitoneal injection of $CdCl_2$, transplantation of SSCs and BMSCs restored fertility, by increasing TFI, in addition to decreasing expression levels of DNMT3A and H4K5ac in germ cells (71, 73). Restoration of fertility following transplantation of SSCs and BMSCs happened based on apoptosis of mitochondrial 3, decrease in expression levels of the apoptosis-associated proteins (Bim, Bax, Cytochrome C, Caspase-3, active-Caspase-3 and AIF), and increased expression levels of Bcl-2 (47). In electron beam irradiated testes of infertility models, BMSCs when transplanted intratesticularly, could recover spermatogenesis and fertility (38). In the Dox-induced model of infertility, transplantation of BMSCs in the testes could restore spermatogenesis by decreasing testicular oxidative stress, and MDA levels, in addition to increasing the antioxidant capacity of BMSCs (74). In contrast, when testicular torsion was utilized to induce male infertility, transplantation of BMSCs into the testis could not cause sperm formation and expression of germ cell-specific markers (like Oct4, Vasa and c-Kit) (75). In a model of autoimmune infertility, BMSC transplantation was found to have immunosuppressive effects on antibody production for antisperm antibody (ASA) (76).

BMSCs were reported to counteract the deleterious effects of cyclophosphamide- (77), Dox- (29), LN- (37) and cdcl2-induced infertilities (78). These cells have a considerable restorative impact on sex hormones, reducing MDA and testicular oxidative stress, increasing antioxidant capacities, amelioration of superoxide dismutase (SOD), glutathione peroxidase and catalase levels, and finally decrease of DNA alteration and fragmentation which can lead to improved spermatogenesis (29, 37, 77, 78). So, BMSCs play an important role in recovering testicular function and reestablishment of spermatogenesis by differentiation into sperm and Leydig cells. It also leads to modulation of serum testosterone, since Leydig cells are responsible for testosterone production (78). Additionally, transplantation of AdSCs was found to counteract the negative impacts of $CaCl_2$ (35) and testicular torsion-induced male infertility (79). These changes happen through reversing the imbalance of glycolysis in sperm and testis, increasing the expression of phosphoglycerate kinase 2, glyceraldehyde-3-phosphate dehydrogenase-spermatogenic, activating Akt, glycogen synthase kinase 3 (GSK3), glycolysis cascades and ATP production, together with increasing serum testosterone secretion and balancing FSH level which ultimately results in improvement of sperm function, particularly in sperm motility and spermatogenesis (35, 39, 79).

Table 2: *In vivo* studies of the treatment of busulfan-induced male infertility using MSCs extracted from various sources and different animal models

Type of stem cell	Other treatments	Animal model	Outcome	Reference
AdSCs	AdSCs-CM	Mouse	Increased number of testis cells, sperm count and motility, and length density of seminiferous tubules, ameliorative effects in mouse testes	(28)
AdSCs	BMSCs, BMSCs-CM	Mouse	Normal morphology of seminiferous tubules, and successful recovery of spermatogenesis, while BMSCs were more favorable than the other choices in the therapy of azoospermia	(51)
AdSCs	TM4 cells, RA, testosterone	Rat	Formation of bigger and tightly packed male germ-like cells feature colonies, up-regulation of expression of male germ cell-related markers (Oct4, Stella, Ddx4, Dazl, PGP9.5, Stra8, and ITGα6) and protein expression levels TGFβ-SMAD2/3, JAK2-STAT3, and AKT pathways	(48)
AdSCs	NA	Guinea pig	Normal spermatogenesis, recovery of spermatogenesis, treatment of azoospermic infertility	(31)
AdSCs	NA	Hamster	Normal repair of epithelial tissue in seminiferous tubules, presence of spermatozoa in epididymis tubes, treatment of azoospermia	(30)
AdSCs	NA	Mouse	Increase in total diameter, cellular diameter, cellular area, cross-sectional area and spermatogenesis index of the seminiferous tubules, restoration of spermatogenesis, treatment of azoospermia	(49)
AdSCs	NA	Rat	Normal morphology of seminiferous tubules, active spermatogenesis, treatment of azoospermia	(45)
AdSCs being GFP^+	NA	Rat	Recovery of spermatogenesis, transdifferentiation into spermatogenetic cells, being $GFP^+/VASA^+$ and $GFP^+/SCP1^+$, treatment of azoospermia	(50)
UCSCs	NA	Mouse	An increase in mRNA levels of genes related to meiosis (*Vasa*, *SCP3*, and *PgK2*), a decrease in FSH and LH levels, restoration of the tubules to normal architecture, improved testicular failure and spermatogenesis	(69)
UCSCs	NA	Mouse	Presence of a round cell shape differentiated spermatids and spermatozoa, migration to the basement of the tubule, expressing germ cell markers octamer-binding transcription factor 4, α6 integrin, C-kit, VASA, improvement in histological features, treatment of azoospermia	(68)
UCSCs	NA	Mouse	Increased levels of spermatogenic gene expression and protein of germ cell-specific genes (miwi, VASA, synaptonemal complex protein Scp3), recovery of spermatogenesis, treatment of azoospermia	(70)
UCPVC	NA	Mouse	Prevention of gonadotoxic drug-induced infertility, recovery of spermatogenesis	(67)
BMSCs	NA	Rat	Enhanced testosterone and estradiol serum levels, up-regulated expression of germ cell-specific genes (miRNA-21, miRNA-34b, miRNA-34c, miRNA-122, miRNA-449a, miRNA-449b, miRNA-449c), down-regulation of expression of miRNA-19b, miRNA-100, miRNA-141, miRNA-146a, miRNA-429, and let-7a, restoration of the disrupted expression of Ccnd1, E2F1, Myc, PLCXD3, ERα and AKT1, treatment of azoospermia	(53)
BMSCs	SSC transplantation by co-transplanting TGFβ1-MSCs	Mouse	Increase in overall TFI and litter sizes, decrease in expression of DNMT3A and H4K5ac in germ cells, restoration of fertility	(54)
BMSCs	NA	Mouse	An increase in cellular and total diameters, cellular and cross-sectional areas, spermatogenesis index and recovered spermatogenesis, treatment of azoospermia	(55)

Table 2: Continue

Type of stem cell	Other treatments	Animal model	Outcome	Reference
BMSCs	SSCs, TGFß1	Mouse	Spermatogenesis, significantly better tubular fertility index TFI, recovery of endogenous SSCs, increase in homing efficiency of the transplanted SSCs, treating infertility	(71)
BMSCs	NA	Hamster	Formation of spermatogonia, primary spermatocytes, spermatids and sperms in seminiferous tubules, treatment of azoospermia	(56)
BMSCs	NA	Guinea pig	Normal appearance of spermatogenesis, restoration of fertility, treatment of azoospermia	(57)
BMSCs	NA	Rat	Survival, migration, homing and differentiation of stem cells at the seminiferous tubules basement membrane, expression of spermatogonia markers (Dazl and Stella), treatment of azoospermia	(58)
BMSCs	NA	Rat	Normal morphology in seminiferous tubules, presence of spermatogenesis, treatment of azoospermic infertility	(10)
BMSCs	NA	Rat	Expression of CD106 and germ cell surface marker (c-kit), transdifferentiation into germ cells, repair of damaged seminiferous tubules, treatment of azoospermia	(59)
BMSCs	NA	Hamster	Normal morphology of epithelial tissue of seminiferous tubules, presence of spermatozoa in epididymis tubes, spermatogenesis in seminiferous tubules, treatment of azoospermic infertility	(60)
BMSCs	Conditioned media derived from cultured testicular Sertoli cells	Rat	Survival and homing at the basement membranes of seminiferous tubules, expression of molecular markers of spermatogonial stem cells and spermatogonia (Vasa, Stella, SMAD1, Dazl, GCNF, HSP90α, integrinβ1, and c-kit), absence of any tumor mass, immune response, or inflammatory reaction, enhancing endogenous fertility, treatment of azoospermia	(52)
BMSCs	NA	Rat	Increased testicular size, recovering androgen hormonal profile levels, resuming spermatogenesis, trans-differentiation into germ cells, restoration of testicular functions, treatment of azoospermia	(61)
BMSCs	NA	Rat	Restoration of spermatogenesis, differentiation to spermatogonia and spermatozoa in seminiferous tubules, and interstitial cells between tubules, treatment of azoospermia	(62)
BMSCs	NA	Mouse	Differentiation into male germ cells, spermatogenesis, treatment of azoospermia	(63)
BMSCs	NA	Rat	Detection of MSCs in the seminiferous tubules, gene expression of a primordial germ cell marker (VASA), stem cell-specific markers (Oct4, SSEA-1 and SSEA-3), specific molecular markers of germ cells (c-Kit, Daz1; premeiotic marker Daz1 and post-meiotic markers c- Kit, Stra 8), presence of spermatocytes and spermatids in testicular tissue, transdifferentiation into germ cells, treatment of azoospermia	(64)
BMSCs- conditioned media (CM)	NA	Mouse	MSC-CM with the most spermatogenic colonies, but no spermatids; higher expressions of the meiosis-associated genes (*Dazl*, *Vasa*, *Miwi*, *Stra8*, *CyclinA1*, *Pgk2* and *Scp3*) in MSC-CM testis; increased levels of Sertoli cell barrier functional factors (ICAM-1 and N-cadherin); significantly improved the short-term restoration of spermatogonial structures	(40)
BMSCs (RFP transfected), SSCs	TGFβ1	Mouse	TFI after SSCT was similar to that after MSC-SSCT, donor-derived TFI after MSC-SSCT was higher after SSCT, litter sizes after SSCT and MSC-SSCT were similar, and significantly reduction of expression of DNMT3A and H4K5ac in transplanted males, but the normal pattern in donor-derived offspring	(34)

Table 2: Continue

Type of stem cell	Other treatments	Animal model	Outcome	Reference
BMSCs (GFP^+)	NA	Mouse	Survival of stem cells within the seminiferous tubules, some with Sertoli cell appearance expressing FSH receptor, some expressing P450scc with appearance of spermatogonia or spermatocytes expressing VASA, treatment of azoospermia	(65)
BMSCs, SSCs	Busulfan testicular gonadotoxicity infertility model	Mouse	showed that BMSCs had no protective effect on fertility after chemotherapy, while after transplantation of SSCs spermatogenesis was observed in 83% of the injected testes	(66)
AFSCs	NA	Rat	Successful homing of AFSCs over the basement membrane of the injured seminiferous tubules, attenuation of degenerative and oxidative changes, re-expression of PCNA in the germ cells, resumption of spermatogenesis, re-appearance of spermatozoa	(41)
PSCs	NA	Mouse	Increase in expression of PCNA of KI67, decrease in apoptotic gene expression levels (*γ-H2AX*, *BRCA1*, *PARP1*), improved semen parameters, increased testosterone levels and testis size, promoting autophagy, protecting against oxidative stress, decrease in testicular oxidative damage, restoration of disrupted spermatogenesis	(42)
USCs	USC-exos	Mouse	Increased spermatogenic gene expression levels (*Pou5f1*, *Prm1*, *SYCP3*, and *DAZL*) and protein UCHL1, restoration of endogenous spermatogenesis, treatment of azoospermia	(43)
AMSCs	NA	Mouse	Restoration of spermatogenesis, elevated testosterone levels, enhanced testicular weight, size, and semen parameters, increased cell proliferation, ameliorated cell apoptosis, repressed oxidative damage, augmented oxidative defense, rise in the expression level of GCS genes (*Dazl*, *Ddx4*, and *Miwi*) and the meiosis genes (*Scp3*, *Cyclin A1*, and *Stra8*)	(44)

AdSCs; Adipose tissue-derived stem cells, AdSCs-CM; Adipose tissue-derived stem cells conditioned medium, AFSCs; Amniotic fluid-derived stem cells, AMSCs; Amnion mesenchymal stem cells, BMSCs; Bone marrow-derived stem cells, BMSCs-CM; Bone marrow-derived stem cells conditioned media, FSH; Follicle-stimulating hormone, GFP^+; Green fluorescent protein-positive, MSCs; Mesenchymal stem cells, NA; Not available, OCT4; Octamer-binding transcription factor 4, P450scc; Cytochrome P450 side chain cleavage enzyme, PCNA; Proliferating cell nuclear antigen, PSCs; Placental mesenchymal stem cells, RA; Retinoic acid, RFP; Red fluorescent protein, Scp3; Synaptonemal complex protein, SSCs; Spermatogonial stem cells, SSCT; Spermatogonial stem cell transplantation, SSEA-1; Stage specific embryonic Antigen-1, TFI; Tubular fertility index, TGFβ1; Transforming growth factor beta 1, UCPVCs; Umbilical cord perivascular cells, USCs; Urine-derived stem cells, USC-exos; Urine-derived stem cells exosomes.

When UCSCs and CD34/CD73-double-positive $CD34^+$/$CD73^+$ testicular stromal cells (TSCs) were used to counteract the deleterious effects of EDS-induced male infertility, restoration of spermatogenesis was noticed via improvement of testosterone level, expression of Leydig cell markers, like cytochrome P450, and polypeptide 1, 3-β-hydroxysteroid dehydrogenase (36, 80). Regarding transplantation of AFSCs in the treatment of the testicular torsion model of male infertility, recovery of spermatogenesis was observed which can be due to the impact of their secretory factors (81). Table 3 presents findings after injection of the various MSC sources into the testis of different animal models of male infertility, induced by methods excepting busulfan.

Cohort studies and case reports of male infertility treated by mesenchymal stem cells

In a cohort study, 105 males with impaired spermatogenesis who received allogeneic stem cell transplantation provided restoration of spermatogenesis (82). In patients undergoing chemo- and radiation therapy with impaired spermatogenesis, autologous bone marrow transplantation (BMT) showed repairing effects for spermatogenesis (83), and finally transplantation of BMSCs and UCSCs could similarly improve spermatogenesis (84). Transplantation of stem cells in these patients improved the hormonal profile of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol, and inhibin B which can describe their therapeutic effects (83, 84). In Table 4, the cohort studies and case reports regarding the treatment of male infertility using MSCs are presented based on autologous and allogenic sources of transplanted stem cells.

Clinical trials in male infertility treated by mesenchymal stem cells

Couto et al. (84) in their screening, during the years 2007-2017 revealed that the median UCSCs dose for local injection was 18.75 million; whereas the median UCSCs was 80 million in the systemic administration. Can et al. (85) in a systemic analysis of clinical trials showed that UCSCs transplantation was successfully used in repairing 25 male infertility suffering from oligospermia. Several factors were mentioned to affect the safety of these interventions, including a good manufacturing practice (GMP), as a gold standard to prevent employment of contaminated cells and inadequate handling or processing methods. So, under internationally recognized GMP conditions, clinical-grade stem cells are manufactured for cell transplantation purposes.

Table 3: Results of transplantation of MSCs from different sources into the testis of animal models of male infertility induced by the other treatment methods except busulfan

Type of stem cell	Induction method of infertility	Animal model	Outcome	Reference
BMSCs	Dox-induced testicular tissue toxicity	Rat	Decrease in testicular oxidative stress, reducing MDA level, increasing antioxidant capacity, recovering testicular atrophy and damages in spermatogenesis, reduction of diameter and germinative cell layer thickness of seminiferous tubules, restoring efficiency of reproductive system	(29)
BMSCs	Gonadotoxicity-induced models exposed to LN	Rat	Amelioration of testosterone level and semen quality, increase in superoxide dismutase, glutathione peroxidase and catalase levels, decrease in genomic DNA alteration and percentage of fragmented DNA, reversing tissue degeneration, necrosis, interstitial edema, reduction in spermatogonia and deformities in the morphology of testis, treatment of infertility	(37)
BMSCs	Electron beam irradiated infertility model	Rabbit	Improved spermatogenesis, recovery of infertility	(38)
BMSCs	$CdCl_2$-induced testicular injury	Rat	Improved pathological changes and expression of apoptosis-associated proteins (Bim, Bax, Cytochrome C, Caspase-3, active-Caspase-3, and AIF), increased Bcl-2, repairing testicular tissue injuries, restoration of fertility	(47)
BMSCs	$CdCl_2$-induced testicular injury	Rat	Increased SOD, LH and total antioxidants of serum and fructose of semen, modulated serum testosterone level, and reestablishment of spermatogenesis by differentiation into sperm, recovery of testicular function	(78)
BMSCs	Cisplatin-induced gonadotoxicity	Rat	Improvement of biochemical and histopathological changes, increase in MDA, TNF-α**,** BAX expression, iNOS, caspase-3, and p38-MAPK and seminiferous tubules atrophy, repairing testicular injury	(72)
BMSCs	$CdCl_2$-induced testicular damage	Rat	Decrease in testicular damages, repairing testicular damages, restoration of infertility	(73)
BMSCs	Dox-induced toxicity in the model of infertility	Rat	Decreased testicular oxidative stress, recovered testicular atrophy and damages of spermatogenesis, reduction of the diameter of seminiferous tubules, significant restoration of structural efficiency of the male reproductive system	(74)
BMSCs	Testicular torsion azoospermia rat model	Rat	Absence of germ cell-specific markers (Oct4, Vasa, and c-Kit), and sperm formation in biopsies	(75)
BMSCs	Traumatic testis rupture autoimmune infertility model	Mouse	Immunosuppressive effects on the production of ASA	(76)
BMSC-exos	Cyclophosphamide-induced testicular spermatogenic dysfunction	Rat	Enhanced cell proliferation, inhibited pathological changes, reduced apoptosis and phosphorylated levels of ERK, AKT, and p38MAPK proteins, a potential treatment for spermatogenic dysfunction	(32)
BMSCs co-transplantation with SSCs, TGFβ1	$CdCl_2$-induced testicular damage	Mouse	Increase in overall TFI and litter sizes, decreasing expression of DNMT3A and H4K5ac in germ cells, restoration of fertility	(71)
BMSCs anti-SCa-1 and CD105	Cyclophosphamide-induced azoospermia	Mouse	Improvement in androgen hormonal profile levels, resuming spermatogenesis, differentiation into germ cells and sperms, restoration of testicular function, treatment of azoospermic infertility	(46)

Table 3: Continue

Type of stem cell	Induction method of infertility	Animal model	Outcome	Reference
BMSCs	Cyclophosphamide-induced azoospermia	Rat	considerable restoration of sex hormone concentrations, enhancement in testicular tissue architecture, improvement of the spermatogenesis cycle	(77)
AdSCs	Cisplatin-induced testicular damage	Rat	Improvement of testicular architecture, increased testosterone level, immune reaction of CD-44, treatment of male infertility	(33)
AdSCs	$CaCl_2$ castration model of infertility	Rat	Improvement in the histological architecture of testicular tissue as well as sperm count and serum testosterone level, treatment of infertility	(36)
AdSCs	Testicular torsion-induced infertility model	Rat	Decreased testicular torsion-detorsion, improvement in sperm function and motility, increased expression levels of phosphoglycerate kinase 2 and glyceraldehyde-3-phosphate dehydrogenase-spermatogenic, activating Akt and GSK3, increase in glycolysis cascades and ATP production, reversing imbalance of glycolysis in sperm and testis, treatment of germ cell injury and infertility	(39)
AdSCs	Testicular torsion-induced infertility model	Rat	Improvement in Johnsen's score, preventing ischemic/reperfusion-induced intrinsic apoptosis, increased serum testosterone secretion, balancing FSH level surrounded Leydig cells by stem cells, rescued infertility	(79)
UCSCs CM-Dil-labeled	EDS-induced male rat hypogonadism model	Rat	Increased testosterone level, expression of Leydig cell markers cytochrome P450, family 11, subfamily A, polypeptide 1, 3-β-hydroxysteroid dehydrogenase treatment of male infertility	(36)
TSCs being CD34/CD73-double-positive $CD34^+$/$CD7^+$	Cavernous nerve crush injury model of infertility	Rat	Restoration of erectile function and fertility	(80)
AFSC	Ischemia/reperfusion injury of twisting the spermatic cord and testicular rotation on its long axis	Mouse	Restoration of normal sperm chromatin condensation, spermatogenesis parameters and histomorphometric organization of seminiferous tubules	(81)

AdSCs; Adipose tissue-derived stem cells, AFSC; Amniotic fluid stem cells, ASA; Antisperm antibody, Bax: B-cell lymphoma-2 Bcl-2 associated X protein, BMSCs; Bone marrow-derived stem cells, BMSCs-CM; Bone marrow-derived stem cells conditioned media, $CaCl_2$; Calcium chloride, $CdCl_2$; Cadmium chloride, Dox; Doxorubicin, EDS; Ethane dimethanesulfonate, FSH; Follicle-stimulating hormone, GSK3; Glycogen synthase kinase 3, iNOS; Inducible nitric oxide synthase, LN; Lead nitrate, MDA; Malondialdehyde, NO; Nitric oxide, SOD; Superoxide dismutase, SSCs; Spermatogonial stem cells, TFI; tubular fertility index, TGFβ1; Transforming growth factor beta 1, TNF-α; Tumor necrosis factor-alpha, TSCs; Testicular stromal cells, and UCSCs; Umbilical cord stem cells.

Can et al. (85) demonstrated that cell isolation and preparation procedures in 93 clinical trials still had a significant gap between the required quality conditions and the de facto status, while just 28% of the trials described standard GMP conditions in the manufacturing of clinical-grade stem cells. Evaluation of cell viability as an important issue in GMP protocols has just been explained in 19% of trials. The immunophenotypic characterization by flow cytometry, on the other hand, was used in 71% of trials. Also in 31% of trials, functional analysis for *in vitro* differentiation assays into three lineages have been undertaken to validate protocols of cell isolation and expansion. In 3% of trials, cell characterization was undertaken by cytokine production assay using an enzyme-linked immunosorbent assay (ELISA) before cell transplantation. So, it seems that the commercialization of MSCs can offer standard manufacturing methods and usage to be applied in clinical trials. As the quality of cells is very sensitive to transportation and storage conditions, local procurement of cells seems mandatory in diverse geographic regions especially when local cell banks provide the off-the-shelf MSCs, as noted in several clinical trials, especially from China.

Can et al. (85) in their investigation reported that 72% of the 93 trials had undertaken the safety of the interventions without any adverse events, and 29% of the interventions

found fever, local pain, headache, and dizziness that disappeared a few days post-transplantation. It is important to carefully monitor short-term signs when they are specific to cell injections or have no direct correlation to cell transplantation. More trials are expected to be published shortly as there are already many registered clinical trial databases. It seems that placebo-controlled, multicenter dose-escalation studies can promote the power of clinical research.

MSCs are generally cultured and expanded in Dulbecco's Modified Eagle's medium (DMEM), DMEM-Ham's F-12 or α-MEM culture media. These media are generally utilized for research purposes because they are frequently enriched with fetal bovine serum (FBS). In Table 4, clinical trials, cohorts and case reports regarding the treatment of male infertility are presented based on the source of stem cells.

Table 4: Cohort and case report studies and undertaken clinical trials using the various sources of MSCs in the treatment of male infertility

Type of study (Year)	Number of patients	Stem cell source, Route	Method	Outcome	Reference
Cohort (2021)	105 adult males with hypogonadism and impaired spermatogenesis	Allogeneic stem cell transplantation	Hormonal assessment (testosterone, FSH, LH, and inhibin B) utilizing IIEF-15 questionnaire and comparison with the general population	Restoration of testicular structure and function	(82)
Case report (2019)	A 36 years-old man after non-Hodgkin T-cell lymphoma treated with chemo- and radiotherapy	Autologous bone marrow	BMT after in mTESE	Showing an absolute restoration of fertility	(83)
Case series (2019)	25 patients with oligospermia	BMSCs, UCSCs	Hormonal profile of follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, testosterone, and findings of semen analysis	Improvement of the emerging evidence contributing to solve problems related to male infertility	(84)
Clinical trial (NA;2013-2015)	10 adult Kleinfelter syndrome patients, in Egypt	Bone marrow	Injection into the testicular tubules and testicular artery, Semen analysis, Hormonal profile	Completed, NA	NCT02414295
Clinical trial (phases I and II;2012-2017)	100 adultazoospermic patients, Egypt	Bone marrow	Injection into rete testis, hormonal assessment	Fertility improvement	NCT02025270
Clinical trial (NA; 2013-2016)	20 child and adult azoospermic patients, in Egypt	Bone marrow	Intra-testicular artery and inside tubules injection, Semen analysis, hormonal assessment	Recruiting	NCT02008799
Clinical trial (phases I and II; 2014-2015)	60 adult azoospermic patients, Egypt	Bone marrow	Injection into testis, hormonal profile, testicular biopsy	Recruiting	NCT02041910
Clinical trial (phases I and II;2015-2020)	50 adult azoospermic patients, Jordan	Bone marrow	Intra-testicular injection, investigating different germ cells in testicles, assessment of testicular morphology, sexual function	Recruiting	NCT02641769
Clinical trial (phase II;2018-2020)	40 adult azoospermic patients, Russia	Adipose tissue	Intra-testicular injection, sperm concentration in ejaculate, spermatozoa in testicular biopsy, hormonal profile	Recruiting	NCT03762967

BMT; Bone marrow transplantation, BMSCs; Bone marrow stem cells, LH; Luteinizing hormone, mTESE; Microdissection testicular sperm extraction, NA; Not available, and UCSCs; Umbilical cord stem cells.

Limitations of using mesenchymal stem cells in the treatment of male infertility

In recent years in modern science and medicine with the development of research on stem cells, increasing evidence was provided describing MSCs as a potential source in the treatment of male infertility. The advances in stem cell biology revealed the 'promiscuity' of MSCs to differentiate, not only into somatic lineages but also into gametic lineages and treatment of male infertility. *In vitro* models were also developed for spermatogenesis and drug screenings to assess gametogenesis. MSC treatments for infertility were divided into two major groups, including the direct transplantation of stem cells or their paracrine factors into reproductive organs, and the *in vitro* differentiation of stem cells into germ cells or gametes and their transplantation (85). In animal models, these therapeutic strategies were found to improve the reproductive potential of the tested animals but there is still few evidences in humans to reveal their conventional use based on the complexity of explored biological processes, the unavailability of proper materials, and ethical considerations. Evolutionary divergence noted in pluripotency among animals and humans still needs caution, when extrapolating the data obtained from murine models to safely apply them to clinics for humans (84, 85).

It seems that more clinical trials based on larger populations and long-term periods are needed to determine the relevance of stem cell therapy, including its efficacy in translational infertility medicine, since a small sample size and a low-quality method can lead to different outcomes. As the storage of stem cells is controversial, the patient is unable to provide his consent or truly understand the implications of the methods. In addition, many technical barriers still exist for most of the stem cell protocols and a well-defined standardized reproducible protocol for storage seems necessary before the use of cell therapies in the clinical landscape. The storage situations in various laboratories for lyophilization, cold chain, and transportation are different which can impact the study results. Moreover, senescent (non-functional) cells can influence the activity of the surrounding healthy cells by releasing several paracrine factors which should be avoided for clinical use to keep the treatment potential of the stem cell batch (84, 85).

Differences in MSC sources, injected cell number, times the cells were transplanted, route of administration, intervals between injections, differences in cell culture laboratories that apply various methods to isolate and purify stem cells, induction method of infertility, type of animal model, assessment methods, and follow-up time were also reported as important factors which can affect survival of the transplanted cells. The donor site for cell isolation can influence proliferation and differentiation potentials of the isolated MSCs which should be noted when cell transplantation is targeted. The genetic stability of transplanted cells is also of great importance and it should be monitored as any acquired mutation can pass to the next progeny. So, the stem cells need to be properly optimized and controlled to prevent any unnecessary cell growth and any probable infection. Furthermore, the intensity of clinical adverse effects can be ignored easily, if a "likely to predict efficacy" is included (85). As there are too many culture media which are specifically designed for human MSCs, there is a strict need for serum-free supplements to avoid using animal products during cell propagation, even if their formulations are expensive (86).

Couto et al. (84) in their screening (between the years 2007 and 2017) found that the cell manufacturing data was often lacking from the published reports. Given the missing variation in the outcomes and the absence of data about manufacturing, they could not identify any trends between outcomes and manufacturing. It should be mentioned that there might be many biases toward the publication of positive results in the literature, as positive findings are more easily and quickly published than negative and null findings (85). Despite these limitations, cell therapies utilizing MSCs remain a tempting strategy to overcome current obstacles in male infertility, because they can lead to regeneration in tissue pathologies. It should be mentioned that some ethical issues may be confronted when transplantation of MSCs is targeted for the treatment of infertility, as MSCs alone may sometimes have a meiotic block during differentiation (8). This may result in a lack of differentiation to SSCs (79). However, well-defined reproducible protocols are necessary for humans to confirm the efficacy of these cells to treat male infertility (87).

Conclusion

The results derived from the transplantation of MSCs in the treatment of male infertility seem encouraging and reveal the safety and efficacy of these cells to recover spermatogenesis; eventhough there is still a need for more stem cell research and clinical application of MSCs in the treatment of male infertility. Undertaking more well-defined, standardized and reproducible protocols, enrolling larger sample sizes and longer follow-up periods can verify the relevance of MSC transplantation in the restoration of spermatogenesis and treatment of male infertility. It seems that developing and utilizing stem cell transplantations, exosomes, scaffold delivery systems and 3D culture methods may open a new window in getting the most benefits from cell therapy in the treatment of men infertility.

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Authors' Contributions

D.I., D.M.; Drafted, Edited and Confirmed the manuscript. D.M., F.K.-B.; Reviewed and Edited the manuscript. All

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