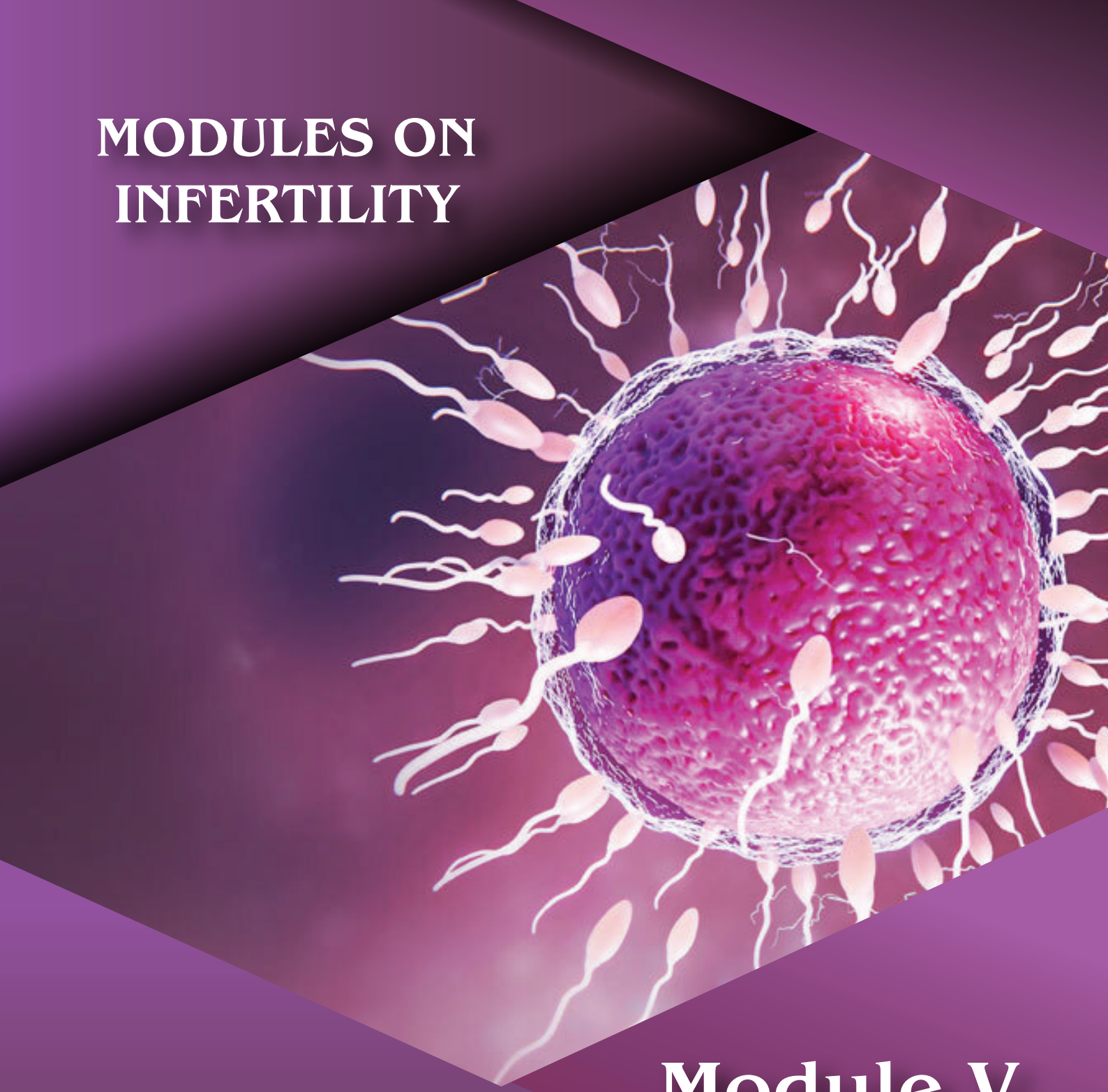
MODULES ON INFERTILITY



Module V

Emerging Technologies and Innovations in Infertility Treatment

CONTENT

Introduction

Infertility, defined as an inability to have children after trying for at least 6 to 12 months, is a global health issue affecting 10% to 15% of couples of reproductive age (1). Recent evidence shows that infertility rates have dramatically risen over the past few decades as more couples are waiting longer to have children. Infertility is a multifactorial condition with contributions from many genetic, environmental, nutritional, and hormonal factors that impair the development and function of reproductive organs. Although advances in the field of assisted reproductive technology (ART) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have improved the chances of infertile couples having children, it has remained an unsuccessful method for some forms of infertility. The success rates of ART may depend on many factors such as the age of the patient, particularly female age, infertility duration, type of infertility, and treatment method. There are risks associated with fertility treatments, including the possible impact of treatment factors, independent of infertility factors, on children. Some studies indicate that children born from ART may have a slightly higher risk of genetic or epigenetic abnormalities. However, reassuringly, the overall risk of birth defects in children born after IVF and ICSI has been reported in studies of large cohorts as very low. A recent retrospective cohort study indicated that increased risk of birth defects could be due to the underlying infertility as opposed to ART technology. Further systematic reviews and stratified meta-analyses are required to elucidate the relative contributions of ART and infertility to risk of birth defects. Unfortunately, IVF technology may not be effective for patients with congenital disorders in the sex organs, particularly those with disorders of sex development, as they are frequently incapable of making the eggs or sperm required for fertilization. Young cancer survivors whose treatment results in destruction of gonadal tissue are similarly faced with premature ovarian or testicular insufficiency without sufficient gametes for having their own children, either spontaneously, or with IVF. Prechemotherapy harvesting of eggs and sperm can be performed for those in the reproductive age, providing a finite number of oocytes in the case of young women and girls, whereas gonadal tissue cryopreservation is the only option for prepubertal children, and this remains experimental in terms of usability for boys, with offspring reported in primate but not human studies to date. In the situation of permanent premature gonadal failure, with no usable gametes, egg or sperm donation pro¬vides the only opportunity for having one's own family.

Stem cells are being explored as a next-generation approach for infertility treatment in different forms of reproductive disorders. Stem cells are undifferentiated cell types that can differentiate into almost all cell types of the mature organism. After identifying their self-renewal characteristic and high capability to produce more specialized cell types, a growing number of studies has been focused on the use of cell-based therapies for infertility treatment in men and women. Moreover, the in vitro generation of human eggs and sperm from stem cells, particularly induced pluripotent stem cells (iPSCs), has provided new hope for infertile couples who are unable to produce gametes, as well as healthy individuals at high risk of transmitting serious diseases to their children.

Development of bioengineering models such as microfluidic systems and 3-dimensional (3D) cultures have allowed researchers to support efficient differentiation of stem cells to reproductive organoids by making appropriate micro-environments, enabling their use for disease modeling and drug screening. Therefore, stem cells can be considered an exciting and promising option for treatment of infertility in the future. In the following sections, we discuss the diversity of stem cells, mechanisms of their action in regenerative processes, their potential and availability for infertility treatment, and the importance of bioengineering models including iPSCs and microfluidics in the context of human reproduction.

Stem Cell Derivation and Types

There are multiple classes of stem cells according to their origin or type (Fig. 1). Embryonic (ESCs) and adult stem cells (ASCs) are the main classes of nonmanipulated stem cells. There is also another classification for stem cells, including totipotent, pluripotent, multipotent, oligopotent, and unipotent, based on their developmental potential (Fig. 2). Totipotent stem cells (eg, zygotes) can differentiate into any cell type, whereas unipotent stem cells (eg, epidermal stem cells) differentiate into only a single cell type. Multipotent (eg, mesenchymal stem cells) and oligopotent stem cells (eg, lymphoid progenitor stem cells) can differentiate into a limited number of specific cells. Pluripotent stem cells (eg, ESCs, iPSCs) have high capacity to produce all cells origin¬ated from any of the 3 germ layers (endoderm, ectoderm, and mesoderm).

Mesenchymal stem cells (MSCs), ESCs, spermatogonial stem cells (SSCs) and iPSCs are among the most studied stem cells in the context of reproductive biomedicine. However, the use of ESCs and SSCs for fertility has some limitations. SSCs are the only germ cells that can self-renew and generate spermatozoa, but their abundance in testes is very low, limiting the feasibility of their isolation, characterization, and culturing. The isolation of SSCs requires an invasive procedure such as needle biopsies, which may incur some risk. ESCs are pluripotent stem cells with unlimited capacity for self-renewal and the potential to differentiate into every cell type; however, ethical issues and tumorigenicity are major concerns regarding the use of these cells in clinical therapy. In contrast, MSCs circumvent most of these limitations for clinical use. They is a group of ASCs with multipotent and immunomodulatory properties, present in most tissues, including the adipose tissue, bone marrow, endometrium, placenta, umbilical cord, and menstrual blood, as well as amniotic fluid, hair follicle, dental pulp, breast milk, and the nose. A significant number of studies has demonstrated that MSCs have the potential to proliferate and differentiate into various cell types such as lung epithelial cells, osteoblasts, chondroblasts, cardiomyocytes, adipocytes, and neuron-like cells. For these reasons, MSCs are considered a potentially useful not only for cell-based therapy of infertility, but also for organoid technology. Induced pluripotent stem cells are an additional of stem cells that are genetically manipulated from somatic cells. Despite major concerns for iPSC-based therapy because of the risk of tumorigenicity, the direct differentiation of these cells to gametes in a Petri dish has become a focus for scientists to help infertile couples have their own biological children. Furthermore, iPSC generation from patients' somatic cells and subsequent differentiation to relevant cells is very important for disease modeling and drug-screening studies. Therefore, according to their origin and characteristics, it is hypothesized that stem cells may help infertile couples through 2 main pathways: (1) regeneration of reproductive organs via cell-based therapy (MSC therapy) and (2) production of reproductive organoids or "gonads in a dish" for therapeutic approaches and diseases modeling. However, more in-depth studies are necessary to consider whether stem cell-derived gametes might carry even higher risks of genetic or epigenetic abnormalities to children.

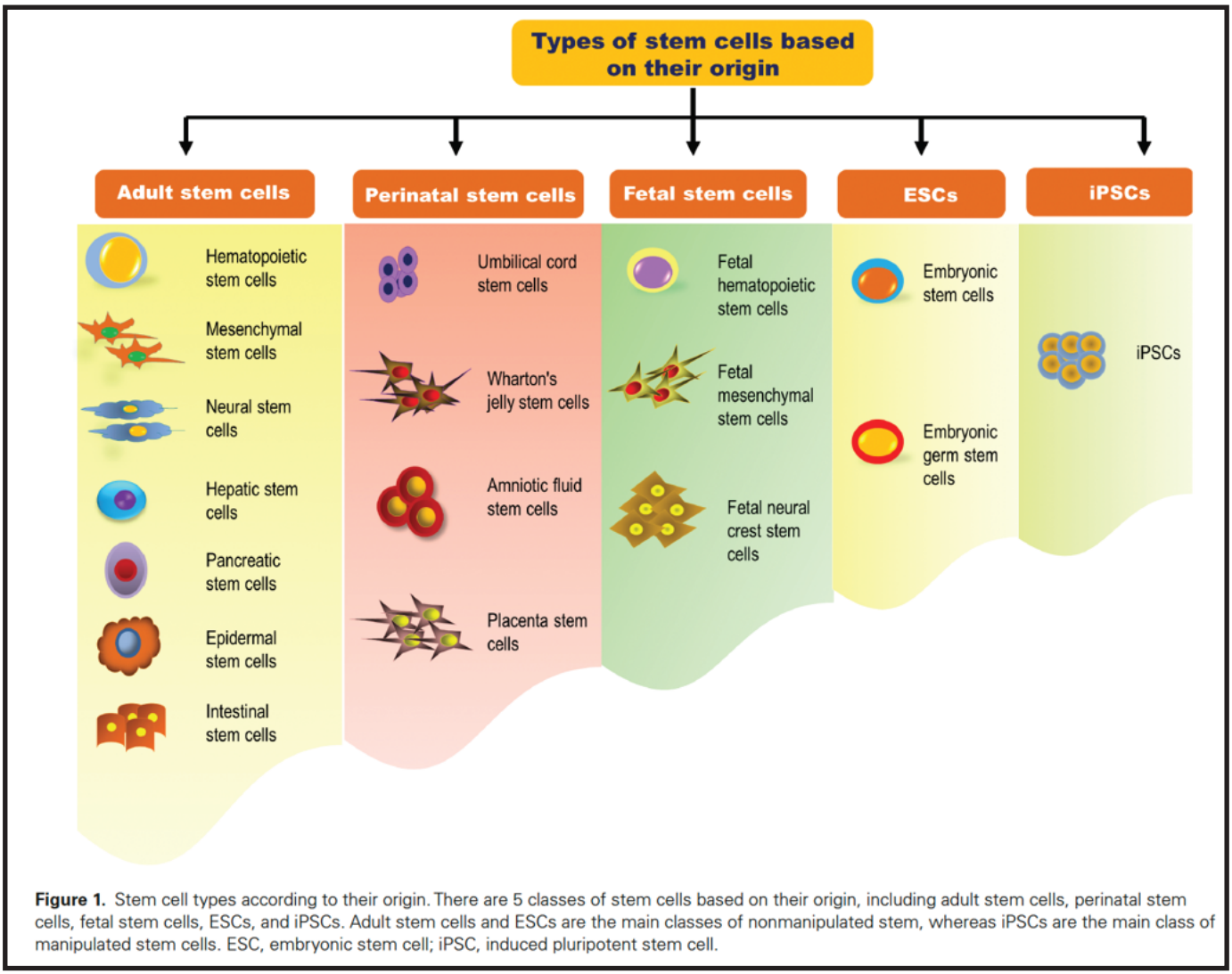


Figure 1. Stem cell types according to their origin. There are 5 classes of stem cells based on their origin, including adult stem cells, perinatal stem cells, fetal stem cells, ESCs, and iPSCs. Adult stem cells and ESCs are the main classes of nonmanipulated stem, whereas iPSCs are the main class of manipulated stem cells. ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

Recently, an additional novel population of pluripotent stem cells, termed very small embryonic-like stem cells (VSELs), was identified in all adult tissues. VSELs exist in adult testis, ovary, and uterus and express receptors for pituitary-derived sex hormones such as LH and FSH. They circulate in a very small number into the peripheral blood under steady-state conditions. VSELs express primordial germ cell (PGC)-specific markers and have a PGC-like epigenetic profile, which support the concept that there is a developmental link between VSELs and PGCs. These stem cells de¬posit in developing organs during embryogenesis and survive throughout life serving as a backup pool for tissue-committed stem cells. VSELs have a self-renewing ability and can be differentiated into all 3 germ layers. In conducive culture media, VSELs collected from mouse bone marrow (BM) can differentiate into all 3 germ layers and hematopoietic cells in vitro. When VSELs are cultured with Sertoli cells in con¬ditioned media supplemented with FSH, they can differentiate into germ cells. Recent evidence has indicated that VSELs may not only give rise to the SSCs or oogonial stem cells (OSCs) that further differentiate and undergo meiosis to form haploid gametes in vivo, but also may be able to produce gametes in vitro and lay the foundation for future cell-based regenerative therapies for infertile patients. However, their scarce nature, small size, and inability to expand in culture limits the current use of VSELs for reproductive research.

Regenerative Mechanisms of Stem Cells

Tissue repair is one of the most important properties of stem cells, allowing the regeneration of injured organs. MSCs are the best-studied stem cells for regeneration because they are an excellent source of the growth factors and cytokines necessary for tissue restoration. MSCs contribute to regeneration through multiple mechanisms (Fig. 3). Their homing and engraftment from peripheral circulation into the injury site (migratory capacity) is the first stage of the regeneration process. MSCs ability to pass through vessel walls to reach injured tissues depends on a variety of chemical factors and chemokines such as stromal derived factor-1, C-X-C motif chemokine receptor 4 (CXCR4), C-C chemokine receptor (CCR) type 1, CCR2, CCR4, CCR7, CCR10, osteopontin, and growth factors. The expression of these factors has been shown to increase in response to injury and inflammation. Structural changes in the extracellular matrix (ECM) that are associated with cellular damage can be considered a mechanical signal for MSC homing. Therefore, any pathological condition and cellular damage may release chemical or mechanical signals that recruit MSCs to the injury sites. When MSCs reach the target area, they induce angiogenesis and regeneration (Fig. 3). Angiogenesis, differentiation potential, immunomodulatory, antiapoptotic, and antifibrotic properties are the major mechanisms of MSC action in regeneration processes.

Mesenchymal Stem Cells in Reproduction

Mesenchymal stem cells, also known as mesenchymal stromal cells, have demonstrated potential for infertility treatment. There is a subclass of multipotent stem cells with self-renewal abilities that originate from the mesoderm. They have high capacity to differentiate into not only mesoderm lineages, but also ectodermal and endodermal cells. For this reason, MSCs are a promising candidate for regeneration or cell-based therapy. However, ethical concerns and the current scarcity of human data prevent the widespread use of MSC transplantation as a routine therapeutic method. The risk of tumorigenesis after stem cell transplantation, their short-lived viability postinjection, and production of allo-antibodies caused by repeated administration of MSCs may be the other restrictions related to the use of MSCs for regenerative ther¬apies. Furthermore, it is still unclear which MSC source has the best therapeutic potential for infertility treatment because of a lack of comparative clinical studies.

Generally, the regenerative properties of MSCs after transplantation may be attributed to cell–cell interaction, secretion of various angiogenic paracrine factors (eg, vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF]2) to promote tissue repair, function, and survival of the resident cells; regulation of local microenvironment or niche; modulation of inflammatory and immune responses; and activation of tissue-specific progenitor cells. Mesenchymal stem cells originating from the BM (BM-MSCs), adipose (Ad-MSCs), menstrual blood (MB-MSCs), umbilical cord (UC-MSCs), and human amniotic (hA-MSCs) tissues are among the most common form of stem cells that have been considered for infertility treatment in men and women. Bone marrow is an invaluable source of MSCs and other stem cell types such as hematopoietic stem cells and endothelial progenitor cells. BM-MSCs represent a heterogeneous group of cells that can differentiate into not only osteoblasts, chondroblasts, chondrocytes, tendon, adipocytes, cardiomyocytes, skeletal muscle cells, endothelial cells, and fibroblasts, but also into male and female reproductive tissue cells. For this reason, BM-MSCs are now considered a potential candidate for treatment of infertility in patients with reproductive dysfunction.

Adipose tissue is a rich source of MSCs that can be used for cell-based therapies. Although Ad-MSCs exhibit the same typical characteristics as BM-MSCs, their harvesting is cheaper and requires a minimally invasive procedure (liposuction) in comparison with BM-MSCs that require isolation via aspiration. Thus, compared with BM-MSCs, autologous Ad-MSCs represent a more practical option in cell-based therapies. For these reasons, several studies reported promising results of Ad-MSCs for infertility treatment, including restoration of endometria and spermatogenesis.

Human UC-MSCs, also known as Wharton jelly MSCs, can be isolated from Wharton jelly of the umbilical cord tissue. These cells have advantages compared with other MSCs. The process of human UC-MSCs isolation is noninvasive because they can be obtained abundantly from discarded UC tissues. These MSCs have low oncogenicity and immunogenicity properties, fast self-renewal abilities, as well as high differentiation potential and proliferation rate.

Furthermore, they have prolonged survival rate after transplantation. Recent studies have indicated that UC-MSCs can differentiate into various tissue-specific cells, endometrial and primordial-like germ cells. Umbilical cord blood banking is increasingly being offered to expecting parents for its potential in treating various conditions including cancer, blood diseases, and immune system disorders. Thus, autologous human UC-MSCs may be a source of stem cells for the future treatment of infertile patients.

Endometrial stem cells (En-SCs), which are located at the basal layer of the endometrium (endometrial MSCs) and lining of the uterus (endothelial progenitor cells), are good candidates for cell-based therapy in regenerative medicine because they exhibit multipotent characteristics of MSCs, including self-renewal and immunoregulation, and have a high capacity for proliferation and differentiation. However, direct access to En-SCs by surgical procedure or biopsy is invasive and may damage the endometrium. Fortunately, recent studies showed easy isolation of endometrial MSCs from menstrual blood. Thus, the isolation and application of MB-MSCs for regenerative therapies is not invasive in contrast to the other source of MSCs. More importantly, recent evidence has revealed that MB-MSCs exhibit MSC characteristics, and their transplantation induces angiogenesis, proliferation, and mor¬phological recovery of the endometrium without autoimmune rejection.

Human MSCs can be also isolated from both the amniotic membrane and amniotic fluid in a large quantity, indicating that amniotic tissue is a rich source of MSCs for clinical therapeutic applications without the ethical con¬cerns of using ESCs. Human A-MSCs have multipotent characteristics such as immunomodulatory potential, rapid proliferation and differentiation properties, and low tumori¬genicity similar to other MSC types. For this reason, some studies have been conducted to use hA-MSCs for treatment of infertility.

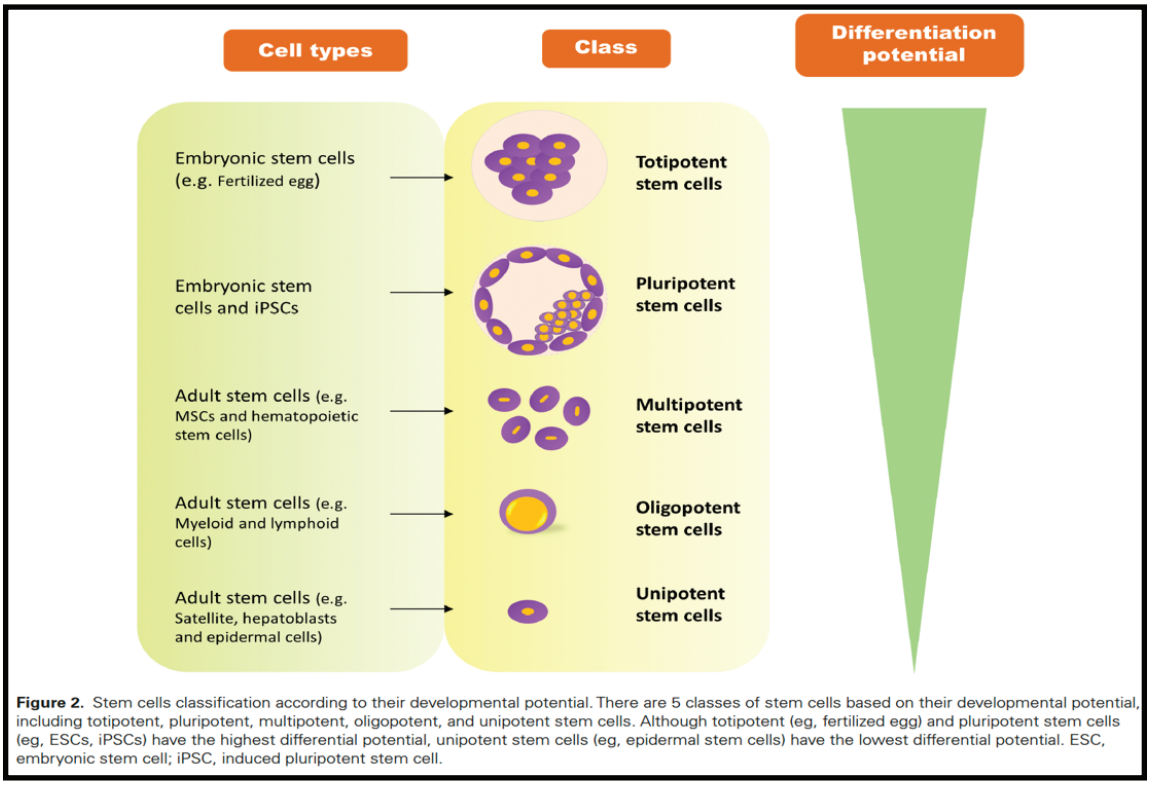


Figure 2. Stem cells classification according to their developmental potential. There are 5 classes of stem cells based on their developmental potential, including totipotent, pluripotent, multipotent, oligopotent, and unipotent stem cells. Although totipotent (eg, fertilized egg) and pluripotent stem cells (eg, ESCs, iPSCs) have the highest differential potential, unipotent stem cells (eg, epidermal stem cells) have the lowest differential potential. ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

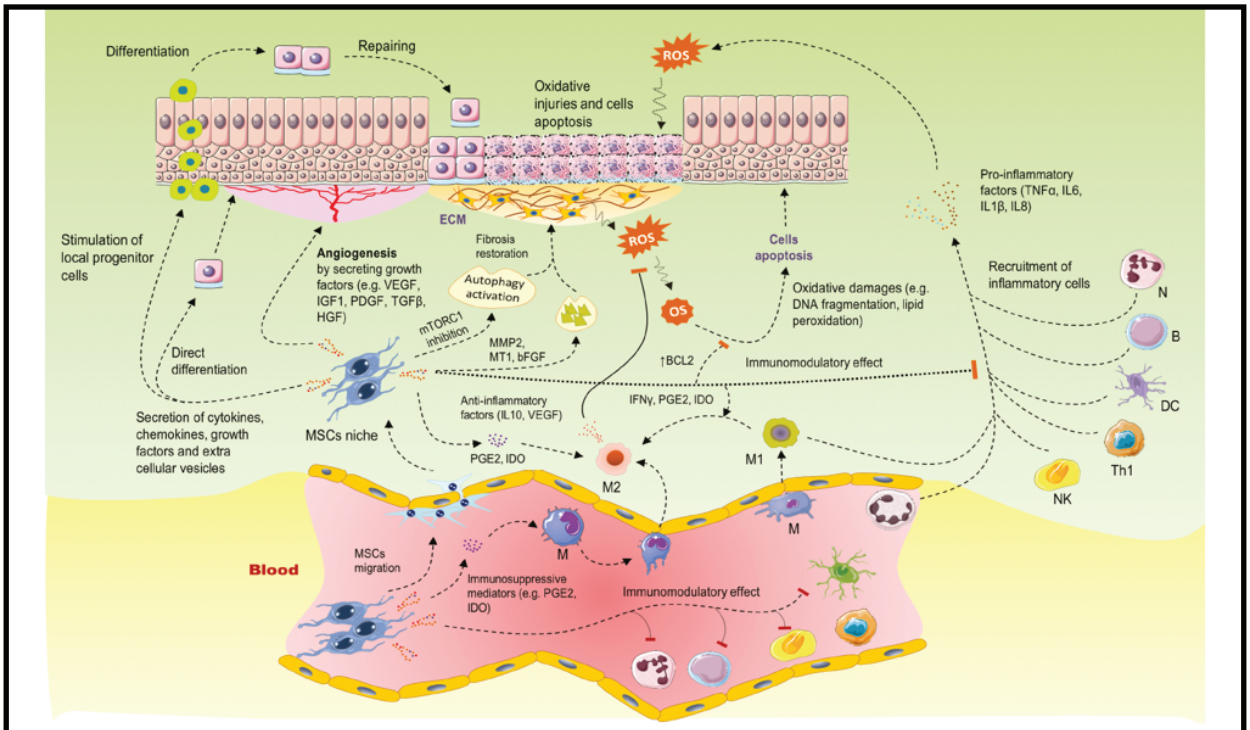


Figure 3. The regenerative mechanisms of mesenchymal stem cells. Injured tissues or cells release chemical and mechanical signals that promote MSC homing and engraftment from peripheral blood into the injury site. Collectively, MSCs contribute to regeneration through multiple mechanisms, including immunomodulatory effect, inducing angiogenesis, direct differentiation to local cells, stimulating local progenitor cells to differentiate into local cells for the repairing process, mitigating the inflammatory reaction, ROS generation and oxidative stress, restoring fibrosis and ECM formation via inhibiting mTORC1 pathway, and activating autophagosomes in accumulated fibroblasts. MSCs also induce differentiation of inflammatory M1 macrophages toward anti-inflammatory M2 phenotype by secreting IDO, PGE2, and IFN-γ. M2 macrophages generate IL-10 that has anti-inflammatory and antioxidative effects. Moreover, MSCs inhibit apoptosis via inducing the expression of Bcl2, as well as mitigating ROS and oxidative stress. Most MSC activities are mediated through their paracrine effects. B, B lymphocytes; Bcl2, B-cell lymphoma 2; bFGF, basic fibroblast growth factor; DC, dendritic cells; ECM, extracellular matrix; HGF, hepatocyte growth factor; IDO, indolamine 2,3-dioxygenase; IFN-γ, interferon gamma; M, monocyte; M1, M1 macrophage; M2, macrophage M2; MMP2, matrix metalloproteinase 2; MSC, mesenchymal stem cell; mTORC1, mammalian target of rapamycin complex 1; N, neutrophil; NK, natural killer cell; OS, oxidative stress; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; Th1, T lymphocytes; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

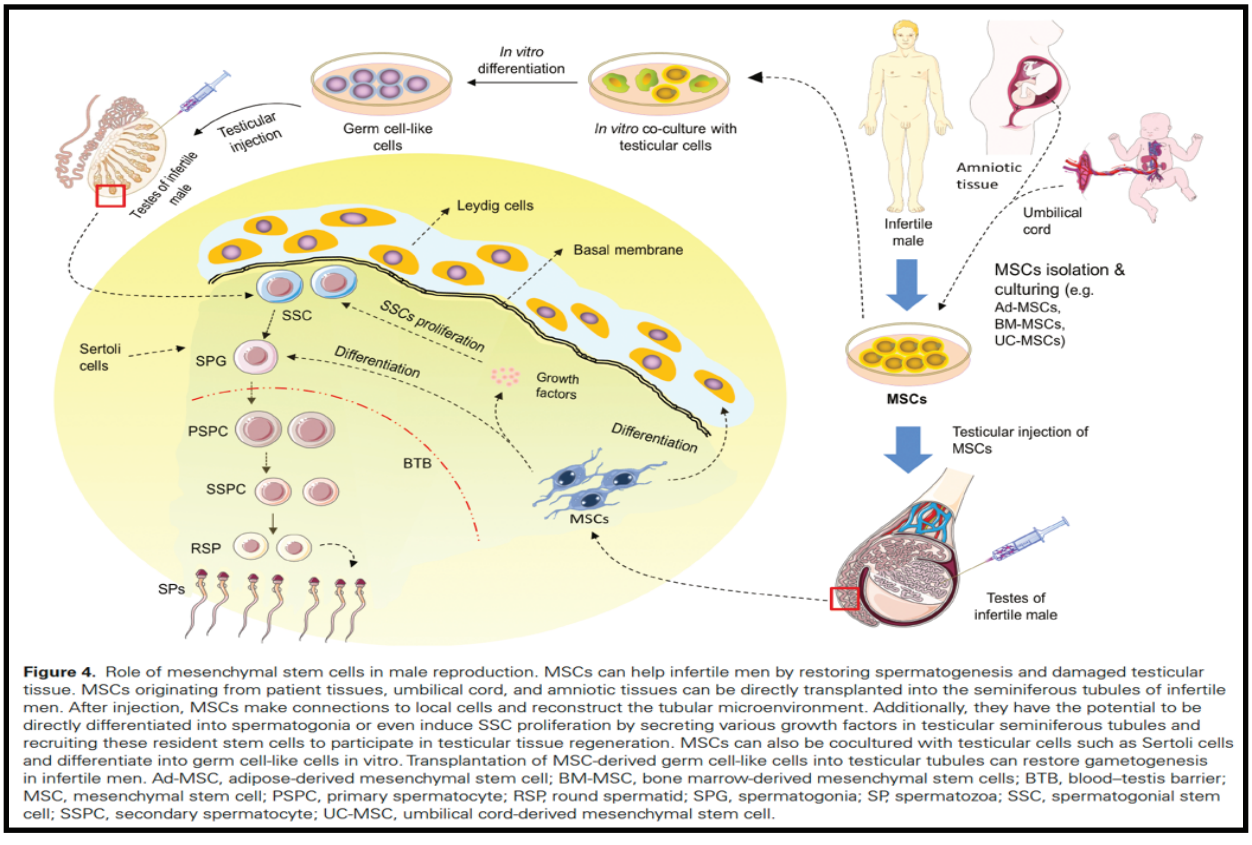


Figure 4. Role of mesenchymal stem cells in male reproduction. MSCs can help infertile men by restoring spermatogenesis and damaged testicular tissue. MSCs originating from patient tissues, umbilical cord, and amniotic tissues can be directly transplanted into the seminiferous tubules of infertile men. After injection, MSCs make connections to local cells and reconstruct the tubular microenvironment. Additionally, they have the potential to be directly differentiated into spermatogonia or even induce SSC proliferation by secreting various growth factors in testicular seminiferous tubules and recruiting these resident stem cells to participate in testicular tissue regeneration. MSCs can also be cocultured with testicular cells such as Sertoli cells and differentiate into germ cell-like cells in vitro. Transplantation of MSC-derived germ cell-like cells into testicular tubules can restore gametogenesis in infertile men. Ad-MSC, adipose-derived mesenchymal stem cell; BM-MSC, bone marrow-derived mesenchymal stem cells; BTB, blood–testis barrier; MSC, mesenchymal stem cell; PSPC, primary spermatocyte; RSP, round spermatid; SPG, spermatogonia; SP, spermatozoa; SSC, spermatogonial stem cell; SSPC, secondary spermatocyte; UC-MSC, umbilical cord-derived mesenchymal stem cell.

Mesenchymal Stem Cells in Male Reproduction

A growing number of studies have revealed the regenerative effect of MSCs on reproductive function and fertility outcomes in men (Table 1); however, this repair mechanism is not well understood. The main mechanisms of MSCs in restoration of spermatogenesis and testicular damage in infertile men involve fusion with the local cells in the affected region, reconstitution of the tubular microenvironment, direct differentiation of MSCs to spermatogonia, or induction of SSC proliferation by the production of growth factors in testicular seminiferous tubules and recruitment of these resident stem cells to participate in tissue regeneration (Fig. 4). SSCs, which are the testicular germ stem cells in the seminiferous epithelium, play a central role in spermatogenesis. SSCs originating from MSCs can proliferate and generate other germ cells, including spermatogonia, spermatocytes, spermatids, and spermatozoids. Interestingly, MSCs can also differentiate into testicular somatic cells such as Sertoli cells in seminiferous tubules and Leydig cells in interstitial connective tissue. Sertoli cells are crucial for testis formation and regulation of spermatogenesis. Not only are they a structural element of the seminiferous tubules, but they also support developing germ cells with nutrients and growth factors and protect them from harmful agents or autoimmune attack by making the blood–testis barrier. Leydig cells, which are positioned in the testicular interstitial tissue, play an important role in reproductive tract development and spermatogenesis via the biosynthesis and secretion of androgens. Therefore, differentiation of transplanted MSCs into these somatic cells provides a supportive microenvironment for SSC proliferation and differentiation to the other spermatogenic cells. There are some studies that have demonstrated that transplantation of MSCs into the atrophic seminiferous tubules of azoospermic rodent models improves fertility outcomes by supporting germ cell self-renewal, SSC colonization and differentiation, and increasing the number of spermatogenic cells during regeneration (Table 1). More importantly, the blood–testis barrier enhances MSC efficacy by protecting them against immunologic responses. BM-MSCs can be differentiated into male germ-like cells in vitro and transplantation of these cells into the seminiferous tubules of busulfan-treated mice can recover spermatogenesis. Many experimental studies have been conducted using different forms of MSCs for infertility treatment (Table 1). For example, testicular injection of BM-MSCs significantly improved impaired spermatogenesis in rats. Injection of Ad-MSCs in seminiferous tubules induced spermatogenesis in azoospermic rats. A recent study has shown that intratubular injection of Ad-MSCs successfully repaired sem-iniferous tubules, induced spermatogenesis, and increased the number of spermatozoa in the epididymis and seminiferous tubules of azoospermic hamsters. A previous study showed that human UC-MSCs transplantation into the lumen of seminiferous tubules in immunocompetent mice not only survived for 120 days, but also migrated to the base of the tubule and successfully differentiated to germ cells. These findings suggest that human UC-MSCs have high capacity to produce germ cells and treat testicular damage or infertility caused by chemotherapy drugs. There are also some studies that have considered the differentiation potential of MSCs toward male germ cells in vitro (Table 2). It has been dem¬onstrated that UC-MSCs have the potential to differentiate into male germ cells in vitro when they are cocultured with Sertoli cells. BM-MSCs cocultured in vitro with Sertoli cells and transplanted into the seminiferous tubules of azoospermic rats not only survived and migrated at the basement membranes, but also successfully transdifferentiated into spermatogenic-like cells.

Mouse BM-MSCs can also be differentiated into male germ-like cells via coculture with testicular cells. These data indicate that Sertoli cells create a unique microenvironment for germ cell colonization and differentiation. Moreover, MSC-derived male germ cells may be used in male infertility treatment. For this to be a useful treatment for infertility, autologous MSCs must be available. Biobanking of UC blood is increasingly being offered because UC-derived stem cells are promising for the treatment of an array of diseases, not just infertility. One of the main limitations of these studies is that the function or fertilization potential of MSC-derived germ cells was not evaluated. However, there are a few experimental studies that have successfully generated functional gonads and live birth through in vitro differentiation of different MSCs (Table 2). Despite the potential of MSCs to restore fertility, further human studies are necessary to draw conclusions.

Testicular microinjection of MSCs appears to be effective technique for infertility treatment with fewer injections required compared with IV injection. MSC homing or fate is a main factor required for regeneration. In systemic administration, homing of MSCs must undergo a multistep process to exit circulation and migrate to the injury site. Furthermore, MSCs interaction with other cells or molecules such as immune cells, inflammatory cytokines, microRNAs, as well as signals released from damaged tissues play important roles in this process. Thus, conducting research to compare the efficacy of intravenous and testicular injection of MSCs in infertility therapy is necessary. Taken together, these data indicate that MSCs not only survive and populate the seminiferous tubules and interstitium, but also play a potential role in restoring spermatogenesis through differentiating into somatic and germinal cell types in testicular seminiferous tubules, although this requires further research for valid¬ation. Furthermore, they provide an ideal microenvironment or niche for activating or proliferating of germinal cells in the testis. For this reason, several experimental studies and clinical trials are now using MSCs for potential application to improve fertility outcomes in males with impaired sperm¬atogenesis (Table 3). Nevertheless, potential risks of the use of stem cells into the human model should be carefully investigated before their introduction into the clinic.

Table 1. Effects of different stem cells on male reproductive function

Stem cell types	Disease	Study model[a]	Type of injection	Main outcomes	Re
Ad-MSCs	Azoospermia	Human (N = 1)[b]	3 sessions IV 1 testicular	↑Spermiogenesis restoration; ↑successful fertilization during IVF; ↑embryos quality; (unsuccessful embryos implantation and no pregnancy)	
	Azoospermia	Rats	Testicular	↑Normal morphology of testes; ↑spermatogenesis; ↑spermatogenetic cells; ↑generations after mating	
	Azoospermia	Rats	Testicular	↑Testes normal morphology; ↑spermatogenesis; ↑spermatogenetic cells	
	Azoospermia	Hamsters	Testicular	↑Repair of seminiferous tubules; ↑spermatogenesis; ↑spermatozoa in epididymis tubes	
	Azoospermia	Guinea pigs	Testicular	↑Testes normal morphology; ↑normal spermatogenesis; ↑primary spermatocytes, ↑spermatids, ↑spermatogonia, ↑spermatozoa	
	C-TD	Rats	Testicular	↑Restoration of testicular architecture; ↑TE level	
	TTIR	Rats	Tail vein	↓Oxidative stress; ↓apoptotic biomarkers; ↓testicular damage scores; ↓mitochondrial damage; ↓fibrotic biomarkers; ↓inflammation; ↑numbers of Sertoli cells; ↑thickness of seminiferous tubule	
	TT	Rats	Testicular	↓MDA; ↑Johnsen score; ↓ROS formation; ↓testicular injury	
BM-MSCs	Azoospermia	Rats	Testicular	↑SSCs, spermatogonia, ↑spermatogenic-like-cells; ↑fertility rate	
	Azoospermia	Mutant mice	Testicular	↑Sertoli cells; ↑Leydig cells, ↑spermatogonia; ↑spermatocytes,	
	Azoospermia	Rats	Testicular	↑Spermatogonia; ↑primary spermatocytes; ↑spermatozoids	
	Azoospermia	Hamsters	Testicular	↑Normal morphology of seminiferous tubules; ↑spermatogenesis restoration; ↑spermatozoa in epididymis tubes	
	Azoospermia	Hamsters	Testicular	↑Spermatogonia, ↑primary spermatocytes, ↑spermatids; ↑sperms in seminiferous tubules	
	Cd-TI	Rats	IV	↓Testicular Cd contents; ↑body weight; ↓pathological changes; ↓expression of apoptosis-associated proteins; ↓mitochondrial apoptosis; ↓testis injury	
UC-MSCs	Azoospermia	Mice	Testicular	↑Germ cells; ↑spermatogonia; ↑spermatids; ↑spermatozoa	
	Azoospermia	Mice	IV	↑Spermatogenesis restoration; ↑sperm counts; ↑sperm motility; ↑sex hormones	
	Azoospermia	Mice	Testicular	↑Normal morphology of seminiferous tubules; ↑spermatogenesis	
hA-MSCs	Azoospermia	Mice	Testicular	↑Spermatogenesis restoration; ↑testosterone levels; ↑testicular weight; ↑testicular size; ↑semen parameters; ↓cell apoptosis; ↓oxidative stress	
SSCs	Azoospermia	Mice, pigs, goats	Testicular	↑Spermatogenesis; ↑attainment of natural fertility	
	Azoospermia	Mice	Testicular	↑Complete spermatogenesis; ↑natural fertility	
	Azoospermia	Mice	Testicular	↑Fertility restoration; ↑offspring production	
	Azoospermia	Macaques	Testicular	↑Spermatogenesis restoration; ↑functional sperm; ↑embryos production	
	Azoospermia	Mice	Testicular	↑Spermatogonia cells; ↑spermatocytes count; ↑spermatids count; ↑spermatogenesis	
	Azoospermia	Rats	Testicular	↑Spermatogenesis; ↑sperm cell production	
hOF-MSCs	TDI	Rats	Testicular	↑TDI improvement; ↑sperm function; ↑sperm ATP; ↑sperm motility; ↓infertility	
	TT	Rats	Testicular	↓Torsion-induced infertility; ↓apoptosis; ↓oxidative stress; ↑serum TE; ↓FSH	

Abbreviations: Ad-MSC, adipose-derived MSC; BM-MSC, bone marrow-derived MSC; Cd-T, cadmium-induced test injury; C-TD, cisplatin-induced testicular damage; hA-MSC, human amniotic-derived MSC; hOF-MSC, human orbital fat tissue-derived MSC; MB-MSC, menstrual blood-derived MS MDA, malondialdehyde; MSC, mesenchymal stem cell; ROS, reactive oxygen species; SSC, spermatogonia stem cell; TDI, torsion-detorsion injury; TE, testosterone; TT, testicular torsion; TTIR, testicular torsion-induced ischemia-reperfusion injury; UC-MSC, umbilical cord-derived MSC.
[a]Note the lack of human studies.
[b]This study reports the outcome for 1 individual who received Ad-MSC injection. The association between restored spermiogenesis has not been prover causal.

Mesenchymal Stem Cells in Female Reproduction

In addition to male infertility treatment, MSCs have also exhibited a potential for treatment of female infertility caused by ovarian dysfunction and endometrial disorders. MSCs have high capacity to penetrate the uterine endometrium and con¬tribute to regeneration of endometrial tissue by secreting paracrine factors or directly differentiating to epithelial, stromal, and endothelial cells. For this reason, a growing number of experimental and clinical trials are now being conducted that use different types of MSCs for treatment of endometrial dysfunction and female infertility (Table 3 and Table 4). Thin endometrium, which is characterized by reduced expression of growth factors (VEGF) and poor epithelial growth and vascular development, is an important cause of implantation failure and poor reproductive outcome in females undergoing IVF, for which there are no conventional treatments of proven benefits. Recent investigations have demonstrated that MSC transplantation improves endometrial thickness in cases with endometrial disorders (Fig. 5). For instance, a recent study using subendometrial injection of autologous Ad-MSCs has been reported to increase the endometrium thickness in most women with thin endometrium (20 of 25 cases), and was associated with a total of 13 pregnancies and 9 live births. This small cohort study requires replication/expansion to draw conclusions. MSCs have also shown promise for the treatment of Asherman syndrome (AS), a gynecological disorder characterized by the progression of intrauterine adhesions and endometrial atrophy causing thin endometrium, menstruation disturbance, recurrent pregnancy loss, and infertility. A clinical trial in women with AS showed that subendometrial implantation of autologous BM-MSCs caused endometrial regeneration tubules, although this requires further research for validation. Furthermore, they provide an ideal microenvironment or niche for activating or proliferating of germinal cells in the testis. For this reason, several experimental studies and clinical trials are now using MSCs for potential application to improve fertility outcomes in males with impaired spermatogenesis (Table 3). Nevertheless, potential risks of the use of stem cells into the human model should be carefully investigated before their introduction into the clinic.

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In addition to male infertility treatment, MSCs have also ex¬hibited a potential for treatment of female infertility caused by ovarian dysfunction and endometrial disorders. MSCs have high capacity to penetrate the uterine endometrium and contribute to regeneration of endometrial tissue by secreting paracrine factors or directly differentiating to epithelial, stromal, and endothelial cells. For this reason, a growing number of experimental and clinical trials are now being conducted that use different types of MSCs for treatment of endometrial dysfunction and female infertility (Table 3 and Table 4). Thin endometrium, which is characterized by reduced expression of growth factors (VEGF) and poor epithelial growth and vascular development, is an important cause of implantation failure and poor reproductive outcome in females undergoing IVF, for which there are no conventional treatments of proven benefit. Recent investigations have demonstrated that MSC transplantation improves endometrial thickness in cases with endometrial disorders (Fig. 5). For instance, a recent study using subendometrial injection of autologous Ad-MSCs has been reported to increase the endometrium thickness in most women with thin endometrium (20 of 25 cases), and was associated with a total of 13 pregnancies and 9 live births.

This small cohort study requires replication/expansion to draw conclusions. MSCs have also shown promise for the treatment of Asherman syndrome (AS), a gynecological disorder characterized by the progression of intrauterine adhesions and endometrial atrophy causing thin endometrium, menstruation disturbance, recurrent pregnancy loss, and infertility. A clinical trial in women with AS showed that subendometrial implantation of autologous BM-MSCs caused endometrial regeneration and restoration of menstruation in 5 of 6 cases. In another clinical trial, autologous MB-MSCs were transplanted into the uterus of 7 patients with severe AS. The endometrial thickness of 5 patients was significantly increased to 7 mm and enabled embryo implantation. Two of 4 patients who underwent frozen embryo transfer became pregnant. One patient had spontaneous pregnancy after the second MB-MSC transplantation. Injection of BM-MSCs into spiral arterioles in the uterus of patients with endometrial atrophy and/or AS was associated with improved uterine cavity, endometrial thickness, density of mature vessels in the endometrium, as well as the duration and intensity of menstruation. Interestingly, some patients successfully became pregnant without any medical intervention. Similar results were also reported in animal models. Some studies on rat models reported that autologous Ad-MSCs injection improves fertilization rates of rats with premature ovarian failure and AS. Therefore, these data indicate that MSC therapy can be considered a good candidate for restoration of female reproductive function.

Several studies have shown that MSCs therapy has the potential to improve endometrial functions; however, the exact mechanism by which MSCs can improve endometrial injury and is not well-elucidated. In addition to direct differentiation of MSCs into the uterine cavity for endometrial regeneration, it appears that MSCs can directly migrate to the uterus and endometrium of female recipients following systemic infu¬sion and facilitate ovarian or endometrial recovery by directly differentiating to endometrial cells or stimulating the secretion of multiple growth factors such as platelet-derived growth factor, hepatocyte growth factor, and TGF-β _ (Fig. 4). Some studies of animal models have reported the direct differentiation of MSCs to endometrial cells and the secretion of various growth factors into the endometrium fol-lowing MSC injection. For example, Ad-MSC transplantation repaired injured endometrium through direct differentiation to endometrial cells in rat models. Furthermore, Ad-MSCs improved conception rate by 60% via increasing the microvessel density, endometrial thickness, and glands. MSC therapy has been also shown to improve the fibrosis of endometrial and uterine tissues (Fig. 5). For example, human UC-MSC injection in a rat model not only restores injured endometrium and its normal morphology, but also improves embryo implantation rate and alleviates endomet-rial fibrosis through up-regulating vascular and growth factors (eg, CK19, VEGFA, MMP9, CD31) and down-regulating proinflammatory factors (eg, interferon-γ, tumor necrosis factor-α _[TNF-α], IL-2). Strikingly, multiple injections of UC-MSCs increased the number of implanted embryos compared with single UC-MSC transplantation. Similarly, IP injection of hA-MSCs in a murine model demonstrated restoration of endometrial morphology and fertility. Transplantation of hA-MSCs significantly improved endometrial thickness and the number of endometrial glands through the up-regulation of endometrial angiogenesis-related factors (VEGF, proliferating cell nuclear antigen, and estrogen receptor) and induction of stromal cell proliferation. Moreover, hA-MSCs decreased endometrial fibrosis through activating the autophagy pathway in this tissue.

The transplantation of hA-MSC has been shown to improve endometrial thickness, degree of fibrosis, and number of endometrial glands through the up-regulation of growth factors (FGF2 and IL-6) and down-regulation of pro-inflammatory cytokines (TNF-α _and IL-1β). Recent studies have revealed that MB-MSCs transplantation can repair endometrial damage and thickness via stimulating the expression of filament proteins (vimentin and keratin), VEGF, and regulation of protein kinase B signaling pathways. Up-regulation of estrogen receptor-α, estrogen receptor-β, and progesterone receptor expression may be another mechanism of BM-MSC trans¬plantation that effectively repairs endometrium injury and regulates the structure and function of ovarian tissue. To support this, studies in animal models have shown that local and/or IV injection of BM-MSCs restores the produc¬tion of ovarian hormones and improve endometrial cell proliferation and ovarian function. Therefore, these findings indicate that MSCs have high capacity to improve endometrial injuries and fibrosis of uterine and endometrial tissues not only by direct differentiation to endometrial cells, but also by promoting the secretion of various growth factors, hormones, and activating autophagy (Fig. 5).

Table 2. Transdifferentiation of different stem cells to germ cells

Stem cell types	Source	Model	Outcomes	Function[a]	I
Ad-MSCs	Rats	In vitro coculture with RA and Sertoli cells; in vivo transplantation into tests	Male G-LCs	NR[b]	(
	Mice	In vitro culture with growth factors and RA	Germ cells	NR	(
	Canine	In vitro culture with bioactive molecules	Male G-LCs	NR	(
BM-MSCs	Mice	In vitro coculture with testicular cells and BMP4	Male G-LCs	NR	(
	Mice	In vitro differentiation	Male G-LCs	NR	(
	Ram	In vitro culture with bioactive molecules	Male G-LCs	NR	(
	Bovine	In vitro culture with bioactive molecules	Male G-LCs	NR	(
	Bovine	In vitro coculture with Sertoli cells	Male G-LCs	NR	(
	Mice	In vitro culture in SCCM	Male germ cells	NR	(
	Mice	In vitro coculture with testicular cells and RA	Male G-LCs	NR	(
	Rams	In vitro culture with growth factors and BMPs	Male germ cells	NR	(
	Rats	In vivo transplantation into tests	Spermatogonia	NR	(
P-MSCs	Mice	In vitro coculture with HFF and HCCM	O-LCs	NR	(
UC-MSCs	Human	In vitro coculture with Sertoli cells	Male G-LCs	NR	(
	Human	In vitro coculture with Sertoli cells	Male G-LCs	NR	(
	Human	In vitro coculture with Sertoli cells	Male G-LCs	NR	(
	Human	In vitro coculture with testicular cells	Male G-LCs	NR	(
	Human	In vitro coculture with HAE, HCP, BMP4, RA	Male G-LCs	NR	(
	Human	In vitro coculture with TCC, PCC, BMP4, RA	Male G-LCs	NR	(
	Human	In vitro culture with BMP4	Female germ cells	NR	(
hA-MSCs	Human	In vitro culture with 5% porcine follicular fluid	O-LCs	NR	(
	Human	In vitro culture with RA	PGC-LCs	NR	(
AM-MSCs	Mice	In vitro culture with BMP4 and RA	Male G-LCs	NR	(
ESCs-SCLCs	Mice	In vitro coculture with Epi-LCs	PGC-LCs	NR	(
ESCs	Human	In vitro differentiation to PGC-LCs	Spermatozoa	NR	(
	Human	In vitro differentiation to PGCs	OF-LCs	NR	(
	Goat	In vitro differentiation to PGC-LCs	O-LCs	Functional	(
	Mice	In vitro differentiation to PGC-LCs	Oocytes	Functional	(
	Mice	In vitro differentiation to PGC-LCs and testicular transplantation	Spermatozoa	Functional	(
	Mice	In vitro differentiation to PGC-LCs and coculture with gonadal somatic cells	SP-LCs	Functional	(
	Mice	In vitro differentiation to PGC-LCs and then transplanted into ovarian bursa	Oocytes	Functional	(
	Mice	In vitro differentiation to PGC-LCs	O-LCs	NR	(
	Mice	In vitro differentiation to PGC-LCs and cocultured with embryonic testicular somatic cells	Spermatogonia-like cells	Functional	(
	Mice	In vitro differentiation to PGC-LCs	Oocytes	Functional	(
iPSCs	Human	In vitro differentiation	Oogonia	NR	(
	Human	In vitro differentiation	PGC-LCs	NR	(
	Human	In vitro differentiation	PGC-LCs	NR	(
	Human	In vitro differentiation to PGC-LCs	Prospermatogonia	NR	(
	Primate	In vitro differentiation to PGC-LCs and testicular transplantation	PGCs	No SPG generation	(
	Mice	In vitro differentiation to PGC-LCs	Oocytes	Functional	(
	Mice	In vitro differentiation to PGC-LCs and cocultured with somatic cells of female embryonic gonads	Oocytes	Functional	(
	Mice	In vitro differentiation to PGC-LCs and testicular transplantation	Spermatozoa	Functional	(
	Mice	In vitro differentiation to PGC-LCs and testicular transplantation	Spermatozoa	Functional	(
	Mice	In vitro differentiation	PGC-LCs	NR	(
	Mice	In vitro differentiation to SSCs and testicular transplantation	Male germ cells	NR	(
	Mice	In vitro differentiation by RA and TE	Male germ cells	NR	(
	Mice	In vitro differentiation	Male germ cells	NR	(
Granulosa cells	Mice	In vitro differentiation to PSCs and then PGC-LCs	Oocytes	Functional	(
VSELs	Mice	In vitro coculture with Sertoli cells supplemented with FSH	Male germ cells	NR	(

Abbreviations: AM-MSC, amniotic membrane-MSC; BMP4, bone morphogenetic protein 4; Epi-LC, epiblast-like cell; ESC-SCLC, embryonic stem cell-derived Sertoli cell-like cell; germ-like cell; HFF, human follicular fluid; HCCM, human cumulus-conditioned medium; MSC, mesenchymal stem cell; OF-LC, ovarian follicle-like cell; O-LC, oocyte-like cell; PC placental culture condition; PGC-LC, primordial germ cell-like cell; PGC, primordial germ cell; P-MSC, peritoneum MSC; RA, retinoic acid; SPG, spermatogonia; SCCM, Sertoli ce condition medium; SP-LC, spermatid-like cell; SSC, spermatogonial stem cell; TCC, testicular culture condition; TE, testosterone; VSEL, very small embryonic-like stem cell.

[a]Note the lack of functional validation performed following the generation of G-LCs.

[b]Not reported.

Table 3. Clinical trials of stem cell therapy for human infertility

Stem cell types	Disease	Patients Number	Type of injection	Outcome measures	Status	ID
Autologous Ad-MSCs	OA, NOA	40	Testicular	Sperm quality; TESA; testosterone; Inhibin-B; FSH	In progress	NCT03762967
UC-MSCs	IUA	26	Uterine cavity	Live birth rate; IUA restoration; endometrial thickness; pregnant rate; menstrual blood volume	Completed[a]	NCT02313415
	TEN	50	Uterine cavity	Endometrial thickness; pregnancy rate; live birth rate; endometrial blood flow; menstrual blood volume; adverse event rate	In progress	NCT03592849
Autologous BM-MSCs	NOA	50	Testicular	Appearance of different germ cells in testicles; semen Analysis; testicular morphology; sexual function	In progress	NCT02641769
	KS, NOA	1	Testicular	Semen analysis; hormonal profile (Inhibin-B, FSH, LH)	Completed[a]	NCT02414295
	AZO	60	Testicular	Serum hormonal profile (testosterone, FSH, LH, and prolactin); testicular size; sexual potency; testicular biopsy	Unknown	NCT02041910
	AZO	20	Testicular	Semen analysis; Inhibin-B hormone	Unknown	NCT02008799
	AZO	100	Testicular	Hormonal assessment (testosterone, FSH, LH, and prolactin); testicular size; sexual potency	Unknown	NCT02025270
	AS AE	46	Endometrial	Endometrial receptivity; pregnancy rate	Completed[a]	NCT03166189
	POF	60	Ovarian	Hormonal assessment (FSH, AMH, and estrogen); ultrasound assessment; menopausal symptoms; pregnancy rate; miscarriage rate	Unknown	NCT02062931
	POF	60	Ovarian	Hormonal assessment (FSH, AMH, and estrogen); menopausal symptoms	Unknown	NCT02043743
	POI POF LOR	3	Ovarian	Hormonal levels (FSH, FSH/LH, AMH, estradiol/ progesterone, and Inhibin-B); resumption of menses; pregnancy rate	Completed[a]	NCT02696889
Autologous SSCs	AZO	65	In vitro differentiation	SSCs isolated from testicular biopsies are matured in vitro for the generation of sperm for infertility treatment	Completed[a]	NCT01375062
	AZO	10	Testicular	Return of spermatogenesis; semen analysis; complications of SSC transplantation	In progress	NCT04452305
hA-ESC	TEN	40	Endometrial	Endometrium thickness; amenorrhea severity	In progress	NCT04676269

Abbreviations: AMH, anti-Mullerian hormone; AS, Asherman syndrome; AE, atrophic endometrium; AZO, azoospermia; hA-ESC, human amnion epithelial stem cells; IUA, intrauterine adhesions; KS, Klinefelter syndrome; LOR, low ovarian reserve; NOA, nonobstructive azoospermia; OA, oligozoospermia; OSC, oogonial stem cell; POF, premature ovarian failure; POI, primary ovarian insufficiency; TEN, thin endometrium; TESA, testicular sperm aspiration.
[a]Although these trials are "completed," their outcome data are not publicly available, raising questions about the study success. This lack of outcome data for human studies is a recurring theme and limits the utility of these therapeutic approaches clinically.

Restoring Ovarian Function

The antiapoptotic property of MSCs is likely another mechanism of cell-based therapy in regenerating female reproductive function. Granulosa cells (GCs), which originate from the ovarian surface epithelium and surround the primordial oocytes, play a central role in female reproduction by stimu¬lating the production of steroids and other paracrine factors, as well as creating a unique environment for oocyte growth and nutrition . Early apoptosis of GCs can be associated with decreased level of estrogen and affects normal development of oocytes. For example, early apoptosis of mural GCs has been associated with poor ovarian response and decreased number of oocytes and embryos generated by ART. Recent studies of animal models of premature ovarian insufficiency have demonstrated that MB-MSC therapy not only mitigates GC apoptosis and fibrosis of the ovarian interstitium, but can also result in fertility restoration by improving folliculogenesis and overall ovarian function (Table 4). For example, it has been reported that BM-MSC therapy can improve ovarian structure and function in an animal model with chemotherapy-induced ovarian failure. In another study, injection of autologous Ad-MSCs improved the quality of freshly grafted ovaries by increasing the expression of VEGF-A and the number of blood vessels in the ovarian tissue of adult female rats. A recent study on perimenopausal rats has shown that injection of human UC-MSCs through tail veins improves ovarian reserve and delays ovarian senescence by inducing the expression of growth factors and cytokines such as IGF-1, hepatocyte growth factor, and VEGF. Overproduction of reactive oxygen species, oxidative stress, and an inflammatory response are probably the main reasons for the early apoptosis of GCs and ovarian dysfunction. Given the immunomodulatory and anti-inflammatory properties of MSCs, it appears that MSC therapy can decrease GC apoptosis by modulating reactive oxygen species production and oxidative stress. In keeping with this notion, a recent study revealed that MB-MSC therapy significantly decreased GC apoptosis and improved ovarian function through down-regulation of Gadd45b
(a cell-cycle arrest, DNA damage- and apoptosis-inducible protein) and up-regulation of cyclinB1 and CDC2 (as regulators of mitosis from G2 to M phase) proteins.

Polycystic Ovary Syndrome Treatment

Polycystic ovary syndrome (PCOS) is a complex endocrine associated with ovulatory dysfunction, endometrial hyperplasia, and infertility. MSCs have been proposed as potential treatment for PCOS patients, but little clinical research has been conducted in this area. Human UC-MSC transplantation has been shown to alleviate uterine fibrosis and improve ovarian polycystic morphology and ovarian function of mice with DHEA-induced PCO. These changes were associated with significantly decreased expression of pro-inflammatory cytokines (interferon-γ, TNF-α, and IL-1β), and enhanced expression of anti-inflammatory factor, IL-10, in the local ovarian and uterine tissues. Interestingly, MSCs reduced M1 macrophages (as source of IL-1β _and TNF-α) and increased M2 macrophages (as source of IL-10) recruitment to this site.

The relevance of PCOS mouse models to human pathology is not well established, but it appears that MSCs may have the potential to improve ovarian function in PCOS patients through inhibition of the systemic and local inflammatory responses (Fig. 5).

Key Challenges

Key Challenges in MSC Therapy for Infertile Patients

Experimental studies have shown some prospect for MSC therapy in tissue regeneration, but there are still multiple concerns regarding the use of these cells in infertility treatment at clinics. First, most studies have used MSC therapy in small animal models that may not reflect the pathophysiology of human infertility. Although most studies did not report tumor mass after MSC transplantation in experimental animal models, the safety and tumorigenic potential of MSC transplantation, especially in case of allogeneic transplantation, still requires long-term observation. Moreover, ethical concerns hinder regenerative therapies in human models. More importantly, it is not clear whether MSC transplantation for infertility treatment may carry even higher risks of genetic or epigenetic abnormalities to offspring. Therefore, further clinical trials must be conducted to confirm the therapeutic effect of MSCs for human infertility treatment. Furthermore, harvesting of BM-MSCs and Ad-MSCs is invasive and needs surgical intervention, which is not welcomed by most patients. Besides, MSC processing for transplantation is relatively time-consuming and costly, and several additional tests such as karyotyping, MSC characterization, and microbial and viral tests should be performed before injections. Because the phenotype, genotype, and differentiation potential of MSCs may change with an increase in passage number, novel MSC-based therapeutic strategies should be performed for patients. This is an important issue for patients who must undergo multiple sessions of MSC therapy to increase the chance of MSC migration and homing to the site of injury or disease. To minimize these limitations, exosome therapy has been suggested as a promising substitute for MSC-based infertility treatment. MSC-derived exosomes (MSC-Ex) are soluble and nano-scale extracellular vesicles (30-150 nm) carrying various functional molecules such as microRNAs, enzymes, DNA fragments, RNA, proteins, and lipids, and play important role in intracellular communication. MSC-Ex administration can mimic MSC therapy and affect cell migration, proliferation, angiogenesis, gene expression, and immune response. Recent studies have shown the potential for stem cell-derived exosomes to promote spermatogenesis, ovarian competence and erectile dysfunction in rodents . For example, a recent study demonstrated that intrabursal injection of human UC-MSC-Ex promoted follicular activation and development, leading to increased mature oocyte yield and quality in aged female mice. Topical administration of Ad-MSC-Ex in the uterus has been shown to promote endometrial regeneration and fertility restoration through remodeling of collagen and up-regulation of leukemia inhibitory factor (LIF), integrin-β3, and VEGF in rats with intrauterine adhesion caused by endometrial injury. However, very few studies have considered the effect of MSC-Ex on infertility treatment, and more in-depth studies are necessary to consider the potential of exosomes therapy for infertility treatment.

The survival rate of MSCs over a longer period is another bottleneck for disease treatment. For this reason, many studies have been focused on application of bioactive polymers or biomaterials to enhance the maintenance and targeted delivery of MSCs to the injury or diseased sites and to maximize their adhesion and engraftment for disease treatment. For instance, collagen-based biomaterials have been suggested as appropriate vehicles of MSC delivery to improve their adhesion, retention, proliferation, and engraftment. Given the important role of MSCs in promoting angiogenesis, their ability to directly differentiate into functional cells, and their paracrine activities, MSC therapy is likely more effective in regeneration of damage caused by non-developmental disorders rather than correction of disorders of developmental origin. This emphasizes that effective MSC therapy closely depends on the type of disease and underlying pathology involved in infertility and are not applicable for treatment of infertility in all patients, particularly for those with developmental disorders.

So, the question remains: how can stem cells help infertile patients with developmental disorders who want a biological child? To treat these patients, a method for generating germ cells from stem cells and differentiating them into gametes in a dish is required. The generation of germ cells from patient stem cells outside the body would provide reproductive potential to individuals affected by disorders of sex development. More importantly, in vitro generation of germ cells and other reproductive organoids from human stem cells provides excellent opportunity for researchers and clinicians to use them for disease modeling and drug screening. For this reason, generation of germ cells and reproductive organoids has been a recent focus of stem cell and reproductive scientists. Recent advances in CRISPR gene editing and hiPSC technology have allowed researchers to generate hiPSC-derived gametes that can be used for experimental and medical purpose. However, there are important practical and ethical consid-erations in the use of CRISPR gene editing, such as scarce human modeling and a lack of long-term data that hinder its use for human gamete production. The development of new 3D culture media and progress in microfluidic systems mean that an ideal microenvironment can theoretic-ally be designed to control differentiation, self-organization, and reproductive organoids on a microfluidic chip.

Table 4. Effects of different stem cells on female reproductive function

Stem cells	Disease	Models	Injection type	Main outcomes
Ad-MSCs	TE	Human	Endometrial	↑Endometrial thickness; ↑successful pregnancies and live births
	POF	Human	Ovarian	↓FSH; ↑return of resumption
	POF	Human	IV	↑Ovarian size; ↑endometrial thickness; ↑ovarian function
	EI	Rats	Endometrial	↑Microvessel density; ↑endometrial thickness; ↑fertility rate; ↑ERα; ↑ERβ; ↑PR; ↓endometrial injury
	OF	Mice	Ovarian	↑Number of follicles; ↑ovarian function
	POI	Rats	Ovarian	↑E2; ↑antral follicles; ↑GCs proliferation; ↑pregnancy rates
	POF	Mice	IV	↑Follicle number; ↑ovulation; ↑ovarian function
	AS	Rats	Uterus	↓Endometrial fibrosis; ↑angiogenesis; ↑endometrium restoring
BM-MSCs	AS	Human	Uterus	↑Endometrial regeneration; ↑successful pregnancies and live births
	AS/EA	Human	Spiral arterioles	↑Uterine cavity; ↑endometrial thickness;↑mature vessel density;↑duration and intensity of menses;↑successful pregnancies with live births
	POF	Human	Ovarian	↑Resumed menstruation after 3 months (2 cases); ↑pregnancy after 11 months and live birth (1 case); ↑EORS
	OF	Rabbits	IV	↓FSH; ↑estrogen; ↑VEGF; ↓Caspase-3; ↑follicle numbers; ↑ovarian function
	OF	Mice	Ovarian	↑Body weight; ↑ovarian weight, ↑weight of estrogen-responsive organs; ↑folliculogenesis; ↑follicle count; ↓FSH; ↑AMH; ↑pregnancies numbers
	OF	Mice	Ovarian	↑Fertility rescue; ↑spontaneous pregnancies; ↑numbers of preovulatory follicles; ↑ovarian vascularization; ↑cell proliferation; ↓apoptosis; ↑follicular growth; ↑E2 secretion
	AS	Rats	Tail vein	↑Reproductive outcomes; ↑number of embryos; ↓fibrosis of damaged endometrium; ↑reconstruction of the injured endometrium
	AS	Murine	Tail vein/uterus	↑Endometrial proliferation
	Ovarian damage	Rats	Ovarian	↑Ovarian function; ↓GC apoptosis; ↑Bcl-2 expression
En-MSCs	AS	Rats	Endometrial	↑Fertility rate; ↑pregnancy outcome; ↑angiogenic and anti-inflammatory factors
	POF/POI	Mice	Tail vein	↑Body weight; ↑estrous cyclicity; ↓GSC apoptosis; ↑ovarian function; ↑fertility rate
	POI	Murine	IV	↑Oocyte production; ↑AMH; ↑ovarian function
UC-MSCS	POF	Human	Ovarian	↑Ovarian function; ↑primordial follicles activation; ↑follicular development; ↑number of antral follicles; ↑successful pregnancy
	POF	Mice	Tail vein	↑Ovarian function; ↓ GC apoptosis; ↑estradiol; ↑number of follicles
	POF	Rats	Ovarian	↑FSH; ↓E2; ↑antral follicle count; ↑folliculogenesis; ↑Caspase-3 expression; ↑ovarian function
	POF	Rats	Ovarian	↑Restoration of estrus cycle; ↑number of ovarian follicles
	PCOS	Mice	Tail vein	↓Pathological changes; ↑ovarian function; fibrosis-related genes; ↓proinflammatory factors; ↓peripheral neutrophils; ↓M1 macrophages; ↓B cells; ↑M2 macrophages; ↑regulatory T cells
	POI	Mice	Ovarian	↑Ovarian functions; ↑ovarian weight; ↑weight of estrogen-dependent organs; ↑follicular number; ↓FSH; ↑AMH; ↑pregnancy numbers; ↑pregnancy rates
	POI	Rats	Laparoscopic	↑Proliferation of OSCs; ↓apoptosis of OSCs; ↑sexual hormone secretion; ↑estrus cycle duration; ↑follicle development; ↑fertility rate
	PMO	Rats	Tail vein	↑E2; ↑AMH; ↓FSH; ↑ovarian structure improvement; ↑follicle number; ↑ovarian reserve function
	EI	Rats	Uterus	↑Endometrial morphology; ↑embryo implantation rates; ↑fertility rate; ↑angiogenesis; ↑endometrial fibrosis; ↑endometrial cell proliferation
hA-MSCs	OPA	Mice	Ovarian	↑Ovary function; ↑follicle numbers; ↑hormone levels; ↑proliferation of ovarian granular cells; ↓ovarian granular cells apoptosis; ↑folliculogenesis activation; ↓DNA damage genes
	NOA	Mice	Ovarian	↑Ovarian function; ↑follicle numbers; ↑HGF; ↑EGF; ↑proliferation rate of ovarian granular cells; ↓apoptosis rate; ↓ovarian aging
	POF	Mice	Ovarian	↑Reproductive ability; ↓follicle atresia; ↑healthy follicles
	POF	Mice	Ovarian	↑Ovarian cells proliferation
	POF	Mice	Ovarian	↑Ovarian follicular atresia; ↓GC apoptosis
	POF	Mice	IV	↑Estrus cycle recovery; ↑estrogen; ↓FSH; ↑ovarian function; ↑GC proliferation; ↑fertility rate; ↑follicle count; ↑fertile offspring
	POF	Mice	Ovarian	↑Ovarian function; ↑regeneration of ovarian tissue; ↑AMH; ↑restored ovaries; ↑restored ovarian morphology; ↑number of oocytes; ↑GC number
En-MSCs	AS	Rats	Endometrial	↑Fertility rate; ↑pregnancy outcome; ↑angiogenic and anti-inflammatory factors
	POF/POI	Mice	Tail vein	↑Body weight; ↑estrous cyclicity; ↓GSC apoptosis; ↑ovarian function; ↑fertility rate
	POI	Murine	IV	↑Oocyte production; ↑AMH; ↑ovarian function
HSCs	AS	Human	Endometrial	↑Endometrial thickness; ↑menstruation restoration
	Cancer	Human	IV	↓Ovarian function; ↓entire follicle pool

Table 4. Continued

Stem cells	Disease	Models	Injection type	Main outcomes	I
hESC-MSCs	POF	Mice	Tail vein	↑Primordial follicle counts; ↓ovarian follicles apoptosis; ↑ovulation; ↑ovary function; ↑live births	(
hP-MSCs	POF	Mice	IV	↑Ovarian function; ↓GC apoptosis; ↑population of Treg cells; ↓serum TGF-β; ↑serum IFN-γ	(
hfL-MSCs	POI	Mice	Tail vein	↓Follicle loss; ↑sex hormone levels; ↓oxidative damage; ↑oxidative protection; ↑antiapoptotic effects; ↓expression of apoptotic genes; ↑GC activity; ↑follicular development	(
ESCs-Ex	POF	Mice	IV	↑Serum sex hormones; ↑number of follicles; ↓number of apoptotic cells; ↑GC proliferation rate; PI3K/AKT signaling pathway; ↑phosphorylated PI3K and AKT expression; ↑ovarian function	(
hA-ECs	POI	Rats	Ovarian	↑Body weight; ↑ovary weight; ↑normal estrous cycles; ↓FSH; ↑AMH; ↑follicle pools; ↑fetus number; ↑up-regulation of angiogenesis molecules, ↓inflammation molecules; ↑ovarian reserve and fertility	(
TSCs	POI	Monkeys	Ovarian	↑Sex hormone levels; ↑follicle development; ↑quality of oocytes; ↑oocyte maturation; ↑fertilization rate	(

Abbreviations: Ad-MSC, adipose-derived MSC; AMH, anti-Mullerian hormone; AS, Asherman syndrome; BM-MSC, bone marrow-derived MSC; E2, estradiol; EA, endometrial atrophy; EI, endometrial injury; ESCs-Ex: embryonic stem cells-derived exosomes; En-MSC, endometrial stem cell; EORS, Edessy ovarian reserve score; GC, granulosa cell; hA-EC, human amniotic epithelial cell; hA-MSC, human amniotic-derived MSC; hESC-MSC, human embryonic stem cells-derived MSC; hfL-MSC, fetal liver MSC; hP-MSC, human placenta-derived MSC; HSC, hematopoietic stem cell; MB-MSC, mens blood-derived MSC; MSC, mesenchymal stem cell; NOA, natural ovarian aging; OF, ovarian failure; OPA, ovarian physiologic aging; OSC, ovarian sor cell; P4, progesterone; PCOS, polycystic ovary syndrome; POF, premature ovarian failure; POI, premature ovarian insufficiency; PMO, peri-menopausa ovary; TC, Theca cell; TE, thin endometrium; UC-MSC, umbilical cord-derived MSC.

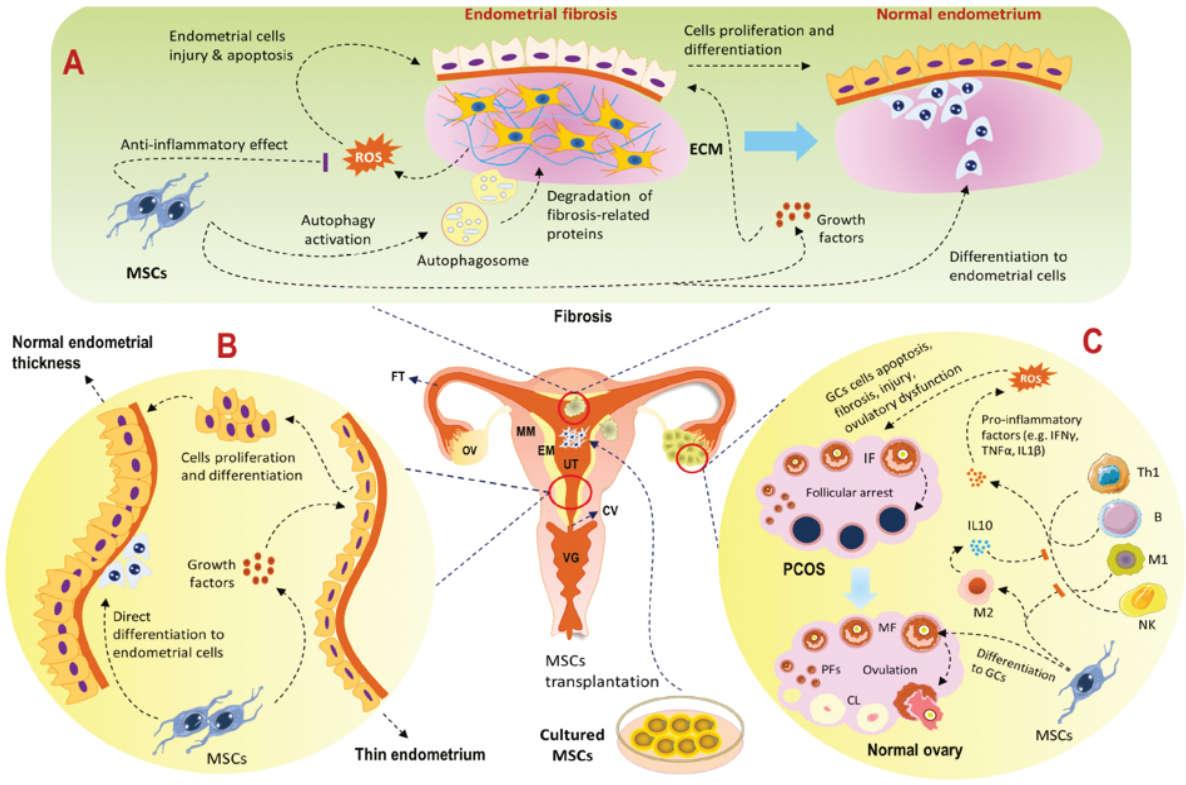

Figure 5. Role of mesenchymal stem cells in female reproduction. MSCs can be transplanted and can penetrate the uterine endometrium and participate in regeneration. (A) MSC therapy can improve endometrial fibrosis through inhibiting the mTORC1 pathway, activating autophagosomes in accumulated fibroblasts and consequently reducing ECM formation. MSC transplantation also protects normal endometrial cells against ROS and oxidative damage produced by accumulated fibroblasts or inflammatory cells via its anti-inflammatory activity. MSCs also repair endometrial fibrosis by direct differentiation to endometrial cells or secreting of paracrine factors. (B) MSC transplantation can improve endometrial thickness in cases with thin endometrium by inducing endometrial cell proliferation either by direct differentiation to endothelial cells or secreting paracrine factors. (C) MSCs can restore folliculogenesis and overall ovarian function in PCOS individuals through modulating macrophage polarization, immunomodulatory properties, anti-inflammatory and anti-oxidative effects, reducing ROS production, mitigating GC apoptosis, and fibrosis of the ovarian interstitium. B, B-lymphocytes; CL, corpus luteum; CV, cervix; ECM, extracellular matrix; EM, endometrium; FT, fallopian tube; IF, immature follicle; GC, granulosa cell; M1, M1 macrophage; M2, macrophage M2; MF, mature follicle; MM, myometrium; MSC, mesenchymal stem cell; NK, natural killer cell; OV, ovary; PCOS, polycystic ovary; PF, primary follicles; ROS, reactive oxygen species; Th1, T lymphocytes; TNF-α, tumor necrosis factor alpha; UT, uterus; VG, vagina.

Essential Points

• Despite advances in assisted reproductive technology (ART), it has remained an unsuccessful method for many forms of infertility.

• Stem cells can help infertile couples through regeneration of reproductive organs via cell-based therapy and/or generation of reproductive organoids or gonads in a dish for therapeutic approaches and diseases modeling.

• Mesenchymal stem cell (MSC) transplantation and exosome therapy may be a safe and effective option for restoring gametogenesis in patients with non-developmental disorders.

• Although very small embryonic-like stem cells (VSELs), spermatogonial stem cells (SSCs) and oogonial stem cells (OSCs) are invaluable cell sources for fertility treatment, their availability for infertility treatment is limited.

• Generating primordial germ cell-like cells (PGC-LCs) from patient-specific induced pluripotent stem cells (iPSCs) is likely the best option to overcome infertility in patients with developmental disorders of reproductive organs and impaired gametogenesis.

• Multi-organs-on-a-chip (MOOAC) platforms have enabled researchers to generate functional gametes following hiPSC-derived PGC-LCs co-culturing with testicular or ovarian somatic cells without adding growth factor

For the use or a Registered Medical Practitioner or a Hospital or a Laboratory only

Developed by:

Weston Medical Education Foundation of India

Office No:- 99, 9th Floor, Kalpataru Avenue, Opp. ESIC Hospital,
Kandivali (East), Mumbai - 400101. M: 9322615653 | W: www.wmefi.co.in