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REVIEW

Cell-Based Therapy Approaches in Treatment of *Non-obstructive Azoospermia*

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Abstract

The rate of infertility has globally increased in recent years for a variety of reasons. One of the main causes of infertility in men is azoospermia that is defined by the absence of sperm in the ejaculate and classified into two categories: obstructive azoospermia and non-obstructive azoospermia. In non-obstructive azoospermia, genital ducts are not obstructed, but the testicles do not produce sperm at all, due to various reasons. Non-obstructive azoospermia in most cases has no therapeutic options other than assisted reproductive techniques, which in most cases require sperm donors. Here we discuss cell-based therapy approaches to restore fertility in men with non-obstructive azoospermia including cell-based therapies of non-obstructive azoospermia using regenerative medicine and cell-based therapies of non-obstructive azoospermia by paracrine and anti-inflammatory pathway, technical and ethical challenges for using different cell sources and alternative options will be described, and then the more effectual approaches will be mentioned as future trends.

Keywords Non-obstructive azoospermia · Cell therapy · Regenerative medicine · Paracrine effect · Inflammation

Introduction

Male factors account for about 50% of couples' causes of infertility. Among these, non-obstructive azoospermia (NOA) constitutes 10–15% of male infertility and 60% of azoospermic men, with an impaired spermatogenesis leading to a lack of sperm in ejaculation. NOA has been shown to occur as a result of congenital or genetic abnormalities, endocrine disorders, varicocele, trauma, exposure to gonadotoxins, infectious agents, chemotherapy drugs, and idiopathic causes [1].

The etiology of NOA is generally categorized into two origins: primary hypogonadism due to primary testicular failure and secondary (hypogonadotropic) hypogonadism due to hormonal abnormality [2].

In secondary hypogonadism, hormone replacement therapy can be administrated to stimulate spermatogenesis and restore fertility with or without the need for surgery [3].

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In primary hypogonadism of NOA the main focus of treatment is on sperm retrieval through testicular sperm extraction.

Therefore, as long as genetic abnormalities such as Klinefelter syndrome and Y chromosome microdeletion do not cause primary testicular failure and are not the main cause of infertility, NOA patients can benefit from hormonal or surgical treatments.

On the other hand, despite hormonal or surgical therapeutic methods, the outcomes of NOA treatments, especially for primary testicular failure, are usually unsatisfactory. In primary hypogonadism of NOA, if sperm could not be retrieved by testicular sperm extraction, the only current option relies on using donor sperm or adoption which deprives couples of having a related biological child.

The larger category of primary testicular failure of NOA is due to idiopathic or inflammatory causes for which there is no effective treatment option [4].

Nowadays, cell transplantation can be used to treat male infertility, especially for the treatment of NOA, in two ways: one from regenerative medicine view and for treatment resulting from failure of germ cells proliferation and differentiation and the other with the help of their paracrine and anti-inflammatory effects for treatment of NOA resulting from idiopathic and inflammatory problems.

Now we will discuss here about the cell-based therapies for NOA using therapeutic approaches of stem/stromal cells based on regenerative medicine and the paracrine mechanisms.

Cell-Based Therapies of NOA Using Regenerative Medicine

From a regenerative medicine view, there are two experimental approaches in which germ cell colonies can be produced for restoring fertility in men with NOA: (1) in vivo approach in which spermatogonial stem cells (SSCs), as the precursors to mature spermatids, are transplanted into the seminiferous tubules of infertile individual. (2) Based on in vitro studies, in addition to SSCs, embryonic stem cells (ESCs) [5], induced pluripotent stem cells (iPSCs) [6], and mesenchymal stem cells (MSCs) [7] can be cultured in vitro and differentiated into male germ cells.

In the following, restoring fertility in men with NOA using mentioned cell sources will be discussed (Fig. 1a).

Spermatogonial Stem Cell Transplantation

SSCs are able to self-renew, differentiate, and regenerate spermatogenesis. To regulate the process, close interactions between SSCs and Sertoli cells surrounding the SSCs, and the creation of a microenvironment called the stem cell niche is essential.

Previously, Lim et al. found SSCs in the testes of NOA patients and isolated and cultured them under exogenous feeder-free culture conditions. After long-term culture, SSCs could be differentiated to male germ cells with developmental potential [8].

Generally, strategies for using SSCs to restore spermatogenesis and fertility include (1) harvest and grafting of testicular tissue and (2) injection of isolated SSCs.

For successful testicular tissue grafting, based on previous studies, SSCs cryopreservation via slow freezing with dimethyl sulfoxide, grafting time after sexual maturity of patients, grafting location in the scrotum, and high levels of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), are ideal requirements.

Although testicular tissue grafting is very optimal in that the natural niche of stem cells is retained, in some cases, such as cancer patients, it needs to be more optimized because of concerns about cancer cell contaminations [9–11].

For injection of isolated SSCs, among the injection targets, including seminiferous tubules, rete testis, and efferent ducts, rete testis injection with guidance of ultrasonography seems to be the most promising injection technique to date [12].

Following transplantation, SSCs migrate to the basement membrane of seminiferous tubules.

One of the advantages of this method over the testicular tissue grafting is that it can provide conception without the need for assisted reproductive techniques (ART) [13].

SSCs can be differentiated into germ cells through in vitro spermatogenesis; in this way, testicular tissues or isolated SSCs can be cultured for days and developed into spermatids. As previously, Lim et al. found SSCs in the testes of NOA patients and isolated and cultured them under exogenous feeder-free culture conditions. After long-term in vitro culture, SSCs could be differentiated to male germ cells with developmental potential [8].

However, despite promising research results of SSC transplantation-based therapy in animals specially in rodent models for restoring infertility, translating this approach to the clinic needs to be optimized in human models from different aspects including SSCs culture condition, cancer cells contamination in cancer patients, cryopreservation of the SSCs, ideal injection site for transplantation, safety of transplantation in recipients, frequency of injection, efficient SSCs volume, and dose for injection [12].

Thus more clinical researches are required to overcome these challenges for SSC transplantation-based therapy for NOA.

Embryonic Stem Cells

Due to the high differentiation potential of ESCs and the ability of these cells to produce germ cells with more efficiency and more autonomy, ESCs are more suitable options of cell therapy for spermatogenesis disorders in NOA.

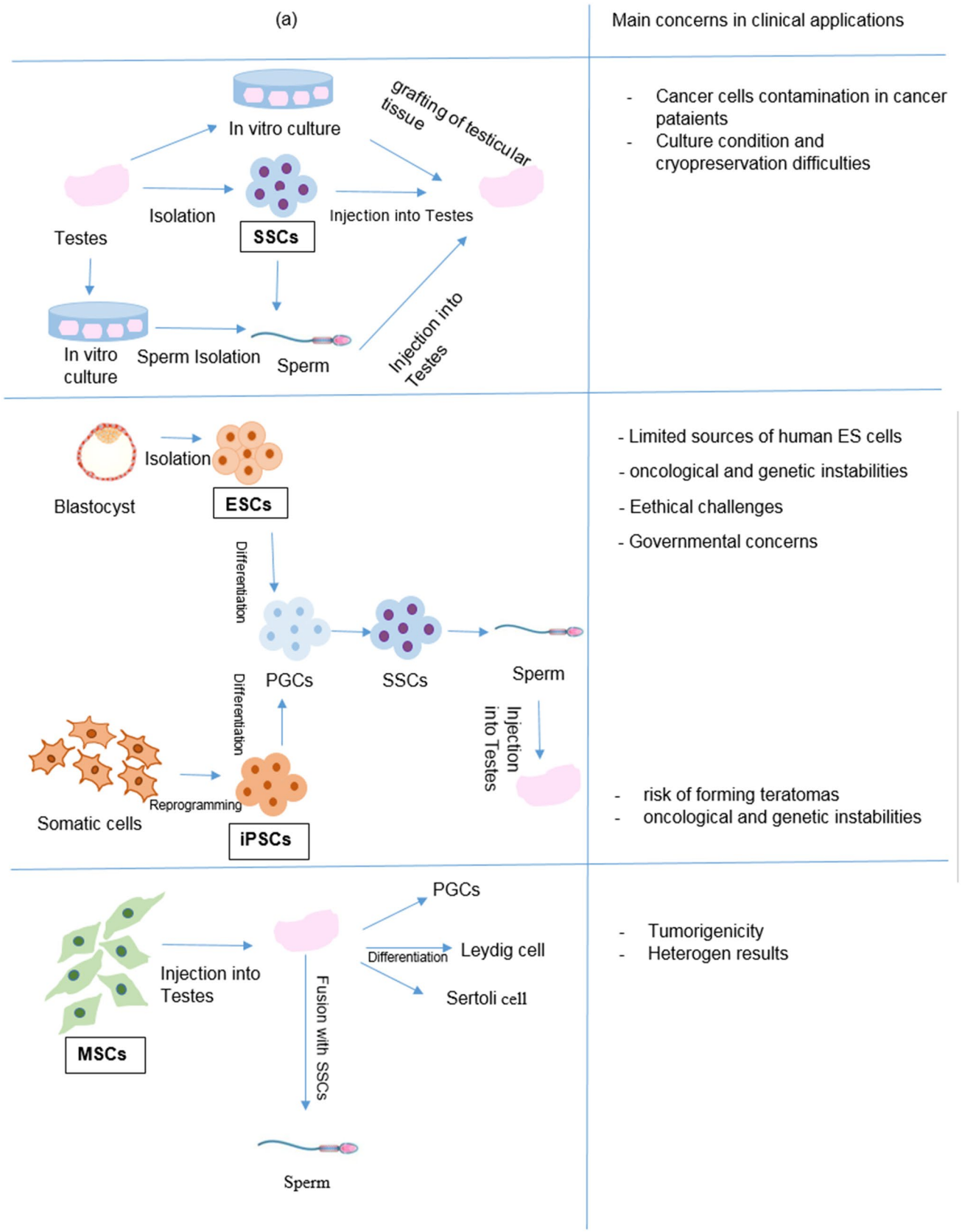

Fig. 1 NOA cell-based therapy. **a** Approaches for cell-based therapies of NOA using regenerative medicine. **b** Cell-based therapies of NOA via anti-inflammatory and paracrine factors secreted by MSCs and DSCs

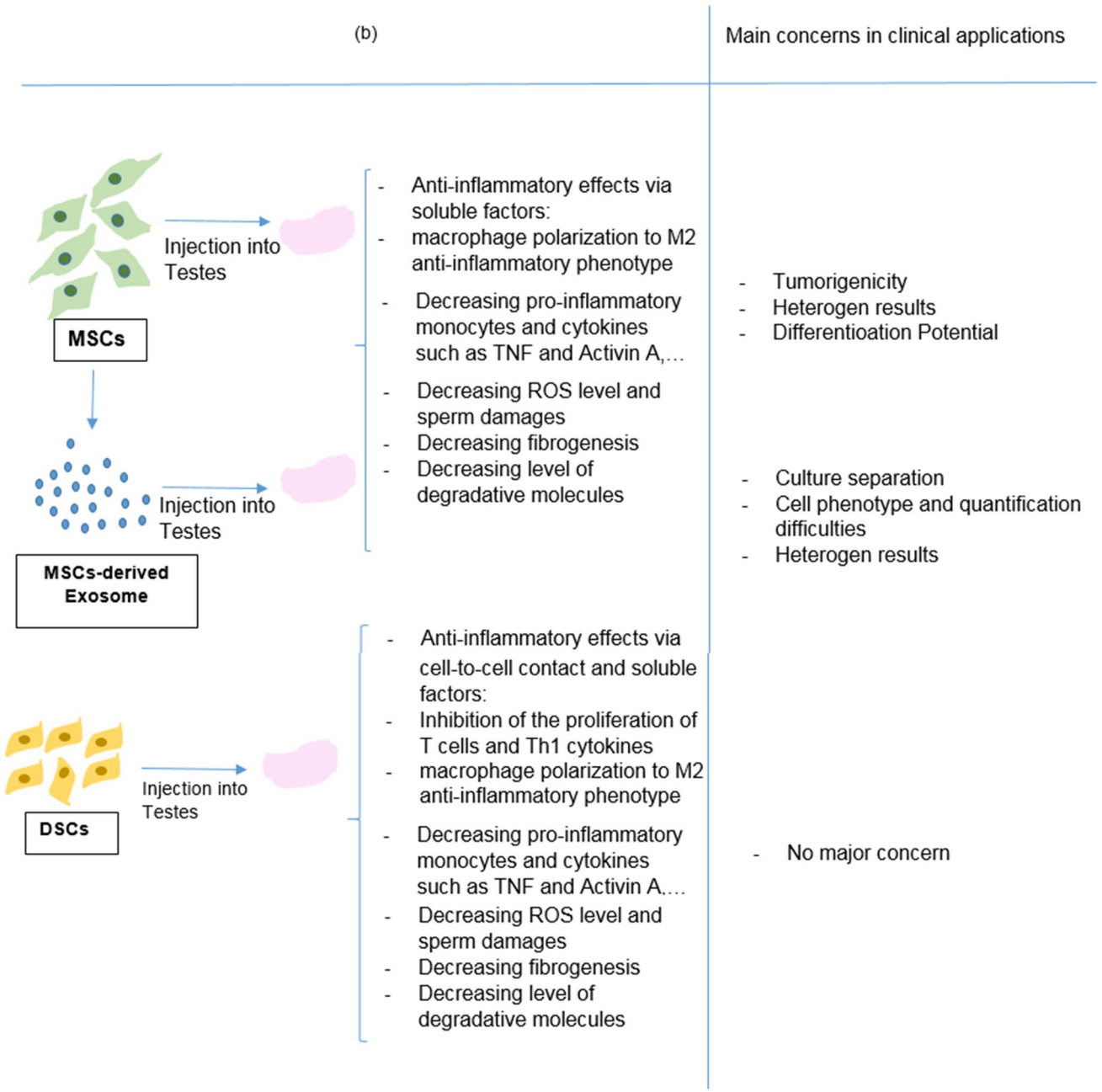

Fig. 1 (continued)

Initially, in mice, it was shown that ES cells can differentiate into male germ cells via embryoid body (EB) formation combined with bone morphogenetic proteins (BMP4) or retinoic acid (RA) induction. After transplantation into mouse testes, ES-derived cells during spermatogenesis generate sperm [14].

Next studies also demonstrated the differentiation potential of ESCs to male germ cells, and that EB microenvironment supports male germ cell development and capacity of fertilizing oocytes [7, 15–17].

Thus, using ES cells to produce male primordial germ cells has promising applications for male infertility treatment in NOA. However, regarding the use of ESCs in infertility treatment, sperms derived from ES cells would be genetically unrelated to the patient. Moreover, limited sources of human ES cells, ethical challenges, and governmental concerns are the main barriers for their clinical applications [18].

Induced Pluripotent Stem Cells

Another source of pluripotent cells in addition to ESCs and SSCs is induced pluripotent stem cells (iPSCs). In comparison with ESCs, iPSCs are free of ethical concerns;

moreover, they have provided powerful means for personalized cell therapies.

Generally, producing male germ cells in vitro from pluripotent stem cells like ESCs, SSCs, and iPSCs can be done via two methods: embryoid body (EB) formation and the monolayer differentiation, in the presence of cytokine and growth factors [19].

Thus, patient-specific iPSCs can be established from NOA patients and recruited for producing male gametes through in vitro culture and transferring into testis tissue to restore spermatogenesis in idiopathic NOA without genetic abnormalities. But if NOA cases are caused by genetic disorders such as Klinefelter syndrome and Y chromosome microdeletion, this platform should be used to produce NOA-specific gametes for modeling and evaluating male infertility rather than for NOA cell therapy because iPSCs generated from NOA patients with genetic disorder showed compromised germ cell development potential [20].

Mesenchymal Stem Cells

Among stem cells with ability to differentiate to or induce proliferation of germ cells like SSCs, ESCs, and iPSCs, different sources of MSC have been used for this purpose. MSC therapy has the potential for direct application in vivo without limitations including immunogenicity, ethical concerns, and source scarcity (as in ESCs) or risk of forming teratomas and oncological and genetic instabilities (as in iPSCs and ESCs) or poor content in the source and isolation and culturing difficulties (as in SSCs) [21–23]. Different sources of MSCs especially MSCs from bone marrow, adipose tissue, and umbilical cord have been utilized for treatment of azoospermia in preclinical and clinical studies (Table 1) [24–30].

The potential of MSC therapy in treatment of male infertility can be exerted in the following mechanisms: (1) differentiation into the spermatozoa or merging with the endogenous SSCs to recover the spermatogenesis and (2) restoration of spermatogenesis via immunomodulatory and paracrine effect through secretion of growth factors and cytokines [31, 32].

As mentioned above, using MSCs for treatment of NOA through differentiation has been performed by different research groups. To date, many studies showed that transplanted bone marrow-derived MSCs (BM-MSCs) into the testis of busulfan-treated infertile animal models could differentiate into male germ cell and also Sertoli and Leydig cells [29, 30, 33, 34]. BM-MSCs are very similar to Sertoli cells and just like them are immune tolerant cells with the same embryonic origin. Transplanted BM-MSCs could reconstitute tubular microenvironment and provide the proliferation of inactivated germinal cells in the host tubules [35].

Although in an in vivo study, in an autoimmune infertility mice model, transplanting allogeneic BM-MSCs showed immunomodulatory effects on antibody production, but that was not a long-lasting immunomodulatory effect [56, 57].

Compared to cells from other sources, BM-MSCs have a high ability to proliferate and differentiate, and like all MSCs, have immunomodulating properties, but their differentiation ability and regenerative effects are greater than their anti-inflammatory and paracrine effects. Therefore, they are more suitable for regenerative purposes, but MSCs originating from fetal and perinatal tissues such as placenta-derived MSCs (PD-MSC), amniotic membrane (AM-MSCs), amniotic fluid (AF-MSCs), fetal membrane (FM-MSC), and umbilical cord blood-derived MSCs (UC-MSCs) have higher properties of immune modulation and are more suitable for the paracrine pathway, especially for the purpose of immune modulation [58, 59].

MSC therapy of NOA via immunomodulatory mechanism has been successful in different animal studies, [55], and is discussed in the next section.

Cell-Based Therapies of NOA by Paracrine and Anti-inflammatory Pathway

It has been shown that immunological factors and inflammatory processes may be responsible for testicular damage and male infertility in about 30% of asymptomatic infertile patients [60, 61]. Studies have shown that infiltration of immune cells has been observed in at least 20% of testicular biopsies of infertile patients with azoospermia, which means inflammatory infertility has a significant contribution to male infertility [62, 63].

Previous studies also showed the presence of immune cell infiltration and corresponding inflammatory conditions in testicular biopsies of all dogs with NOA including M1 pro-inflammatory phenotype macrophages and pro-inflammatory monocytes and cytokines [64].

In previous studies, biopsies from NOA-affected men showed inflammatory lesions (including lymphocytes and monocytes/macrophages) associated with impaired spermatogenesis, while specimen from patients with OA indicated intact spermatogenesis without inflammation [65, 66]. That was also indicated that inflammatory effects can result in damage to the testicles and epididymis and that the levels of inflammation mediators such as tumor necrosis factor (TNF) and Activin A were elevated in human testicular biopsies with impaired spermatogenesis [67]. NOA has been also observed in 10% of men with acute epididymitis [68]

As mentioned in previous section, MSCs have been shown to have immunomodulatory, anti-inflammatory, anti-apoptotic, and proliferative effects through secretion of cytokines and growth factors.

MSC-derived exosomes, as part of their paracrine factors, also have similar functions to MSCs but with the superior

Table 1 Preclinical and clinical studies using different sources of MSC for treatment of non-obstructive azoospermia

Preclinical studies				
MSC source	Non-obstructive azoospermia	Animal model	Transplant type	Reference
Adipose tissue	Busulfan-induced NOA	Hamster	Allotransplant	[28]
Adipose tissue	Busulfan-induced NOA	Rat	Allotransplant	[25, 29, 36]
Adipose tissue	Cisplatin-induced NOA	Rat	Allotransplant	[37]
Adipose tissue	Torsion-induced NOA	Rat	Xenotransplant (human)	[38]
Amnion	Busulfan-induced NOA	Mouse	Allotransplant	[39]
Bone marrow	Busulfan-induced NOA	Guinea pig	Allotransplant	[24]
Bone marrow	Busulfan-induced NOA	Hamster	Allotransplant	[26]
Bone marrow	Busulfan-induced NOA	Mouse	Allotransplant	[30, 40]
Bone marrow	Cisplatin-induced NOA	Mouse	Allotransplant	[41]
Bone marrow	Busulfan-induced NOA	Rat	Allotransplant	[27, 33, 42–46]
Bone marrow	Doxorubicin-induced NOA	Rat	Allotransplant	[47]
Bone marrow	Nitrate-induced NOA	Rat	Allotransplant	[48]
Bone marrow	Torsion-induced NOA	Rat	Allotransplant	[49]
Bone marrow	Busulfan-induced NOA	Mouse	Xenotransplant (Goat)	[50]
Umbilical cord	Busulfan-induced NOA	Mouse	Xenotransplant (Human)	[51–53]
Bone marrow	cadmium-induced NOA	Rat	Allotransplant	[54]
Bone marrow	food ad libitum-induced NOA	Mouse	Allotransplant	[55]
Clinical trials				
MSC source	Year/status	Location	Transplant type	References
Bone marrow	2015/completed	Egypt	Autotransplant	NCT02414295
Bone marrow	2014/1 and 2	Egypt	Autotransplant	NCT02025270
Bone marrow	2013/recruiting	Egypt	Autotransplant	NCT02008799
Bone marrow	2014/1 and 2	Egypt	Autotransplant	NCT02041910
Bone marrow	2015/1 and 2	Jordan	Autotransplant	NCT02641769
Adipose tissue	2018/recruiting	Russia	Autotransplant	NCT03762967
Adipose tissue	2019/recruiting	Iran	Autotransplant	IRCT20190519043634N1

properties that is mentioned in the relevant section. In addition to MSCs and their exosomes, stromal cells isolated from decidua, known as decidua stromal cells (DSCs), have similar properties to MSCs with more potent immunomodulatory and anti-inflammatory effects.

In the following sections, the potentials for NOA cell therapy using MSCs, MSC-derived exosomes, and DSCs through their immunomodulatory and anti-inflammatory effects are described (Fig. 1b).

Mesenchymal Stem Cells

Among different source of MSCs including bone marrow, adipose tissue, umbilical cord, endometrium, dental pulp, and menstrual blood, some of them have superior characteristics to others, making them in higher priority for cell therapy. For instance, endometrium and placenta-derived MSCs and adipose tissue-derived MSCs (AT-MSCs) have superior properties compared to bone marrow (BM)-MSCs, including greater immunomodulatory effects, higher secretion of cytokines and growth factors, and higher proliferation rate. However, many studies that have used MSCs for azoospermia have not confirmed that MSCs differentiate into spermatozoa or only through paracrine effects can induce reconstitution of the testis and epididymis tubes and recovery of spermatogenesis [28, 29, 42, 43, 48, 52, 69].

On the other hand, other studies have shown that MSCs are able both to differentiate into germ cells, in vitro and in vivo, and to improve the testicular tissue via paracrine effects [38, 47, 51, 52, 54].

Some studies have also shown that BM-MSCs were not capable of differentiation into spermatozoa [70].

However, it is not yet clear whether transplanted stem cells differentiate into spermatocytes, but it can be concluded that if different sources of MSCs are not able to differentiate into sperm, they may improve testicular tissues and recover spermatogenesis through their paracrine secretions [55, 71].

In proportion to this purpose, using some MSCs sources such as placenta-derived MSCs due to their better immunomodulatory effects are more compatible for using in NOA

cell therapy through paracrine and immunomodulatory pathway. Different mechanisms of action by which MSCs can induce spermatogenesis in the inflammatory environment have been summarized in Fig. 2.

There are promising and valuable results from preclinical researches and clinical trials using placenta-derived MSC (PD-MSCs) for treatment of infertility-related disorders. Placenta-derived MSC as a non-surgical treatment in men with Peyronie's disease [72] and erectile dysfunction [73] were evaluated and resulted in outstanding results.

Therefore, it is better to utilize the appropriate MSC source purposefully, depending on the etiology of NOA and which pathway of treatment is to be used.

Preclinical studies showed that autologous MSCs could be transplanted into the testis and migrate and settle down in the seminiferous tubules of the basement membrane. Then, they can proliferate and differentiate into spermatogonia in some seminiferous tubules of the animal model. Also they could ameliorate testicular damage through paracrine effects such as anti-inflammatory, antioxidative, and anti-apoptotic factors [33, 41, 42].

Up to now, not many clinical trials have been recorded for cell therapy of NOA (Registered trials: NCT02414295, NCT02025270, NCT02008799, NCT02041910, NCT02641769, NCT03762967, RCT20190519043634N1), and none of the registered trials have been fully published to treat NOA yet.

Regarding cell-based therapies of NOA using regenerative and differentiation approach, the autologous source of MSCs is preferred so that the male germ cell produced by MSCs are genetically related. When there is a genetic disease in the parent, MSC therapy of NOA via regenerative and differentiation approach may be preferable using allogeneic source of MSC. In this regard, the only challenge is producing genetically unrelated male germ cells, which in many cases is not accepted by couples. On the other hand, along with aging, differentiation potential, viability, and the reservoir of MSCs decrease. Therefore, in NOA cell-based therapies by the paracrine and anti-inflammatory pathway, other options such as exosomes and DSC (low differentiation potential compared to MSC) are suggested as suitable alternatives for this purpose [74].

MSC-Derived Exosomes

MSC-derived exosomes, as part of extracellular vesicles (EVs), have similar properties and functions to MSCs but have very low immunogenicity and tumorigenicity compared to MSCs, with no differentiation potential, and they are well tolerated, easier, and more practical to use in vivo. Conditioned media (CM) of MSC culture contains the EVs with the same properties of MSCs [75].

In Zhankina et al. study, the effect of EV-contained CM in comparison with MSCs was studied for the first time to treat non-obstructive azoospermia in the NOA mice models. The results showed successful recovery of spermatogenesis in all therapy groups with more favorable results in MSCs compared with the CM group [55].

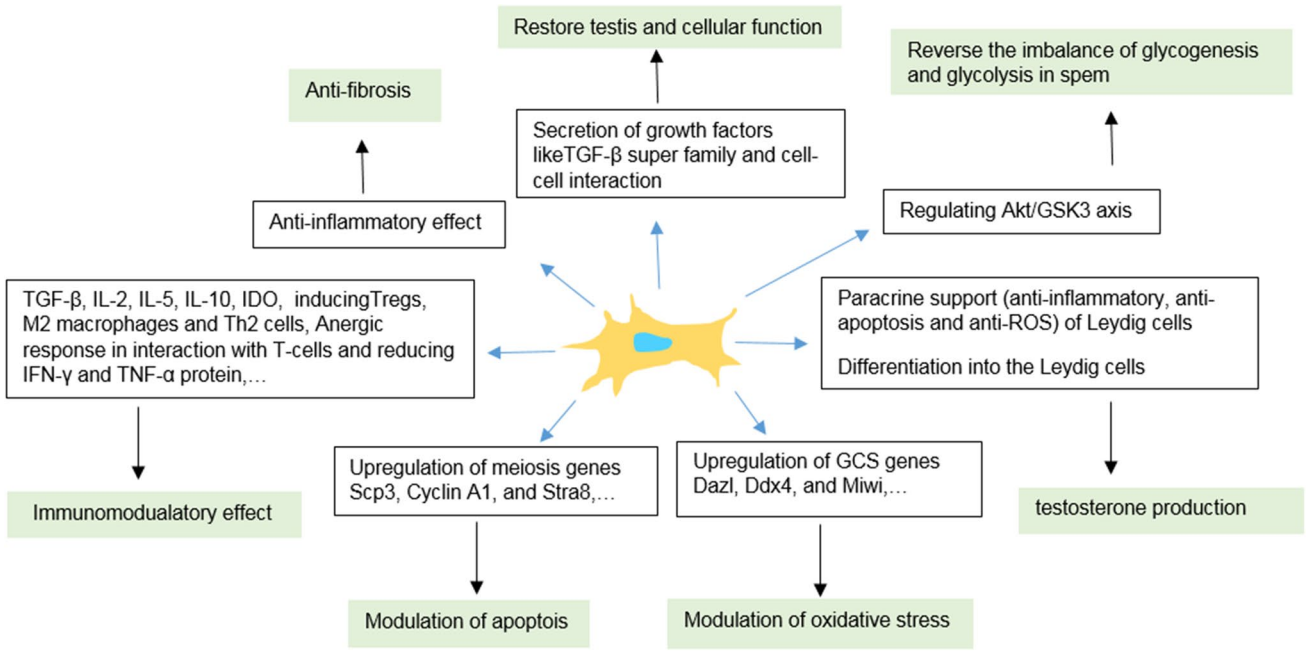

Fig. 2 Different mechanisms of action for MSCs in the inflammatory environment to retrieve spermatogenesis. IDO, indoleamine 2,3-dioxygenase; GCS, germ cell-specific

Other study showed that exosomes isolated from urine-derived stem cells could facilitate the recovery of spermatogenesis in busulfan-induced NOA mice [76].

Exosomes can exert their paracrine effects through carriage of lipids, proteins, miRNAs, and mRNAs into target cells [77, 78].

They have also proregenerative effects in damaged regions directly, just like stem cells [79]. In addition, they can regulate the function of target cell through regulation of target protein/gene expression [80].

As demonstrated in Guo et al. study, bone marrow MSC exosomes could restore spermatogenesis in NOA mice model through inhibiting the p38MAPK/ERK and AKT signaling pathways [81].

In other studies, exosomes derived from other cell types could improve spermatogenesis through above-mentioned mechanisms. In Mobarak et al. study, amniotic fluid-derived exosomes could ameliorate sperm quality and spermatogenesis in NOA rat models. Using exosomes resulted in significant increased OCT-3/4 + cells in NOA rats [82].

.Sertoli-derived exosomes could also improve spermatogenesis through the regulation of oxidative stress in NOA mice models.

Based on different preclinical studies, using paracrine MSCs-derived exosomes for NOA clinical studies seems to be promising [55, 76, 83].

Decidua Stromal Cells

During the start of preparation for pregnancy, significant changes in endometrium stromal cells occur following decidualization process. The transformed stromal cells are called decidual stromal cells that are specialized morphologically and functionally. DSCs play role in identification, selection, and acceptance of allogeneic embryos and the in development of immune tolerance and protection of semi-allogeneic fetus [84].

DSCs have similar properties to MSCs, but the ability of DSCs in preventing alloreactivity is significantly better than other sources of stromal cells, and they have stronger immunomodulatory effect in comprise to other sources of MSCs [85, 86].

The priorities of DSCs over other sources of MSCs include smaller size, higher proliferation rate, higher resistance to oxidative conditions, higher expression of homing markers in order to achieve inflammatory target areas, higher ability to suppress immunity, much lower differentiation potential (highly compatible for using in cell therapy of NOA through paracrine and anti-inflammatory pathway), no tumorigenesis report, more therapeutic effect, higher survival rate after freezing, easier access, and the need for cell-to-cell contact to induce immunomodulatory effects [87–91].

DSCs have been shown to have even higher proliferative capacity and greater immunomodulatory properties than stromal cells from neonatal tissues such as the amnion and chorion [86, 92].

Relying on the above characteristics, DSCs seem to be better therapeutic candidates for cell therapy of NOA through paracrine and immunomodulatory approach than regeneration pathway compared to MSC and other stromal cells.

Therefore, DSCs may have higher potential especially for treatment of inflammation-related NOA.

Heretofore, DSCs have been used in clinical trials to treat graft-versus-host disease (GVHD) and hemorrhagic cystitis [93–95] and *COVID-19*-induced acute respiratory distress syndrome (*ARDS*), and in preclinical settings to treat recurrent spontaneous abortion, and have yielded promising results in both settings [96].

It also seems that DSCs have a higher potential for fighting inflammation in inflammatory environments, as their location and activity is in such an environment with higher oxidative stress and inflammatory mediators. Therefore, stromal cells from placenta are more suitable candidates for the treatment of various inflammatory disorders [97].

To date, mesenchymal-like cells isolated from different parts of human placenta including amnion, chorion, and decidua have been used in preclinical and clinical studies to treat various diseases [98, 99]. Among these, placenta-derived MSCs (PD-MSCs) have been used for treatment of infertility-related diseases such as premature ovarian failure (POF) [100–103], testicular failure [104], and male sexual problems such as Peyronie's disease [72] and erectile dysfunction (ED) [73] and have promising results.

MSCs seem to be preferred for use in the regenerative pathway due to their superior differentiation properties over DSCs, but DSCs are more potent in their immunomodulatory properties and are better options for the treatment of idiopathic NOA associated with inflammation.

As mentioned in previous section, MSC-derived exosomes have very low immunogenicity and tumorigenicity compared to MSCs, with no differentiation potential and other superiorities. However, exosomes still have their own challenges like culture separation, cell phenotype, and quantification in clinical applications.

Thus, DSCs may be preferred candidates for cell-based therapies of NOA by paracrine and anti-inflammatory pathway, and using this strategy for the NOA treatment in clinical settings is strongly supported.

Scientists stand still at the beginning of the therapeutic path using DSCs for inflammatory disorders, and there is a need for more preliminary, preclinical, and clinical studies for this purpose. However, given the outstanding preclinical and clinical results following the use of DSCs for the treatment of GVHD, and hemorrhagic cystitis following HSCT, and ARDS caused by COVID-19, as well as the results from preclinical studies in animal models of male and female infertility, and on the other

hand, with the superiority of DSCs over MSCs, we can hope to have a higher potential of DSCs to treat infertility-related disorders that are associated with inflammation like idiopathic and inflammation-related NOA.

It is noteworthy that, for the sake of safety, the frequency of injections, the injected cell dose, and the post-injection anticoagulation therapy such as heparin infusion should be based on the previous clinical trials similar to those of GVHD and COVID-19 ARDS.

Prospects for Future Direction

Currently, the use of new diagnostic and therapeutic technologies like genomics, proteomics, and artificial intelligence, along with conventional therapeutic techniques of surgery and hormone therapy, has been promising in the treatment of NOA. However, in many cases, this severe form of male infertility requires other promising treatments. Cell-based therapies of NOA, depending on the etiology, which is structural defects or idiopathic (one of the main causes of which is related to inflammatory factors), can be potentially used through differentiation of stem cells (SSCs, ESCs, iPSCs, and MSCs) or immunomodulatory effects (MSCs, their exosomes, and DSCs), respectively.

Among these, based on the results of in vitro, animal model studies, and a clinical trial, MSCs are in the top priority of regenerative medicine for treatment of NOA due to their high differentiation capacity, high proliferative potential, and similarity to embryonic stem cells of the testes [105].

These fibroblast-like MSCs have also paracrine actions and are able to secrete growth factors and signaling molecules to restore spermatogenesis especially through anti-inflammatory pathway. However, according to in vitro data, preclinical experiences, and recent clinical trials for treatment of inflammation-related disorders such as GVHD and ARDS, DSCs have stronger immunomodulatory properties and some other priorities over MSCs such as higher proliferation rate, higher resistance to oxidative conditions, smaller size, higher expression of homing markers in order to achieve Inflammatory target areas, lower differentiation potential, higher survival rate after freezing, easier access, and safer and more reliable on the target site due to the need for cell-to-cell contact to induce immunomodulatory effects [87, 90, 106–108].

As mentioned, regarding MSC-derived exosomes, they have anti-inflammatory and paracrine effects just like the parents, but they still have their own limitations such as culture isolation, cell phenotype, and quantification in clinical applications [109].

After all, DSCs may be preferred candidates for the treatment of inflammation-related NOA, and using this approach in clinical trials for the treatment of NOA is strongly supported. Regarding to the safety issue, the injected DSCs doses, injection frequencies, and anticoagulant therapy after injection must be optimized based on previous clinical trials using DSCs in other inflammatory-related disorders like GVHD.

Conclusion

Current therapies for patients with non-obstructive azoospermia, if not treated with surgery and hormone therapy, are limited and need to retrieve normal and mature spermatozoa. By cell-based therapies of NOA using two approaches based on regenerative medicine and the paracrine mechanisms, male germ cell could be produced from different cell sources such as SSCs, iPSCs, ESCs, and MSCs in vitro/in vivo (regenerative medicine), or spermatogenesis could be recovered using paracrine effects of secreting stromal cells/stromal cell derivatives such as MSCs, MSC-derived exosomes, and DSCs. In the regenerative pathway, each source of cells that male germ cell differentiate from has their own challenges.

In the NOA cell-based therapies by regenerative pathway, choosing the right MSC source may not be easy since some sources are not capable to differentiate to sperm, but in the paracrine pathway, MSCs that have higher immunomodulatory effects, such as placenta-derived MSC and endometrium MSC, appear to be appropriate sources for this purpose. DSCs can be a preferred candidate for NOA cell-based therapies using paracrine and anti-inflammatory approach due to superior priorities of DSCs over other sources of MSCs and their higher immunomodulatory effects. However, there should be more comparing studies between the different cell sources for treatment of NOA in vitro and in vivo and clinical trials to translate preclinical results to the clinic. On the other hand, challenges regarding methods for cell isolation, culture, and complications of achieving an appropriate and safe cell source should be somewhat resolved in order to take firm and serious steps in cell therapy of NOA.

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Author Contribution All listed authors contributed significantly to the creation of this manuscript, each having fulfilled criteria as.

1. contributions to the conception of the work; data gathering and interpretation of data for the work
2. drafting the work or revising it critically for important intellectual content
3. final approval of the version to be published
4. agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

We confirm that the manuscript has been read and approved by all named authors.

We confirm that the order of authors listed in the manuscript has been approved by all named authors.

Declarations

Conflict of Interest The authors declare no competing interests.

References

1. Berookhim BM, Schlegel PN. Azoospermia due to spermatogenic failure. Urol Clin. 2014;41:97–113.
2. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol. 1989;142:62–5.
3. Alkandari MH, Zini A. Medical management of non-obstructive azoospermia: a systematic review. Arab J Urol. 2021;19:215–20.
4. Vij SC, Sabanegh E Jr, Agarwal A. Biological therapy for non-obstructive azoospermia. Expert Opin Biol Ther. 2018;18:19–23.
5. Bucay N, Yebra M, Cirulli V, Afrikanova I, Kaido T, Hayek A, et al. A novel approach for the derivation of putative primordial germ cells and sertoli cells from human embryonic stem cells. Stem cells. 2009;27:68–77.
6. Yang W, Mills JA, Sullivan S, Liu Y, French DL, Gadue P. iPSC reprogramming from human peripheral blood using Sendai virus mediated gene transfer. 2012; In: StemBook [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008–. PMID: 23785736.
7. Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathsack K, Drusenheimer N, et al. In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev Cell. 2006;11:125–32.
8. Lim J, Sung SY, Kim H, Song SH, Hong J, Yoon T, et al. Long-term proliferation and characterization of human spermatogonial stem cells obtained from obstructive and non-obstructive azoospermia under exogenous feeder-free culture conditions. Cell Prolif. 2010;43:405–17.
9. Marc Luetjens C, Stukenborg J-B, Nieschlag E, Simoni M, Wistuba J. Complete spermatogenesis in orthotopic but not in ectopic transplants of autologously grafted marmoset testicular tissue. Endocrinology. 2008;149:1736–47.
10. Goossens E, Van Saen D, Tournaye H. Spermatogonial stem cell preservation and transplantation: from research to clinic. Human Reproduction. 2013;28: 897–907.
11. Keros V, Rosenlund B, Hultenby K, Aghajanova L, Levkov L, Hovatta O. Optimizing cryopreservation of human testicular tissue: comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants. Hum Reprod. 2005;20:1676–87.
12. Gul M, Hildorf S, Dong L, Thorup J, Hoffmann ER, Jensen CFS, et al. Review of injection techniques for spermatogonial stem cell transplantation. Hum Reprod Update. 2020;26:368–91.
13. Hermann B, Sukhwani M, Winkler F, Pascarella J, Peters K, Sheng Y, et al. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. Cell Stem Cell. 2012;11:715–26.
14. Toyooka Y, Tsunekawa N, Akasu R, Noce T. Embryonic stem cells can form germ cells in vitro. Proc Natl Acad Sci. 2003;100:11457–62.
15. Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, Daley GQ. Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature. 2004;427:148–54.
16. West JA, Park I-H, Daley GQ, Geijsen N. In vitro generation of germ cells from murine embryonic stem cells. Nat Protoc. 2006;1:2026–36.
17. Kerkis A, Fonseca SA, Serafim RC, Lavagnolli TM, Abdelmassih S, Abdelmassih R, et al. In vitro differentiation of male mouse embryonic stem cells into both presumptive sperm cells and oocytes. Cloning Stem Cells. 2007;9:535–48.
18. Lin C-Y, Lee B-S, Liao C-C, Cheng W-J, Chang F-M, Chen M-H. Transdifferentiation of bone marrow stem cells into acinar cells using a double chamber system. J Formos Med Assoc. 2007;106:1–7.
19. Zhu Y, Hu H-L, Li P, Yang S, Zhang W, Ding H, et al. Generation of male germ cells from induced pluripotent stem cells (iPS cells): an in vitro and in vivo study. Asian journal of andrology. 2012;14:574.
20. Fang F, Li Z, Zhao Q, Ye Z, Gu X, Pan F, et al. Induced pluripotent stem cells derived from two idiopathic azoospermia patients display compromised differentiation potential for primordial germ cell fate. Frontiers Cell Dev Biol. 2020;8:432.
21. Danner S, Kajahn J, Geismann C, Klink E, Kruse C. Derivation of oocyte-like cells from a clonal pancreatic stem cell line. Mol Hum Reprod. 2007;13:11–20.
22. Wuputra K, Ku C-C, Wu D-C, Lin Y-C, Saito S, Yokoyama KK. Prevention of tumor risk associated with the reprogramming of human pluripotent stem cells. J Exp Clin Cancer Res. 2020;39:1–24.
23. Nayernia K, Lee JH, Drusenheimer N, Nolte J, Wulf G, Dressel R, et al. Derivation of male germ cells from bone marrow stem cells. Lab Invest. 2006;86:654–63.
24. Hajihoseini M, Vahdati A, Ebrahim Hosseini S, Mehrabani D, Tamadon A. Induction of spermatogenesis after stem cell therapy of azoospermic guinea pigs. Veterinarski arhiv. 2017;87:333–50.
25. Mehrabani D, Hassanshahi MA, Tamadon A, Zare S, Keshavarz S, Rahmanifar F, et al. Adipose tissue-derived mesenchymal stem cells repair germinal cells of seminiferous tubules of busulfan-induced azoospermic rats. J Human Reproductive Sci. 2015;8:103.
26. Tamadon A, Mehrabani D, Rahmanifar F, Jahromi AR, Panahi M, Zare S, et al. Induction of spermatogenesis by bone marrow-derived mesenchymal stem cells in busulfan-induced azoospermia in hamster. Int J Stem Cells. 2015;8:134–45.
27. Rahmanifar F, Tamadon A, Mehrabani D, Zare S, Abasi S, Keshavarz S, et al. Histomorphometric evaluation of treatment of rat azoosper-mic seminiferous tubules by allotransplantation of bone marrow-derived mesenchymal stem cells. Iran J Basic Med Sci. 2016;19:653.
28. Karimaghai N, Tamadon A, Rahmanifar F, Mehrabani D, Jahromi AR, Zare S, et al. Spermatogenesis after transplantation of adipose tissue-derived mesenchymal stem cells in busulfan-induced azoospermic hamster. Iran J Basic Med Sci. 2018;21:660.
29. Cakici C, Buyrukcu B, Duruksu G, Haliloglu AH, Aksoy A, Isik A, et al. Recovery of fertility in azoospermia rats after injection of adipose-tissue-derived mesenchymal stem cells: the sperm generation. Biomed Res Int. 2013;2013:1–18.
30. Lue Y, Erkkila K, Liu PY, Ma K, Wang C, Hikim AS, et al. Fate of bone marrow stem cells transplanted into the testis: potential implication for men with testicular failure. Am J Pathol. 2007;170:899–908.
31. Tamadon A, Zhan-Byrbekuly U, Kairgaliyev I, Khoradmehr A. Mesenchymal stem cell therapy of male infertility. Male Reproductive Health, IntechOpen. 2019;105–112.
32. Kinnaird T, Stabile E, Burnett M, Lee C, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94:678–85.

33. Monsefi M, Fereydouni B, Rohani L, Talaei T. Mesenchymal stem cells repair germinal cells of seminiferous tubules of sterile rats. Iran J Reprod Med. 2013;11:537–44.
34. Vahdati A, Fathi A, Hajihoseini M, Aliborzi G, Hosseini E. The regenerative effect of bone marrow-derived stem cells in spermatogenesis of infertile hamster. World journal of plastic surgery. 2017;6:18.
35. Mital P, Kaur G, Dufour JM. Immunoprotective sertoli cells: making allogeneic and xenogeneic transplantation feasible. Reproduction. 2010;139:495–504.
36. Luo Y, Xie L, Mohsin A, Ahmed W, Xu C, Peng Y, et al. Efficient generation of male germ-like cells derived during co-culturing of adipose-derived mesenchymal stem cells with Sertoli cells under retinoic acid and testosterone induction. Stem Cell Res Ther. 2019;10:1–18.
37. Meligy FY, Abo Elgheed AT, Alghareeb SM. Therapeutic effect of adipose-derived mesenchymal stem cells on Cisplatin induced testicular damage in adult male albino rat. Ultrastructural Pathol. 2019;43:28–55.
38. Hsiao CH, Ji AT, Chang CC, Cheng CJ, Lee LM, Ho JH. Local injection of mesenchymal stem cells protects testicular torsion-induced germ cell injury. Stem Cell Res Ther. 2015;6:113. https://doi.org/10.1186/s13287-015-0079-0.
39. Qian C, Meng Q, Lu J, Zhang L, Li H, Huang B. Human amnion mesenchymal stem cells restore spermatogenesis in mice with busulfan-induced testis toxicity by inhibiting apoptosis and oxidative stress. Stem Cell Res Ther. 2020;11:1–12.
40. Kadam P, Ntemou E, Baert Y, Van Laere S, Van Saen D, Goossens E. Co-transplantation of mesenchymal stem cells improves spermatogonial stem cell transplantation efficiency in mice. Stem Cell Res Ther. 2018;9:1–11.
41. Sherif IO, Sabry D, Abdel-Aziz A, Sarhan OM. The role of mesenchymal stem cells in chemotherapy-induced gonadotoxicity. Stem Cell Res Ther. 2018;9:196. https://doi.org/10.1186/s13287-018-0946-6.
42. Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad MB, Sayahpour F. Transplantation of autologous bone marrow mesenchymal stem cells into the testes of infertile male rats and new germ cell formation. Int J Stem Cells. 2016;9:250–63. https://doi.org/10.15283/ijsc16010.
43. Zhang D, Liu X, Peng J, He D, Lin T, Zhu J, et al. Potential spermatogenesis recovery with bone marrow mesenchymal stem cells in an azoospermic rat model. Int J Mol Sci. 2014;15:13151–65. https://doi.org/10.3390/ijms150813151.
44. Badawy AA, El-Magd MA, AlSadrah SA, Alruwaili MM. Altered expression of some miRNAs and their target genes following mesenchymal stem cell treatment in busulfan-induced azoospermic rats. Gene. 2020;737:144481.
45. Zahkook S, Atwa A, Shahat M, Mansour AM, Bakry S. Mesenchymal stem cells restore fertility in induced azoospermic rats following chemotherapy administration. J Reprod Infertil. 2014;5:50–7.
46. Aziz MTA, Mostafa T, Atta H, Asaad S, Fouad HH, Mohsen G, et al. In vitro and in vivo lineage conversion of bone marrow stem cells into germ cells in experimental azoospermia in rat. Stem Cell Studies. 2011;1:e15–e15.
47. Abdelaziz MH, Salah El-Din EY, El-Dakdoky MH, Ahmed TA. The impact of mesenchymal stem cells on doxorubicin-induced testicular toxicity and progeny outcome of male prepubertal rats. Birth Defects Res. 2019;111:906–19. https://doi.org/10.1002/bdr2.1535.
48. Hassan AI, Alam SS. Evaluation of mesenchymal stem cells in treatment of infertility in male rats. Stem Cell Res Ther. 2014;5:131. https://doi.org/10.1186/scrt521.
49. Sabbaghi MA, Bahrami AR, Feizzade B, Kalantar SM, Matin MM, Kalantari M, et al. Trial evaluation of bone marrow derived mesenchymal stem cells (MSCs) transplantation in revival of spermatogenesis in testicular torsion. Middle East Fertility Society Journal. 2012;17:243–9.
50. Fang W, Chao L, Zhang S-S, Liu W-S, Hua J-L. Transplantation of goat bone marrow mesenchymal stem cells (gMSCs) help restore spermatogenesis in endogenous germ cells-depleted mouse models. J Integrative Agric. 2013;12:483–94.
51. Abdallah SH, Pasha HF, Abdelrahman AA, Mazen NF. Molecular effect of human umbilical cord blood CD34-positive and CD34-negative stem cells and their conjugate in azoospermic mice. Mol Cell Biochem. 2017;428:179–91. https://doi.org/10.1007/s11010-016-2928-2.
52. Chen H, Tang QL, Wu XY, Xie LC, Lin LM, Ho GY, et al. Differentiation of human umbilical cord mesenchymal stem cells into germ-like cells in mouse seminiferous tubules. Mol Med Rep. 2015;12:819–28. https://doi.org/10.3892/mmr.2015.3528.
53. Yang R-F, Liu T-H, Zhao K, Xiong C-L. Enhancement of mouse germ cell-associated genes expression by injection of human umbilical cord mesenchymal stem cells into the testis of chemical-induced azoospermic mice. Asian J Androl. 2014;16:698.
54. Wang Y-J, Yan J, Zou X-L, Guo K-J, Zhao Y, Meng C-Y, et al. Bone marrow mesenchymal stem cells repair cadmium-induced rat testis injury by inhibiting mitochondrial apoptosis. Chem Biol Interact. 2017;271:39–47. https://doi.org/10.1016/j.cbi.2017.04.024.
55. Zhankina R, Afshar A, Farrar Z, Khoradmehr A, Baghban M, Suleiman M et al. Restoration of spermatogenesis in azoospermic mice by bone marrow mesenchymal stromal/stem cells conditioned medium. 2022; https://doi.org/10.21203/rs.3.rs-169243/v2.
56. Aghamir SMK, Salavati A, Yousefie R, Tootian Z, Ghazaleh N, Jamali M, et al. Does bone marrow–derived mesenchymal stem cell transfusion prevent antisperm antibody production after traumatic testis rupture? Urology. 2014;84:82–6.
57. Yu Y, Valderrama AV, Han Z, Uzan G, Naserian S, Oberlin E. Human fetal liver MSCs are more effective than adult bone marrow MSCs for their immunosuppressive, immunomodulatory, and Foxp3+ T reg induction capacity. Stem Cell Res Ther. 2021;12:1–18.
58. ArefNezhad R, Motedayyen H, Mohammadi A. Therapeutic aspects of mesenchymal stem cell-based cell therapy with a focus on human amniotic epithelial cells in multiple sclerosis: a mechanistic review. Int J Stem Cells. 2021;14:241–51.
59. Lee JM, Jung J, Lee H-J, Jeong SJ, Cho KJ, Hwang S-G, et al. Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. Int Immunopharmacol. 2012;13:219–24.
60. Fijak M, Pilatz A, Hedger MP, Nicolas N, Bhushan S, Michel V, et al. Infectious, inflammatory and 'autoimmune' male factor infertility: how do rodent models inform clinical practice? Hum Reprod Update. 2018;24:416–41.
61. Kauerhof AC, Nicolas N, Bhushan S, Wahle E, Loveland KA, Fietz D, et al. Investigation of activin A in inflammatory responses of the testis and its role in the development of testicular fibrosis. Hum Reprod. 2019;34:1536–50. https://doi.org/10.1093/humrep/dez109.
62. Schuppe HC, Meinhardt A, Allam J, Bergmann M, Weidner W, Haidl G. Chronic orchitis: a neglected cause of male infertility? Andrologia. 2008;40:84–91.
63. Hasan H, Bhushan S, Fijak M, Meinhardt A. Mechanism of inflammatory associated impairment of sperm function, spermatogenesis and steroidogenesis. Front Endocrinol. 2022;13:897029.
64. Pröbstl C, Umbach A, Beineke A, Körber H, Goericke-Pesch S. Immune cell characterization in spontaneous autoimmune orchitis in dogs. Theriogenology. 2022;187:219–26. https://doi.org/10.1016/j.theriogenology.2022.05.010.

65. Bergmann M, Kliesch S. Testicular Biopsy and Histology. In Nieschlag E, Behre HM, Nieschlag S (eds) Andrology 2010:155–167. Springer, Berlin.
66. Hauptman D, Perić MH, Marić T, Bojanac AK, Sinčić N, Zimak Z et al. Leydig cells in patients with non-obstructive azoospermia: do they really proliferate? Life (Basel). 2021;11. https://doi.org/10.3390/life11111266
67. Nicolas N, Michel V, Bhushan S, Wahle E, Hayward S, Ludlow H, et al. Testicular activin and follistatin levels are elevated during the course of experimental autoimmune epididymo–orchitis in mice. Sci Rep. 2017;7:42391. https://doi.org/10.1038/srep42391.
68. Rusz A, Pilatz A, Wagenlehner F, Linn T, Diemer T, Schuppe H, et al. Influence of urogenital infections and inflammation on semen quality and male fertility. World J Urol. 2012;30:23–30.
69. Cassim MI, Tasneem M. A novel therapy for the treatment of malefactor infertility due to non-obstructive azoospermia: a case report. Crescent Journal of Medical and Biological Sciences. 2019;6:129–131. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=775944
70. Van Saen D, Goossens E, De Block G, Tournaye H. Bone marrow stem cells transplanted to the testis of sterile mice do not differentiate into spermatogonial stem cells and have no protective effect on fertility. Fertil Steril. 2009;91:1549–52. https://doi.org/10.1016/j.fertnstert.2008.09.036.
71. Bader R, Ibrahim J-N, Mourad A, Moussa M, Azoury J, Azoury J, et al. Improvement of human sperm vacuolization and DNA fragmentation co-cultured with adipose-derived mesenchymal stem cell secretome: in vitro effect. Int J Stem Cells. 2019;12:388–99. https://doi.org/10.15283/ijsc19047.
72. Levy JA, Marchand M, Iorio L, Zribi G, Zahalsky MP. Effects of stem cell treatment in human patients with Peyronie disease. J Am Osteopath Assoc. 2015;115:e8-13. https://doi.org/10.7556/jaoa.2015.124.
73. Levy JA, Marchand M, Iorio L, Cassini W, Zahalsky MP. Determining the feasibility of managing erectile dysfunction in humans with placental-derived stem cells. J Am Osteopath Assoc. 2016;116:e1-5. https://doi.org/10.7556/jaoa.2016.007.
74. Ganguly P, El-Jawhari JJ, Giannoudis PV, Burska AN, Ponchel F, Jones EA. Age-related changes in bone marrow mesenchymal stromal cells: a potential impact on osteoporosis and osteoarthritis development. Cell Transplant. 2017;26:1520–9. https://doi.org/10.1177/0963689717721201.
75. Park KS, Bandeira E, Shelke GV, Lässer C, Lötvall J. Enhancement of therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Stem Cell Res Ther. 2019;10:288. https://doi.org/10.1186/s13287-019-1398-3.
76. Deng C, Xie Y, Zhang C, Ouyang B, Chen H, Lv L, et al. Urine-derived stem cells facilitate endogenous spermatogenesis restoration of busulfan-induced nonobstructive azoospermic mice by paracrine exosomes. Stem Cells Dev. 2019;28:1322–33. https://doi.org/10.1089/scd.2019.0026.
77. Ibrahim A, Marbán E. Exosomes: fundamental biology and roles in cardiovascular physiology. Annu Rev Physiol. 2016;78:67–83. https://doi.org/10.1146/annurev-physiol-021115-104929.
78. Jiang N, Xiang L, He L, Yang G, Zheng J, Wang C, et al. Exosomes mediate epithelium-mesenchyme crosstalk in organ development. ACS Nano. 2017;11:7736–46. https://doi.org/10.1021/acsnano.7b01087.
79. Basu J, Ludlow JW. Exosomes for repair, regeneration and rejuvenation. Expert Opin Biol Ther. 2016;16:489–506. https://doi.org/10.1517/14712598.2016.1131976.
80. Auyeung CL, Co NN, Tsuruga T, Yeung TL, Kwan SY, Leung CS, et al. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nat Commun. 2016;7:11150. https://doi.org/10.1038/ncomms11150.
81. Guo XB, Zhai JW, Xia H, Yang JK, Zhou JH, Guo WB, et al. Protective effect of bone marrow mesenchymal stem cell-derived exosomes against the reproductive toxicity of cyclophosphamide is associated with the p38MAPK/ERK and AKT signaling pathways. Asian J Androl. 2021;23:386–91. https://doi.org/10.4103/aja.aja_98_20.
82. Mobarak H, Heidarpour M, Rahbarghazi R, Nouri M, Mahdipour M. Amniotic fluid-derived exosomes improved spermatogenesis in a rat model of azoospermia. Life Sci. 2021;274:119336. https://doi.org/10.1016/j.lfs.2021.119336.
83. Zhankina R, Baghban N, Askarov M, Saipiyeva D, Ibragimov A, Kadirova B, et al. Mesenchymal stromal/stem cells and their exosomes for restoration of spermatogenesis in non-obstructive azoospermia: a systemic review. Stem Cell Res Ther. 2021;12:229. https://doi.org/10.1186/s13287-021-02295-9.
84. Okada H, Tsuzuki T, Murata H. Decidualization of the human endometrium. Reprod Med Biol. 2018;17:220–7. https://doi.org/10.1002/rmb2.12088.
85. Karlsson H, Erkers T, Nava S, Ruhm S, Westgren M, Ringdén O. Stromal cells from term fetal membrane are highly suppressive in allogeneic settings in vitro. Clin Exp Immunol. 2012;167:543–55. https://doi.org/10.1111/j.1365-2249.2011.04540.x.
86. Ringden O, Erkers T, Nava S, Uzunel M, Iwarsson E, Conrad R, et al. Fetal membrane cells for treatment of steroid-refractory acute graft-versus-host disease. Stem cells. 2013;31:592–601.
87. Abumaree MH, Abomaray F, Alshehri N, Almutairi A, AlAskar A, Kalionis B, et al. Phenotypic and functional characterization of mesenchymal stem/multipotent stromal cells from decidua parietalis of human term placenta. Reprod Sci. 2016;23:1193–207.
88. Abomaray FM, Aljumah MA, Alsaad KO, Jawdat D, Alkhaldi A, Alaskar AS, et al. Phenotypic and functional characterization of mesenchymal stem/multipotent stromal cells from decidua basalis of human term placenta. Stem Cells Int. 2016;2016:5184601. https://doi.org/10.1155/2016/5184601.
89. Intanker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells. 2004;22:1338–45. https://doi.org/10.1634/stemcells.2004-0058.
90. Kazemi S, Parivar K, Roudbari NH, Yaghmaei P, Sadeghi B. Growth kinetic comparison of human mesenchymal stem cells from bone marrow, adipose tissue and decidua. Med Sci. 2020;24:223–34.
91. Semenov OV, Koestenbauer S, Riegel M, Zech N, Zimmermann R, Zisch AH, et al. Multipotent mesenchymal stem cells from human placenta: critical parameters for isolation and maintenance of stemness after isolation. Am J Obstet Gynecol. 2010;202:193.e191-193.e113. https://doi.org/10.1016/j.ajog.2009.10.869.
92. Erkers T, Nava S, Yosef J, Ringdén O, Kaipe H. Decidual stromal cells promote regulatory T cells and suppress alloreactivity in a cell contact-dependent manner. Stem Cells Dev. 2013;22:2596–605.
93. Erkers T, Kaipe H, Nava S, Molldén P, Gustafsson B, Axelsson R, et al. Treatment of severe chronic graft-versus-host disease with decidual stromal cells and tracing with 111Indium radiolabeling. Stem cells Dev. 2015;24:253–63.
94. Ringden O, Baygan A, Remberger M, Gustafsson B, Winiarski J, Khoein B, et al. Placenta-derived decidua stromal cells for treatment of severe acute graft-versus-host disease. Stem Cells Transl Med. 2018;7:325–31.

95. Baygan A, Aronsson-Kurttila W, Moretti G, Tibert B, Dahllöf G, Klingspor L, et al. Safety and side effects of using placenta-derived decidual stromal cells for graft-versus-host disease and hemorrhagic cystitis. Front Immunol. 2017;8:795.
96. Ringdén O, Solders M, Erkers T, Nava S, Molldén P, Hultcrantz M, et al. Successful reversal of acute lung injury using placenta-derived decidual stromal cells. J Stem Cell Res Ther. 2014;4:1–5.
97. Alshareef GH, Mohammed AE, Abumaree M, Basmaeil YS. Phenotypic and functional responses of human decidua basalis mesenchymal stem/stromal cells to lipopolysaccharide of gram-negative bacteria. Stem Cells Cloning: Adv App. 2021;14:51.
98. Lublin FD, Bowen JD, Huddlestone J, Kremenchutzky M, Carpenter A, Corboy JR, et al. Human placenta-derived cells (PDA-001) for the treatment of adults with multiple sclerosis: a randomized, placebo-controlled, multiple-dose study. Multiple Sclerosis Related Disorders. 2014;3:696–704.
99. Gustafsson B, Frisk P, Szakos A, Sadeghi B, Ringdén O, Frost BM. Successful treatment with placenta-derived decidual stromal cells in a pediatric patient with life-threatening acute gastrointestinal graft-versus-host disease. Pediatr Transplant. 2017;21:e12990.
100. Kim T-H, Choi JH, Jun Y, Lim SM, Park S, Paek J-Y, et al. 3D-cultured human placenta-derived mesenchymal stem cell spheroids enhance ovary function by inducing folliculogenesis. Sci Rep. 2018;8:1–11.
101. Yin N, Zhao W, Luo Q, Yuan W, Luan X, Zhang H. Restoring ovarian function with human placenta-derived mesenchymal stem cells in autoimmune-induced premature ovarian failure mice mediated by Treg cells and associated cytokines. Reprod Sci. 2018;25:1073–82.
102. Choi JH, Seok J, Lim SM, Kim TH, Kim GJ. Microenvironmental changes induced by placenta-derived mesenchymal stem cells restore ovarian function in ovariectomized rats via activation of the PI3K-FOXO3 pathway. Stem Cell Res Ther. 2020;11:1–13.
103. Seok J, Park H, Choi JH, Lim J-Y, Kim KG, Kim GJ. Placenta-derived mesenchymal stem cells restore the ovary function in an ovariectomized rat model via an antioxidant effect. Antioxidants. 2020;9:591.
104. Lu J, Liu Z, Shu M, Zhang L, Xia W, Tang L, et al. Human placental mesenchymal stem cells ameliorate chemotherapy-induced damage in the testis by reducing apoptosis/oxidative stress and promoting autophagy. Stem Cell Res Ther. 2021;12:1–10.
105. Zhankina R, Baghban N, Askarov M, Saipiyeva D, Ibragimov A, Kadirova B, et al. Mesenchymal stromal/stem cells and their exosomes for restoration of spermatogenesis in non-obstructive azoospermia: a systemic review. Stem Cell Res Ther. 2021;12:1–12.
106. Abomaray F, Aljumah M, Alsaad K, Jawdat D, Alkhaldi A, Alaskar AS, et al. Phenotypic and functional characterization of mesenchymal stem/multipotent stromal cells from decidua basalis of human term placenta. Stem Cells Int. 2016;2016:5184601.
107. Intanker PS, Scherjon SA, Kleijburg-Van Der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem cells. 2004;22:1338–45.
108. Semenov OV, Koestenbauer S, Riegel M, Zech N, Zimmermann R, Zisch AH, et al. Multipotent mesenchymal stem cells from human placenta: critical parameters for isolation and maintenance of stemness after isolation. Am J Obstet Gynecol. 2010;202:19. e391-193.e113.
109. Hettich BF, Ben-Yehuda Greenwald M, Werner S, Leroux JC. Exosomes for wound healing: purification optimization and identification of bioactive components. Advanced Science. 2020;7:2002596.