# Stem Cell Therapy for Infertility

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Stem cells are a subtype of cells that remain in undifferentiated form in embryos and in adult tissues and can self-renew and differentiate as and when required. Stem cells in differentiated organs contribute to the restoration of function through organ damage repair. According to their origin, stem cells are classified as embryonic stem cells (ESC), adult stem cells (includes mesenchymal stem cells MSC), induced pluripotent stem cells (iPSC), spermatogonial stem cells (SSCs), and ovarian stem cells. Stem Cells can be applicable for several disorders including infertility both in male and female.

infertility | mesenchymal stem cell | induced pluripotent stem cell | spermatogonial stem cell | assisted reproduction technology

## 1. Introduction

According to the international glossary, infertility is defined as “A disease characterized by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person’s capacity to reproduce either as an individual or with his/her partner.” Fertility interventions may be initiated in less than 1 year based on medical, sexual, and reproductive history, age, physical evaluation, and diagnostic testing. In developing countries, 1 in 4 couples suffers from infertility (WHO 2004). To overcome the shortcomings of the current treatment methods and considering the ability of stem cells to replenish damaged tissues via their self-renewal properties and ability to differentiate into multiple lineages, stem cells could be considered as a novel therapeutic measure for infertility [1][2].

## 2. Conventional Treatment

Conventional treatments improve fertilization rates inside the womb. Conventional treatments for male infertility are mainly focused on improving sperm quality, which depends on etiologic factors. Evidence-based medicine revealed a significant improvement in pregnancy rate after treatment with antioestrogens and gonadotropins therapy in cases of idiopathic male infertility [3]. Administration of antioxidants such as vitamin E and zinc along with assisted reproduction technology enhanced the rate of live offspring in infertile couples [4][5].

Previously, human menopausal gonadotropins, a mixture of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), were used. However, at present, more expensive but purer forms of recombinant gonadotropins are recommended. The risk of multiple pregnancies with injectable gonadotropins is much higher (30%) as compared

to orally administered clomiphene citrate or letrozole (7%). After the administration of ovulation-inducing drugs, regular follicular monitoring is carried out using ultrasonography in order to identify the time of formation of the dominant follicle (18–20 mm), which would be followed by ovulation trigger by intramuscular injection of βhCG [6].

Intrauterine insemination (IUI) is a conventional treatment used for both male and female infertility. After collection of semen from the male partner, highly motile sperm with normal morphology are isolated. The highest quality isolated sperm are injected within the uterine cavity with the help of a thin malleable catheter bypassing the cervix approximately 36 h after natural ovulation or triggered ovulation [6].

## 3. Implications for Stem Cells in Infertility

Even after recent progress in ART, many couples are unable to parent healthy babies except through gamete donation or adoption. Infertility due to gamete deficiency resulting from genetic defects does not benefit from ART. However, most couples seeking infertility treatment wish to have their own genetically related issues resolved [7], which could be less invasive and more cost-effective compared to ART. In this respect, stem cells have shown new hope to overcome the issues related to infertility in the form of cell-based therapies in various experimental preclinical and clinical models [8][9][10][11][12][13][14][15][16].

Stem cells are a subtype of cells that remain in undifferentiated form in embryos and in adult tissues and can self-renew and differentiate as and when required. Stem cells in differentiated organs contribute to the restoration of function through organ damage repair. According to their origin, stem cells are classified as embryonic stem cells (ESC), adult stem cells (includes mesenchymal stem cells MSC), induced pluripotent stem cells (iPSC), spermatogonial stem cells (SSCs) [17], and ovarian stem cells [18]. Stem cells collected from various organs explored to treat infertility are listed below:

ESCs play an important role in regenerative medicine because of their indefinite capacity to self-renew, ability to differentiate into all three lineages (ectoderm, endoderm, and mesoderm) and ability to maintain the normal karyotype during growth. It was documented that both mouse and human ESC can differentiate into primordial germ cells in vitro and subsequently undergo meiosis and give rise to male and female gametes [19]. the feasibility of developing functional sperm by using gene repaired ESC isolated from cloned blastocysts that were originated from nuclear transferred somatic cells (ntESC) using gene repair technology [20]. However, because of ethical concerns, even after initial derivation, it is not so commonly used in cell replacement therapy.

Various studies have been conducted for the differentiation of iPSCs to male germ cells in vitro. Next, they harvested the cells in the presence of forskolin, human recombinant leukemia inhibiting factor (LIF), and the CYP26 inhibitor R115866 for 2, 3, and 4 more weeks [21]. They documented that iPSCs transplanted into seminiferous tubules differentiated into germ cell-like cells (GCLCs), whereas cells outside the tubule could not result in GCLCs. They isolated the human PGCs from other cells with the help of markers present on PGCs such as EpCAM

In their studies, at first, they differentiated somatic cells into iPSC by reprogramming and then generated incipient mesoderm-like cells (iMeLCs) in the presence of Activin A and Chiron. These hPGCLCs were mixed with gonadal cells isolated from female mouse embryonic ovaries and cultured for 4 months to obtain oogonia by in vitro gametogenesis. This technique will bring a revolution in reproductive medicine through the generation of oogonia from human pluripotent stem cells. [22], on the other hand, documented the mechanistic explanation of differentiation of human pluripotent stem cells to heterogeneous mesoderm-like cells in the presence of cytokines followed by the generation of PGC-like cells (PGCLCs), and the PGCLCs are committed to developing gametes (sperm or eggs) that were associated with a unique expression of PRDM14 [22].

In spite of promising results obtained from experimental models, the teratogenic potentiality of iPSCs and their derivatives resulting from reprogramming by induction with oncogenes (for example, c-myc) and use of nucleic acid integration procedures restrict their clinical application towards personalized cell-based therapy [23]. Moreover, the persistence of residual epigenetic impressions and gene silencing, as well as genomic instability, might be a potential hurdle that further limits their therapeutic application [24][25].

Although the iPSC technology does not destroy the embryos, the potentiality to exploit the embryo generated from gametes developed after iPSCs reprogramming demand ethical clearance from the institutional review board as well as the collection of informed consent from the cells or tissue donor prior to acquiring any sample for development of iPSC for clinical trials as well as research purpose. In addition, for their application in animal models, approval is required from IACUC.

According to the mesenchymal and tissue stem cell committee of the International Society for Cellular Therapy, MSCs are defined as cells that have plastic-adhesion properties, express CD105, CD73 and CD90 as surface markers, and can differentiate into osteoblasts, adipocytes and chondroblasts [26]. Depending on their origin, MSCs are categorized as bone marrow stromal cells, adipose-derived stem cells, menstrual-blood-derived MSCs and umbilical-cord-derived MSCs [2], amniotic-fluid-derived MSCs, placental-tissue-derived MSCs, salivary-gland-derived MSCs, and dental-pulp-derived MSCs [1]. MSCs travel to the damaged ovary and settle there to restore ovarian function through the release of various cytokines by paracrine mechanisms. Cytokines such as vascular endothelial growth factor, insulin-like growth factor, and hepatocyte growth factor induce the formation of new vessels, prevent apoptosis as well as fibrosis and thus ameliorate ovarian dysfunction.

On the contrary, fetal subpopulations of MSCs exhibit a higher proliferation rate leading to rapid expansion and efficient differentiation to multilineages as well as strong immunomodulatory properties [27]. Augmented telomerase activity might contribute to better performance and prolonged survival of the fetal MSCs (FMSC) compared to adult MSCs [27]. [28] documented that FMSCs could ameliorate ovarian follicular function in a cyclophosphamide-induced mice model via modulation of melatonin membrane receptor 1 and antioxidant enzyme activity. Some of the sources used to derive MSCs used for the treatment of infertility are listed below:

After its initial documentation in 1988 by Owen and Friedenstein [29], the isolation of MSCs from bone marrow was carried out by density gradient centrifugation and then incubated in a growth medium for expansion [30]. These

BMSCs are not only differentiated to mesodermal lineages but also committed to the development of endometrial and follicular cells in rat models [31][32].

[33] explored whether BMSCs could contribute to endometrial regeneration in the experimental rat model. They transplanted BMSCs through rat tail intravenous injection and observed a significant increase in thickness of endometrium along with enhanced expression of various markers, cytokeratin, vimentin, integrin αγβ3, and leukemia inhibitor factor. It was observed that in both cases, BMSCs could effectively heal the endometrial injury, as evidenced by the increase in the number of endometrial glands and decrease in the fibrotic area through upregulation of estrogen and progesterone receptor expression [34]. [10] conducted a prospective, experimental pilot study on patients with Asherman's syndrome/endometrial atrophy and reported that injection of CD133+ BMSCs into the spiral arterioles of recruited patients could improve endometrial thickness, the intensity of matured blood vessels, and the nature of menstruation of infertile patients.

Menstrual-blood-derived MSCs (MB-MSCs) have the potential to proliferate and differentiate into multiple lineages and can self-renew similar to other stem cells. Moreover, the collection of these cells is noninvasive, safe, and easy, without ethical issues and with minimal immune reactions, facilitating their clinical application in reproductive medicine compared to other tissue-derived stem cells.

[35] reported the impact of MB-MSCs on cyclophosphamide-induced premature ovarian failure (POF) in a mouse model. They have shown that MB-MSC treated groups of animals showed an increase in the number of the normal ovarian follicles and restoration of normal ovarian function represented by a higher level of ovarian hormones such as estradiol, antimullerian hormone and inhibin α/β compared to control. In their studies, they documented that MB-MSC improved ovarian function through localization of MSCs into granulosa cells and by augmentation of the expression of FSH receptors as well as restoration of hormone levels [11]. It was documented that MSC derived from menstrual blood could resume fertility in an animal model of damaged endometrium through induction of angiogenesis and release of anti-inflammatory factors [12].

Moreover, MB-MSCs could improve epirubicin (broad-spectrum anti-cancer drug)-induced ovarian damage and thus promote the multiplication of granulosa cells through upregulation of Cyclin B1 and CDC2 protein expression and downregulation of Gadd45b protein expression [36].

A clinical study conducted by Tan et al. [37] reported on seven patients with resistant Asherman syndrome treated with MB-MSC transplantation followed by hormonal therapy; out of these seven patients, five patients attained endometrial thickness of 7 mm, one out of these five patients achieved pregnancy spontaneously and two conceived after assisted reproduction technology [36]. The role of the OCT-4 transcription factor on differentiation of MSCs documented in animal models was supported by the decreased expression of OCT-4 observed in MB-MSCs isolated from patients with severe intrauterine adhesion [38].

Following the initial introduction of endometrial stem cells by Prianishnikov and his colleagues in 1978 [39], Chan and his colleagues in 2004 [40] isolated stem cells from endometrial tissues and identified the colonization

properties of these cells in vitro. The EndSC microenvironment is comprised of stromal cells, epithelial progenitor cells and endothelial cells. However, in the presence of inflammation or damage to the endometrium, these stem cells were directed to the injured site following interaction between chemokines and CXCR4, which is expressed on stem cells, and CXCL12, which is expressed by the epithelium of endometrial tissues [41]. Endometrial stem cells act as the origin of cells that help in replenishing the endometrium, such as mesenchymal stem cells (MSCs), epithelial stem cells, endometrial side population cells (ESP), and endometrial regenerative cells (ERC)

Therapeutic implications of endometrial stem cells have been documented in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced model of Parkinson's Disease [42] to observe if it can restore dopamine release and in a murine model of diabetes mellitus [43]. Mechanistic explanations and roles of endometrial stem cells in the restoration of fertility are summarized in **Figure 1**. Modified from references [44][45].

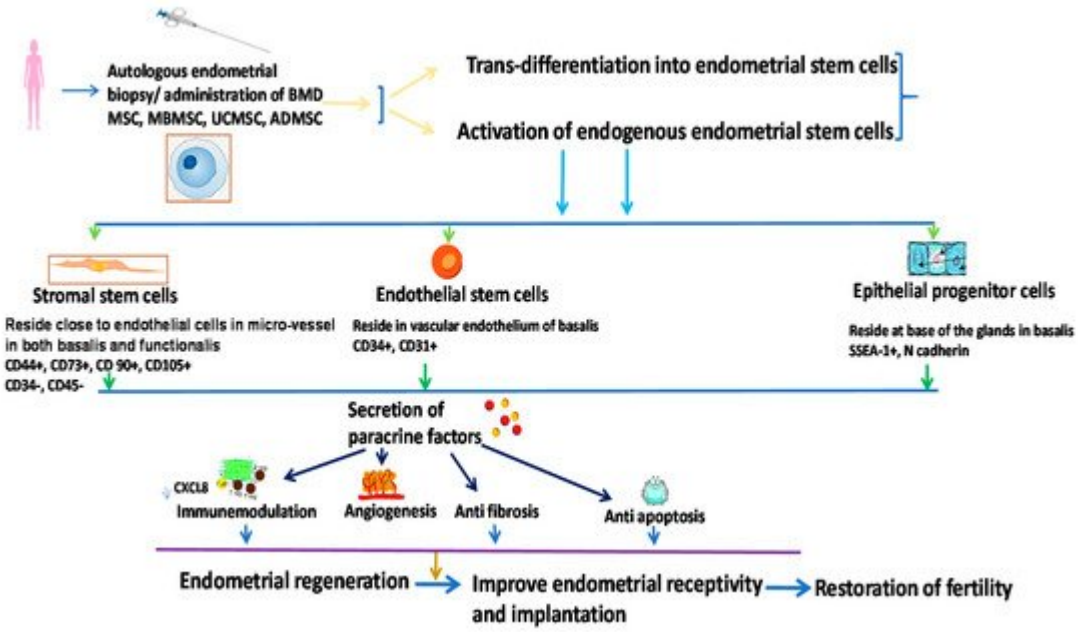


**Figure 1.** The mechanistic explanation of stem cell therapy for restoration of fertility via endometrial regeneration; BMDMSC: Bone-marrow-derived mesenchymal stem cell; MB MSC: menstrual blood mesenchymal stem cell; UC MSC: umbilical cord mesenchymal stem cell; ADMSC: adipose-tissue-derived mesenchymal stem cell; CXCL: C-X-C motif chemokine ligand; SSEA-1: Stage-specific embryonic antigen-1; CD: Cluster of differentiation. Modified from references [44][41].

Wharton's jelly of umbilical-cord-derived MSCs showed the presence of CD29, CD44, CD73, CD90, and CD105 expression, with the absence of CD31, CD45, and HLA-DR85. Because of low tumorigenicity, rapid self-renewal capacity committed to various mesodermal cell types, easy availability from usually-discarded resources, minimum ethical concerns, and low immunogenicity, UC-MSCs have emerged as a popular cell-based therapeutic approach for restoration of fertility [2].

In various experimental animal models of premature ovarian insufficiency, UC-MSCs restored ovarian function through its antiapoptotic activity against granulosa cells and modulation of hormone levels, for example, reducing

FSH levels while augmenting estrogen and progesterone levels [46][47]. UC-MSCs could restore ovarian function in paclitaxel-induced ovarian dysfunction through the provocation of ovarian surface epithelium stability and modulation of the expression of Cyto Keratin 8/18, TGF-ß, and proliferating cell nuclear antigen (PCNA), which are critical regulators of follicular synthesis and the antiapoptotic activity of UC-MSCs [48]. UC-MSCs also emerged to be beneficial in the revival of infertility in a mouse model of PCOS induced by dehydroepiandrosterone via inactivation of proinflammatory cytokines, for instance, IL- 1β, tumor necrosis factor alpha (TNFα), and interferon gamma (IFN-γ)

The beneficial effect of UC-MSCs in the improvement of fertility might involve various signaling pathways. UC-MSCs prevent apoptosis of granulosa cells to improve fertility via the activation of mitogen-activated protein kinase signaling pathway, G protein-coupled receptor, and insulin signaling pathways [49]. UC-MSCs restored fertility in an animal model of damaged endometrium through the increase and decrease in vascular and inflammatory factors, respectively [50]. Similarly, transplantation of UC-MSCs on a collagen scaffold in a rat model of uterine injury could rescue fertility via activation of matrix metalloproteinase 9 [51].

Administration of UC-MSCs on a collagen scaffold could restore ovarian function in patients suffering from premature ovulatory failure (POF) as confirmed by the increase in follicle number through activation of primordial follicles via phosphorylation of transcription factor forkhead box protein O3a (FOXO3a) and FOXO1 [52]. Yang et al. [15] documented that Wharton's jelly-derived MSC could improve proliferation and prevent apoptosis of mifepristone-induced damage in endometrium collected from patients undergoing hysterectomy via upregulation of VEGF expression and downregulation of caspase 3 and 8 [15]. [19] conducted a single-center, prospective, randomized, double-blind, placebo-controlled phase II clinical trial and observed the safety and efficacy of intramuscular injection of UC-MSCs for the treatment of uterine scars caused by caesarean section.

Their immune regulatory properties, lack of ethical concern, plus ability to differentiate into various cell types such as osteocytes, muscle, and adipocytes make the AFSCs highly useful for regenerative medicine. Various animal studies have documented the therapeutic role of AFSCs in muscular and bone-related disorders [53][54]. Previous studies revealed that AFSCs could ameliorate infertility through the restoration of ovarian function via the activation of various signaling molecules such as VEGF, TGFα and β, epidermal growth factor (EGF), and bone morphogenic protein (BMP) [49]. Various studies on a mouse model of POF induced by chemotherapy documented the role of AFSCs, especially having CD4C/CD105+ markers, on the prevention of follicular atresia, reestablishment of healthy oocytes, and thus preserving ovarian function [55][56].

The restorative effect of ADMSCs on ovarian dysfunction in chemotherapy-mediated animal models of POF has been documented in various studies [57][58]. AmDMSCs restore ovarian function by attenuating apoptosis and improving the multiplication of granulosa cells and neovascularization in the surrounding environment to some extent by paracrine mechanisms. Moreover, AmDMSC treatment accomplished its function via suppression of inflammation by inactivating cytokines IL-1β, IL-6 and TNFα. [59] documented that surprisingly, human ADMSCs, after treatment with low-intensity pulsed ultrasound, become more efficient in the revival of ovarian function in a rat model of POF.

Various studies conducted on an animal model of POF revealed that PDMSCs produced their beneficial effects on folliculogenesis through modulation of cytokines and various hormone levels such as estradiol, LH, AMH, and FSH and their receptors' expression by activation of PI3K/Akt signaling pathway [60][61]. On the other hand, Li et al. (IRE1)α signaling pathway in PDMSC improved ovarian dysfunction [62]. [63] demonstrated in ovariectomized rats that PDMSCs are more effective in 3D spheroid formation to produce their restorative function [63].

ADMSCs are a novel type of MSCs having almost similar biological properties to MSC derived from other sources but having more advantages compared to BMSC because such tissues can be collected with less invasive procedures and in larger quantity [64]. Therefore, these are widely used for a broad spectrum of clinical conditions in reparative medicine [65][66].

One study observed the impact of ADMSCs on ovarian grafts in a female animal model and revealed that ADMSCs restored ovarian function faster through augmentation of VEGF gene expression and promotion of angiogenesis in the grafted tissue [67]. ADMSCs could restore ovarian function in chemotherapy-mediated ovarian damage in a mouse model through neovascularization and promotion of ovarian follicular proliferation [68]. However, administration of ADMSCs on a collagen scaffold in an ovarian insufficiency rat model revealed better retention of ADMSCs, giving rise to the longer resumption of ovarian function and better pregnancy rate compared to ADMSC injection alone [69]. Hence, ADMSCs could be considered an effective alternative therapeutic tool for the restoration of fertility for infertile couples, depicted in **Figure 1**.

The invention in findings of the rate of follicular atresia along with the death of oocytes and depletion of ovarian reserve in mice brought the concept of the existence of OSCs. Subsequently, they also documented that these cells were able to induce synthesis of follicles after xenotransplantation into diabetic immunocompromised mice [70]. However, these cells remained unidentified for a long time, probably because of their very low number, constituting only 0.0145% of the total cell population in mouse ovary that declines gradually with age. From a previous study on the isolation of OSCs from old mice and subsequent folliculogenesis following transplantation in young mice [71], it could be speculated that these OSCs could show new hope to those with idiopathic premature ovarian failure.

The therapeutic implication of OSCs include restoration of fertility and giving birth to live offspring documented in age-associated infertility and premature ovarian failure in childhood cancer survivors following exposure to chemotherapy [18][72].

SSCs play a crucial role in maintaining the highly productive complex process known as spermatogenesis in the seminiferous tubule through self-renewal and unlimited differentiation into spermatogonia followed by haploid spermatozoa [73]. In an experimental model, it was observed that SSCs are derived from primitive germ cells that travel to the gonadal ridge during embryonic development and then lodge into the seminiferous tubules of adult testicles to help in lifelong sperm generation [74][75]. Various biomedically active factors, either alone or in combination with others, have been used for in vitro induction of potential stem cells into germ cells: retinoic acid for proper timing in the initiation of meiosis, CYP26 inhibitor for control of meiosis through modulation of the STRA8

gene [76], testosterone for the potential release of stem cell factor to induce germ cell differentiation [77], forskolin to promote proliferation of germ cells [78], and leukemia inhibiting factor (LIF) to keep gonocytes alive [79]. Some studies carried out both in vitro induction of stem cells and subsequent transplantation into male mice that were rendered infertile to prove the ability of SSCs to form colonies within the testes as well as to differentiate into male gametes through highly coordinated spermatogenesis [80].

The main drawback of this cell-based therapy in reproductive medicine is that this procedure may disturb the environment of the testes, resulting in a decline in acceptability of SSC transplantation, giving rise to treatment failure [81].

## 4. Stem Cell Therapy in Some Known Syndromes

Syndromes leading to infertility are shown in **Figure 2** and discussed below:

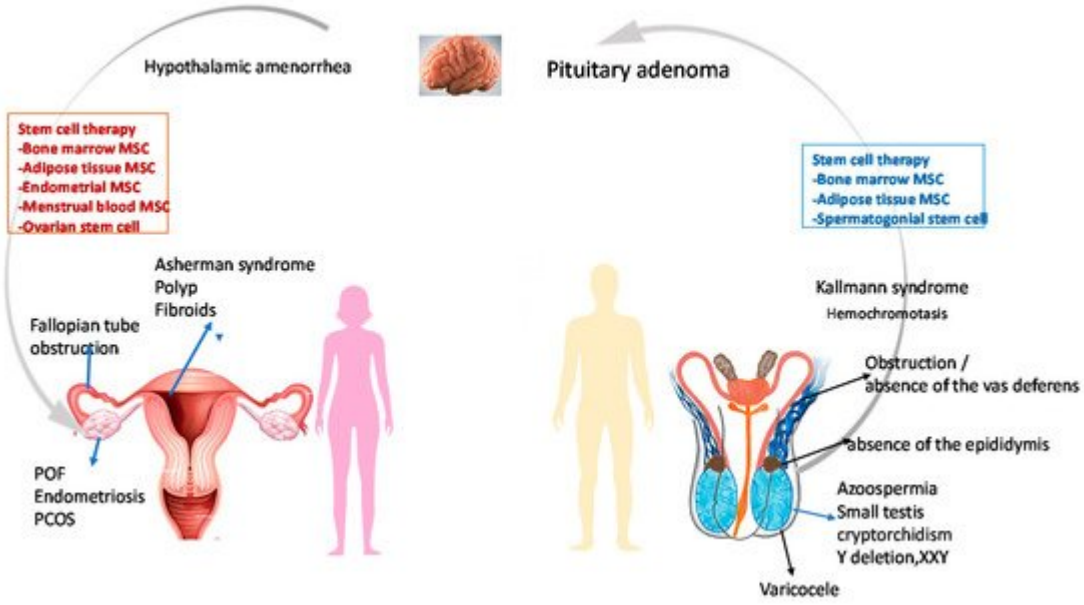


**Figure 2.** Some probable causes of infertility in male and female POF: premature ovarian failure, PCOS: Polycystic ovarian syndrome, XXY: Klinefelter syndrome, MSC: Mesenchymal stem cell. Modified from references Lindsay and Virtikas [2] and Zhao et al. [82].

Various diagnostic approaches are available to diagnose this syndrome, including hysterosalpingography (HSG), 3D ultrasonography, hysteroscopy, and magnetic resonance imaging (MRI), with hysteroscopy being the gold standard. Common treatments of infertility resulting from AS include restoration of the uterine cavity through hysteroscopic adhesiolysis and replenishment of normal endometrial lining with hormonal therapy [83][84]. Surgical treatment of AS has been found to be associated with various obstetric complications such as preterm delivery [85]. Hence, the use of stem cell therapy to treat AS has been evaluated.

The use of stem cells derived from bone marrow [86], autologous menstrual blood [37], as well as mesenchymal stem cells [87] for the replenishment of endometrium, has been worked out in various animal models [88] as well as clinical studies [89]. It was observed that endometrial MSCs derived from menstrual blood could improve fertility in an animal model of damaged endometrium [12]. Various studies reported that women with AS attained regular menstruation [90] and endometrial regeneration following transplantation of menstrual-blood-derived stem cells [37].

With the increase in cancer survivors, the incidence of POI is also increased proportionately. However, in these cases, restoration of fertility has been tried by cryopreservation of oocytes prior to gonadotoxic treatment (Ethics Committee of American Society for Reproductive Medicine, 2013). Several studies have documented that fertility could be preserved in POI cases by ovarian vitrification followed by in vitro activation by stimulation of the AKT pathway followed by re-transplantation [91][92][93]. However, the successful demonstration of ovarian stem cells both in experimental animal and human models might bring revolution in the potential therapy for restoration of infertility in patients with POI [94]

PCOS, a gynecological disorder of reproductive-aged women resulting from hormonal imbalance, was initially diagnosed by Rotterdam using two criteria: “(1) presence of high levels of androgens, and (2) ovulatory dysfunction, or polycystic ovaries”. Patients with PCOS commonly present with irregular menstruation, obesity, abnormal hair growth on the body and infertility/subfertility [95]. Hormonal contraceptives and clomiphene citrate are the first-line therapy for restoration of normal menstruation and fertility, respectively [95]. MSCs could attenuate DHEA-mediated PCOS in mouse [96] and human theca cells from PCOS patients [97] by suppression of inflammation via the release of anti-inflammatory cytokines.

Endometriosis is an estrogen-dependent complex disorder affecting women of reproductive age; approximately 10% of women suffer from endometriosis, and approximately 50% of those women with endometriosis have trouble achieving pregnancy [98]. According to The Practice Committee of the American Society for Reproductive Medicine, 2012, the fecundity rate is significantly decreased in couples with endometriosis compared to normal fertile couples [99]. The principal speculations related to the pathogenesis of endometriosis include retrograde menstruation, coelomic metaplasia and metastatic spread, altered immunity, stem cells, and genetics [100]. Endometriosis with its inflammatory response and increased release of cytokines could affect fertility via alteration of ovum formation, gamete transport through impairment of tubal movement, and endometrial development through modulation of the Wnt pathway [101].

A previous study revealed that initial treatment with GnRH agonist followed by IVF with or without ICSI could significantly improve pregnancy rates in infertile couples with endometriosis [102]. [103] did not find significant improvement in fertility with the initial administration of hormonal therapy [103]. Surgical treatment alone or in combination with medical treatment showed different responses. However, recent experimental evidence in both animal and human models has documented promising results of stem-cell-based therapy for the restoration of fertility in patients with endometriosis [100]

Azoospermia, characterized by the absence of mature morphologically normal and functional sperm in the ejaculate, contributes approximately 15% of infertility solely related to male factors [104]. Based on history, detailed physical examination, hormonal assay and genetic testing, azoospermia can be divided into two subtypes: obstructive (OA) and nonobstructive (NOA) azoospermia. However, NOA results in testicular failure, which could be due to pathology primarily in the testis or secondary due to decreased release of gonadotropin from the pituitary. In the case of NOA, the success rate of ART by intracytoplasmic injection is very poor because of the difficulty in retrieving functional sperm.

Spermatogonial stem cells (SSCs), having self-renewal properties, can differentiate into haploid spermatids and finally undergo maturation to spermatozoa and thus might play an important role in promoting sperm production to solve this prevalent cause of male factor infertility [105]. [106] demonstrated that SSCs injected into the rete testes of pre-pubertal rhesus monkeys undergo a transformation into mature spermatozoa along with the maturation of the monkey; the spermatozoa present in the ejaculate and have fertilization capability [106]. However, SSC transplantation could not be replicated in humans because of challenges associated with the identification of SSCs in the testes, lack of proper culture and preservation protocol for SSCs, and safety concerns for the recipients following transplantation [107]. Other than SSCs, embryonic and adult stem cells were demonstrated to have the ability to be differentiated to germ cells [108] and have the ability to fertilize in animal models [109][110].

Various clinical trials (NCT02025270, NCT02641769, NCT02414295) have been performed or are underway on injection of bone-marrow-derived MSCs to the rete testis of azoospermia patients to assess hormonal levels as well as testicular size along with sexual potency (see **Table 1**).

**Table 1.** Clinical trials related stem cell therapy performed or underway for improvement of infertility.

| Trial Identifier | Est. # of Subjects | Status | Site | Conditions | Interventions | Outcome of Trial |
|---|---|---|---|---|---|---|
| NCT04706312 | 12 | Not yet recruiting | Nanjing Medical University | Diminished Ovarian Response | Human Amniotic Mesenchymal Stem Cells (Hamscs) Transplantation | No results posted |
| NCT04676269 | 40 | Recruiting | Indonesia University | Thin Endometrium Infertile Patients | Amnion Bilayer and Stem Cell Combination Therapy | No results posted |
| NCT03207412 | 20 | Unknown | Chongqing Medical University, China | Premature Ovarian Failure | Human Amniotic Epithelial Cells | No results posted |
| NCT02696889 | 3 | Active | University of Illinois at | Primary Ovarian | Autologous Stem Cell | Report of 2 cases revealed |

| Trial Identifier | Est. # of Subjects | Status | Site | Conditions | Interventions | Outcome of Trial |
|---|---|---|---|---|---|---|
| | | | Chicago | Insufficiency, Low Ovarian Reserve | Therapy | a significant improvement in clinical features related to POI. There was an increase in size as well as estrogen production in the MSC engrafted ovary [111] |
| NCT02713854 | 240 | Recruiting | The University of Hong Kong | Subfertility | Human Embryonic Stem-Cell-Derived Trophoblastic Spheroid (Bap-Eb) as a Predictive Tool | No results posted |
| NCT03592849 | 50 | Enrolling by invitation | Nanjing Drum Tower Hospital, China | Infertile Women with Thin Endometrium or Endometrial Scarring | Procedure: Collagen Scaffold Loaded with Umbilical-Cord-Derived Mesenchymal Stem Cells Therapy | No results posted |
| NCT03166189 | 46 | Completed | D.O. Ott Research Institute of Obstetrics, Gynecology, Russian Federation | Infertility of Uterine Origin Asherman Syndrome | Biological: Bone Marrow-Derived Msc and Hrt<br>Other: Hormonal Replacement Therapy | No Results Posted |
| NCT02313415 | 26 | Completed | Nanjing Drum Tower Hospital, China | Infertility with Intrauterine Adhesions | Procedure: Umbilical Cord Mesenchymal Stem Cells | Phase 1 trial revealed that transplantation of clinical grade human UC MSC could improve the proliferative |

| Trial Identifier | Est. # of Subjects | Status | Site | Conditions | Interventions | Outcome of Trial |
|---|---|---|---|---|---|---|
| | | | | | | and differentiation efficiency of endometrium [112] |
| NCT02025270 | 100 | Unknown | Al Azhar University, Egypt | Azoospermic Patients | Bone-Marrow-Derived Mesenchymal Stem Cells | No results posted |
| NCT02641769 | 50 | Recruiting | Stem Cells of Arabia, Amman, Jordan | Non-obstructive Azoospermia | Intratesticular Transplantation of Autologous Stem Cells | No results posted |
| NCT02414295 | 1 | Completed | Man Clinic for Andrology and male infertilit, Cairo, Egypt | Klinefelter Syndrome Azoospermia | Mesenchymal Stem Cell Injection | No Results Posted |
| NCT02062931 | 60 | Unknown | Al-Azhar University hospitals, Egypt | Premature Ovarian Failure | Biological: Stem Cell Preparation and Injection | No results posted |
| NCT02603744 | 9 | Unknown | Royan Institute | Premature Ovarian Failure | Intraovarian Injection of Adipose-Derived Stromal Cells (Adscs) | Intraovarian engrafting of ADSCs were found to be safe and feasible and linked to reduction in FSH level [113] |
| NCT02204358 | 30 | Unknown | Nanjing University Medical School | Intrauterine Adhesions, Endometrial Dysplasia | Collagen Scaffold Loaded with Autologous Bone Marrow Stem Cells | No results posted |
| | | | | | Testicular Injection of Autologous | |

| Trial Identifier | Est. # of Subjects | Status | Site | Conditions | Interventions | Outcome of Trial |
|---|---|---|---|---|---|---|
| | | | | | Human Bone Marrow | |
| NCT02041910 | 60 | Unknown | Hesham Saeed Elshaer, El-Rayadh Fertility Centre | Azoospermia | Derived Stem Cells | No results posted |
| NCT02151890 | 10 | Completed | Al Azhar University, Cairo, Egypt | Premature Ovarian Failure | Biological: Stem Cell | No results posted |
| NCT02372474 | 112 | Completed | Al Azhar University, Cairo, Egypt | Premature Ovarian Failure | Biological: Stem Cell | No results posted |
| NCT01742533 | 40 | Unknown | Shenzhen People's Hospital, Shenzhen, Guangdong, China | Premature Ovarian Failure | Biological: Human Umbilical Cord Mesenchymal Stem Cells and Human Cord Blood Mononuclear Cells | No results posted |
| | | | | | Drug: Hormone Replacement Therapy | |
| NCT03069209 | 50 | Active, not recruiting | Stem Cells Arabia, Amman, Jordan | Premature Ovarian Failure | Biological: Stem Cells | No results posted |
| NCT00429494 | 60 | Completed | UT MD Anderson Cancer Center, United States | Amenorrhea | Procedure: Hematopoietic Stem Cell Transplantation (Hsct) | Phase II trial revealed that Leuprolide could not preserve ovarian function in HSCT patients [114] |
| | | | | Premature Ovarian Failure | Drug: Leuprolide Acetate | |
| | | | | Ovarian Function | Behavioral: Questionnaire | |

| Trial Identifier | Est. # of Subjects | Status | Site | Conditions | Interventions | Outcome of Trial |
|---|---|---|---|---|---|---|
| | | | | Insufficiency | | |
| NCT04009473 | 100 | Enrolling by invitation | Multicenter | Ovarian Failure<br>Premature Ovarian Failure | Combination Product: SEGOVA Procedure Includes Stem Cell Therapy, Growth Factor, and Platelet Plasma Rich Therapy | No results posted |
| NCT02240823 | 30 | Unknown | Odense University Hospital | Erectile Dysfunction After Prostatectomy | Adipose-Derived Stem Cells (ADMSC) | Intracavernous injection of ADMSC is a safe procedure and resulted in improvement of erectile function [115] |
| NCT02414308 | 20 | Unknown | Man Clinic for Andrology, Male Infertility, and Sexual Dysfunction | Erectile Dysfunction Peyronie' Disease | Adipose Tissue Stem Cell Injection | No results posted |
| NCT02008799 | 20 | Recruiting | Man Clinic for Andrology, Male Infertility, and Sexual Dysfunction | Azoospermia | Intratesticular Artery Injection of Bone Marrow Stem Cell | No result posted |

## References


1. Esfandyari, S.; Chugh, R.M.; Park, H.-S.; Hobeika, E.; Ulin, M.; Al-Hendy, A. Mesenchymal stem cells as a bio organ for treatment of female infertility. Cells 2020, 9, 2253.
2. Zhao, Y.-X.; Chen, S.-R.; Su, P.-P.; Huang, F.-H.; Shi, Y.-C.; Shi, Q.-y.; Lin, S. Using mesenchymal stem cells to treat female infertility: An update on female reproductive diseases. Stem Cells Int. 2019.

3. Attia, A.M.; Abou-Setta, A.M.; Al-Inany, H.G. Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst. Rev. 2013.
4. Showell, M.G.; Mackenzie-Proctor, R.; Brown, J.; Yazdani, A.; Stankiewicz, M.T.; Hart, R.J. Antioxidants for male subfertility. Cochrane Database Syst. Rev. 2014.
5. Ayeleke, R.O.; Asseler, J.D.; Cohlen, B.J.; Veltman, S.M. Intra-uterine insemination for unexplained subfertility. Cochrane Database Syst. Rev. 2020.
6. Dodson, W.C.; Haney, A. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of infertility. Fertil. Steril. 1991, 55, 457–467.
7. Fang, F.; Li, Z.; Zhao, Q.; Li, H.; Xiong, C. Human induced pluripotent stem cells and male infertility: An overview of current progress and perspectives. Hum. Reprod. 2018, 33, 188–195.
8. Abd-Allah, S.H.; Shalaby, S.M.; Pasha, H.F.; Amal, S.; Raafat, N.; Shabrawy, S.M.; Awad, H.A.; Amer, M.G.; Gharib, M.A.; El Gendy, E.A. Mechanistic action of mesenchymal stem cell injection in the treatment of chemically induced ovarian failure in rabbits. Cytotherapy 2013, 15, 64–75.
9. Mohamed, S.A.; Shalaby, S.M.; Abdelaziz, M.; Brakta, S.; Hill, W.D.; Ismail, N.; Al-Hendy, A. Human mesenchymal stem cells partially reverse infertility in chemotherapy-induced ovarian failure. Reprod. Sci. 2018, 25, 51–63.
10. Santamaria, X.; Cabanillas, S.; Cervello, I.; Arbona, C.; Raga, F.; Ferro, J.; Palmero, J.; Remohí, J.; Pellicer, A.; Simón, C. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman’s syndrome and endometrial atrophy: A pilot cohort study. Hum. Reprod. 2016, 31, 1087–1096.
11. Manshadi, M.D.; Navid, S.; Hoshino, Y.; Daneshi, E.; Noory, P.; Abbasi, M. The effects of human menstrual blood stem cells-derived granulosa cells on ovarian follicle formation in a rat model of premature ovarian failure. Microsc. Res. Tech. 2019, 82, 635–642.
12. Domnina, A.; Novikova, P.; Obidina, J.; Fridlyanskaya, I.; Alekseenko, L.; Kozhukharova, I.; Lyublinskaya, O.; Zenin, V.; Nikolsky, N. Human mesenchymal stem cells in spheroids improve fertility in model animals with damaged endometrium. Stem Cell Res. Ther. 2018, 9, 50.
13. Wang, S.; Yu, L.; Sun, M.; Mu, S.; Wang, C.; Wang, D.; Yao, Y. The therapeutic potential of umbilical cord mesenchymal stem cells in mice premature ovarian failure. Biomed Res. Int. 2013.
14. Fan, D.; Wu, S.; Ye, S.; Wang, W.; Guo, X.; Liu, Z. Umbilical cord mesenchyme stem cell local intramuscular injection for treatment of uterine niche: Protocol for a prospective, randomized, double-blinded, placebo-controlled clinical trial. Medicine 2017, 96, e8480.
15. Yang, X.; Zhang, M.; Zhang, Y.; Li, W.; Yang, B. Mesenchymal stem cells derived from Wharton jelly of the human umbilical cord ameliorate damage to human endometrial stromal cells. Fertil. Steril. 2011, 96, 1029–1036.E4.

16. Zhu, S.F.; Hu, H.B.; Xu, H.Y.; Fu, X.F.; Peng, D.X.; Su, W.Y.; He, Y.L. Human umbilical cord mesenchymal stem cell transplantation restores damaged ovaries. J. Cell. Mol. Med. 2015, 19, 2108–2117.
17. Wang, J.; Liu, C.; Fujino, M.; Tong, G.; Zhang, Q.; Li, X.-K.; Yan, H. Stem cells as a resource for treatment of infertility-related diseases. Curr. Mol. Med. 2019, 19, 539–546.
18. Dunlop, C.E.; Telfer, E.E.; Anderson, R.A. Ovarian stem cells—Potential roles in infertility treatment and fertility preservation. Maturitas 2013, 76, 279–283.
19. Kehler, J.; Hübner, K.; Garrett, S.; Schöler, H.R. Generating oocytes and sperm from embryonic stem cells. Semin. Reprod. Med. 2005, 23, 222–233.
20. Yuan, Y.; Zhou, Q.; Wan, H.; Shen, B.; Wang, X.; Wang, M.; Feng, C.; Xie, M.; Gu, T.; Zhou, T. Generation of fertile offspring from Kit w/Kit wv mice through differentiation of gene corrected nuclear transfer embryonic stem cells. Cell Res. 2015, 25, 851–863.
21. Eguizabal, C.; Montserrat, N.; Vassena, R.; Barragan, M.; Garreta, E.; Garcia-Quevedo, L.; Vidal, F.; Giorgetti, A.; Veiga, A.; Belmonte, J.I. Complete meiosis from human induced pluripotent stem cells. Stem Cells 2011, 29, 1186–1195.
22. Sugawa, F.; Araúzo-Bravo, M.J.; Yoon, J.; Kim, K.P.; Aramaki, S.; Wu, G.; Stehling, M.; Psathaki, O.E.; Hübner, K.; Schöler, H.R. Human primordial germ cell commitment in vitro associates with a unique PRDM14 expression profile. EMBO J. 2015, 34, 1009–1024.
23. Lee, A.S.; Tang, C.; Rao, M.S.; Weissman, I.L.; Wu, J.C. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. Nat. Med. 2013, 19, 998–1004.
24. Wu, P.; Deng, G.; Sai, X.; Guo, H.; Huang, H.; Zhu, P. Maturation strategies and limitations of induced pluripotent stem cell-derived cardiomyocytes. Biosci. Rep. 2020.
25. Fernandez, T.d.S.; de Souza Fernandez, C.; Mencalha, A.L. Human induced pluripotent stem cells from basic research to potential clinical applications in cancer. BioMed Res. Int. 2013.
26. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8, 315–317.
27. Chen, P.-M.; Yen, M.-L.; Liu, K.-J.; Sytwu, H.-K.; Yen, B.-L. Immunomodulatory properties of human adult and fetal multipotent mesenchymal stem cells. J. Biomed. Sci. 2011, 18, 49.
28. Huang, B.; Qian, C.; Ding, C.; Meng, Q.; Zou, Q.; Li, H. Fetal liver mesenchymal stem cells restore ovarian function in premature ovarian insufficiency by targeting MT1. Stem Cell Res. Ther. 2019, 10, 362.

29. Owen, M.; Friedenstein, A. Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Found. Symp. 1988, 136, 42–60.
30. Gnecchi, M.; Melo, L.G. Bone marrow-derived mesenchymal stem cells: Isolation, expansion, characterization, viral transduction, and production of conditioned medium. In Stem Cells in Regenerative Medicine; Springer: Berlin/Heidelberg, Germany, 2009; pp. 281–294.
31. Gao, L.; Huang, Z.; Lin, H.; Tian, Y.; Li, P.; Lin, S. Bone marrow mesenchymal stem cells (BMSCs) restore functional endometrium in the rat model for severe Asherman syndrome. Reprod. Sci. 2019, 26, 436–444.
32. Besikcioglu, H.E.; Sarıbas, G.S.; Ozogul, C.; Tiryaki, M.; Kilic, S.; Pınarlı, F.A.; Gulbahar, O. Determination of the effects of bone marrow derived mesenchymal stem cells and ovarian stromal stem cells on follicular maturation in cyclophosphamide induced ovarian failure in rats. Taiwan. J. Obstet. Gynecol. 2019, 58, 53–59.
33. Jing, Z.; Qiong, Z.; Yonggang, W.; Yanping, L. Rat bone marrow mesenchymal stem cells improve regeneration of thin endometrium in rat. Fertil. Steril. 2014, 101, 587–594.E3.
34. Wang, J.; Ju, B.; Pan, C.; Gu, Y.; Zhang, Y.; Sun, L.; Zhang, B.; Zhang, Y. Application of bone marrow-derived mesenchymal stem cells in the treatment of intrauterine adhesions in rats. Cell. Physiol. Biochem. 2016, 39, 1553–1560.
35. Liu, T.; Huang, Y.; Zhang, J.; Qin, W.; Chi, H.; Chen, J.; Yu, Z.; Chen, C. Transplantation of human menstrual blood stem cells to treat premature ovarian failure in mouse model. Stem Cells Dev. 2014, 23, 1548–1557.
36. Yan, Z.; Guo, F.; Yuan, Q.; Shao, Y.; Zhang, Y.; Wang, H.; Hao, S.; Du, X. Endometrial mesenchymal stem cells isolated from menstrual blood repaired epirubicin-induced damage to human ovarian granulosa cells by inhibiting the expression of Gadd45b in cell cycle pathway. Stem Cell Res. Ther. 2019, 10, 4.
37. Tan, J.; Li, P.; Wang, Q.; Li, Y.; Li, X.; Zhao, D.; Xu, X.; Kong, L. Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome. Hum. Reprod. 2016, 31, 2723–2729.
38. Zheng, S.X.; Wang, J.; Wang, X.L.; Ali, A.; Wu, L.M.; Liu, Y.S. Feasibility analysis of treating severe intrauterine adhesions by transplanting menstrual blood-derived stem cells. Int. J. Mol. Med. 2018, 41, 2201–2212.
39. Prianishnikov, V. On the concept of stem cell and a model of functional-morphological structure of the endometrium. Contraception 1978, 18, 213–223.
40. Chan, R.W.; Schwab, K.E.; Gargett, C.E. Clonogenicity of human endometrial epithelial and stromal cells. Biol. Reprod. 2004, 70, 1738–1750.

41. Lapidot, T.; Petit, I. Current understanding of stem cell mobilization: The roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp. Hematol. 2002, 30, 973–981.
42. Wolff, E.F.; Mutlu, L.; Massasa, E.E.; Elsworth, J.D.; Eugene Redmond, D., Jr.; Taylor, H.S. Endometrial stem cell transplantation in MPTP-exposed primates: An alternative cell source for treatment of P arkinson's disease. J. Cell. Mol. Med. 2015, 19, 249–256.
43. Santamaria, X.; Massasa, E.E.; Feng, Y.; Wolff, E.; Taylor, H.S. Derivation of insulin producing cells from human endometrial stromal stem cells and use in the treatment of murine diabetes. Mol. Ther. 2011, 19, 2065–2071.
44. de Miguel-Gómez, L.; López-Martínez, S.; Francés-Herrero, E.; Rodríguez-Eguren, A.; Pellicer, A.; Cervelló, I. Stem cells and the endometrium: From the discovery of adult stem cells to pre-clinical models. Cells 2021, 10, 595.
45. Azizi, R.; Aghebati-Maleki, L.; Nouri, M.; Marofi, F.; Negargar, S.; Yousefi, M. Stem cell therapy in Asherman syndrome and thin endometrium: Stem cell-based therapy. Biomed. Pharmacother. 2018, 102, 333–343.
46. Mohamed, S.A.; Shalaby, S.; Brakta, S.; Elam, L.; Elsharoud, A.; Al-Hendy, A. Umbilical cord blood mesenchymal stem cells as an infertility treatment for chemotherapy induced premature ovarian insufficiency. Biomedicines 2019, 7, 7.
47. Song, D.; Zhong, Y.; Qian, C.; Zou, Q.; Ou, J.; Shi, Y.; Gao, L.; Wang, G.; Liu, Z.; Li, H. Human umbilical cord mesenchymal stem cells therapy in cyclophosphamide-induced premature ovarian failure rat model. BioMed Res. Int. 2016.
48. Elfayomy, A.K.; Almasry, S.M.; El-Tarhouny, S.A.; Eldomiaty, M.A. Human umbilical cord blood-mesenchymal stem cells transplantation renovates the ovarian surface epithelium in a rat model of premature ovarian failure: Possible direct and indirect effects. Tissue Cell 2016, 48, 370–382.
49. Zhang, C. The Roles of Different Stem Cells in Premature Ovarian Failure. Curr. Stem Cell Res. Ther. 2020, 15, 473–481.
50. Zhang, L.; Li, Y.; Guan, C.-Y.; Tian, S.; Lv, X.-D.; Li, J.-H.; Ma, X.; Xia, H.-F. Therapeutic effect of human umbilical cord-derived mesenchymal stem cells on injured rat endometrium during its chronic phase. Stem Cell Res. Ther. 2018, 9, 36.
51. Xu, L.; Ding, L.; Wang, L.; Cao, Y.; Zhu, H.; Lu, J.; Song, T.; Hu, Y.; Dai, J. Umbilical cord-derived mesenchymal stem cells on scaffolds facilitate collagen degradation via upregulation of MMP-9 in rat uterine scars. Stem Cell Res. Ther. 2017, 8, 84.
52. Ding, L.; Yan, G.; Wang, B.; Xu, L.; Gu, Y.; Ru, T.; Cui, X.; Lei, L.; Liu, J.; Sheng, X. Transplantation of UC-MSCs on collagen scaffold activates follicles in dormant ovaries of POF patients with long history of infertility. Sci. China Life Sci. 2018, 61, 1554–1565.

53. Steigman, S.A.; Ahmed, A.; Shanti, R.M.; Tuan, R.S.; Valim, C.; Fauza, D.O. Sternal repair with bone grafts engineered from amniotic mesenchymal stem cells. J. Pediatric Surg. 2009, 44, 1120–1126.
54. Kunisaki, S.M.; Fuchs, J.R.; Kaviani, A.; Oh, J.-T.; LaVan, D.A.; Vacanti, J.P.; Wilson, J.M.; Fauza, D.O. Diaphragmatic repair through fetal tissue engineering: A comparison between mesenchymal amniocyte–and myoblast-based constructs. J. Pediatric Surg. 2006, 41, 34–39.
55. Xiao, G.-Y.; Liu, I.-H.; Cheng, C.-C.; Chang, C.-C.; Lee, Y.-H.; Cheng, W.T.-K.; Wu, S.-C. Amniotic fluid stem cells prevent follicle atresia and rescue fertility of mice with premature ovarian failure induced by chemotherapy. PLoS ONE 2014, 9, e106538.
56. Liu, T.; Huang, Y.; Guo, L.; Cheng, W.; Zou, G. CD44+/CD105+ human amniotic fluid mesenchymal stem cells survive and proliferate in the ovary long-term in a mouse model of chemotherapy-induced premature ovarian failure. Int. J. Med. Sci. 2012, 9, 592.
57. Ling, L.; Feng, X.; Wei, T.; Wang, Y.; Wang, Y.; Wang, Z.; Tang, D.; Luo, Y.; Xiong, Z. Human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation improves ovarian function in rats with premature ovarian insufficiency (POI) at least partly through a paracrine mechanism. Stem Cell Res. Ther. 2019, 10, 46.
58. Feng, X.; Ling, L.; Zhang, W.; Liu, X.; Wang, Y.; Luo, Y.; Xiong, Z. Effects of human amnion–derived mesenchymal stem cell (hAD-MSC) transplantation In Situ on primary ovarian insufficiency in SD rats. Reprod. Sci. 2020, 27, 1502–1512.
59. Ling, L.; Feng, X.; Wei, T.; Wang, Y.; Wang, Y.; Zhang, W.; He, L.; Wang, Z.; Zeng, Q.; Xiong, Z. Effects of low-intensity pulsed ultrasound (LIPUS)-pretreated human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation on primary ovarian insufficiency in rats. Stem Cell Res. Ther. 2017, 8, 283.
60. Zhang, H.; Luo, Q.; Lu, X.; Yin, N.; Zhou, D.; Zhang, L.; Zhao, W.; Wang, D.; Du, P.; Hou, Y. Effects of hPMSCs on granulosa cell apoptosis and AMH expression and their role in the restoration of ovary function in premature ovarian failure mice. Stem Cell Res. Ther. 2018, 9, 20.
61. Yin, N.; Wang, Y.; Lu, X.; Liu, R.; Zhang, L.; Zhao, W.; Yuan, W.; Luo, Q.; Wu, H.; Luan, X. hPMSC transplantation restoring ovarian function in premature ovarian failure mice is associated with change of Th17/Tc17 and Th17/Treg cell ratios through the PI3K/Akt signal pathway. Stem Cell Res. Ther. 2018, 9, 37.
62. Li, H.; Zhao, W.; Wang, L.; Luo, Q.; Yin, N.; Lu, X.; Hou, Y.; Cui, J.; Zhang, H. Human placenta-derived mesenchymal stem cells inhibit apoptosis of granulosa cells induced by IRE1α pathway in autoimmune POF mice. Cell Biol. Int. 2019, 43, 899–909.
63. Kim, T.-H.; Choi, J.H.; Jun, Y.; Lim, S.M.; Park, S.; Paek, J.-Y.; Lee, S.-H.; Hwang, J.-Y.; Kim, G.J. 3D-cultured human placenta-derived mesenchymal stem cell spheroids enhance ovary function

by inducing folliculogenesis. Sci. Rep. 2018, 8, 15313.

64. Lee, R.H.; Kim, B.; Choi, I.; Kim, H.; Choi, H.S.; Suh, K.; Bae, Y.C.; Jung, J.S. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell. Physiol. Biochem. 2004, 14, 311–324.
65. Yang, J.-A.; Chung, H.-M.; Won, C.-H.; Sung, J.-H. Potential application of adipose-derived stem cells and their secretory factors to skin: Discussion from both clinical and industrial viewpoints. Expert Opin. Biol. Ther. 2010, 10, 495–503.
66. Ra, J.C.; Jeong, E.C.; Kang, S.K.; Lee, S.J.; Choi, K.H. A prospective, nonrandomized, no placebo-controlled, phase I/II clinical trial assessing the safety and efficacy of intramuscular injection of autologous adipose tissue-derived mesenchymal stem cells in patients with severe buerger's disease. Cell Med. 2017, 9, 87–102.
67. Damous, L.L.; Nakamuta, J.S.; de Carvalho, A.E.S.; Carvalho, K.C.; Soares, J.M., Jr.; de Jesus Simões, M.; Krieger, J.E.; Baracat, E.C. Does adipose tissue-derived stem cell therapy improve graft quality in freshly grafted ovaries? Reprod. Biol. Endocrinol. 2015, 13, 108.
68. Sun, M.; Wang, S.; Li, Y.; Yu, L.; Gu, F.; Wang, C.; Yao, Y. Adipose-derived stem cells improved mouse ovary function after chemotherapy-induced ovary failure. Stem Cell Res. Ther. 2013, 4, 80.
69. Su, J.; Ding, L.; Cheng, J.; Yang, J.; Li, X.; Yan, G.; Sun, H.; Dai, J.; Hu, Y. Transplantation of adipose-derived stem cells combined with collagen scaffolds restores ovarian function in a rat model of premature ovarian insufficiency. Hum. Reprod. 2016, 31, 1075–1086.
70. White, Y.A.; Woods, D.C.; Takai, Y.; Ishihara, O.; Seki, H.; Tilly, J.L. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat. Med. 2012, 18, 413.
71. Niikura, Y.; Niikura, T.; Tilly, J.L. Aged mouse ovaries possess rare premeiotic germ cells that can generate oocytes following transplantation into a young host environment. Aging 2009, 1, 971.
72. Anderson, R.A.; Wallace, W.H.B. Fertility preservation in girls and young women. Clin. Endocrinol. 2011, 75, 409–419.
73. Kita, K.; Watanabe, T.; Ohsaka, K.; Hayashi, H.; Kubota, Y.; Nagashima, Y.; Aoki, I.; Taniguchi, H.; Noce, T.; Inoue, K. Production of functional spermatids from mouse germline stem cells in ectopically reconstituted seminiferous tubules. Biol. Reprod. 2007, 76, 211–217.
74. Kanatsu-Shinohara, M.; Lee, J.; Inoue, K.; Ogonuki, N.; Miki, H.; Toyokuni, S.; Ikawa, M.; Nakamura, T.; Ogura, A.; Shinohara, T. Pluripotency of a single spermatogonial stem cell in mice. Biol. Reprod. 2008, 78, 681–687.
75. McLean, D.J. Spermatogonial stem cell transplantation and testicular function. Cell Tissue Res. 2005, 322, 21–31.

76. Koubova, J.; Menke, D.B.; Zhou, Q.; Capel, B.; Griswold, M.D.; Page, D.C. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. Proc. Natl. Acad. Sci. USA 2006, 103, 2474–2479.

77. Griswold, M.D. The central role of Sertoli cells in spermatogenesis. In Seminars in Cell & Developmental Biology; Academic Press: Cambridge, MA, USA; pp. 411–416.

78. Byskov, A.G.; Fenger, M.; Westergaard, L.; Andersen, C.Y. Forskolin and the meiosis inducing substance synergistically initiate meiosis in fetal male germ cells. Mol. Reprod. Dev. 1993, 34, 47–52.

79. Kanatsu-Shinohara, M.; Inoue, K.; Ogonuki, N.; Miki, H.; Yoshida, S.; Toyokuni, S.; Lee, J.; Ogura, A.; Shinohara, T. Leukemia inhibitory factor enhances formation of germ cell colonies in neonatal mouse testis culture. Biol. Reprod. 2007, 76, 55–62.

80. Zhu, Y.; Hu, H.-L.; Li, P.; Yang, S.; Zhang, W.; Ding, H.; Tian, R.-H.; Ning, Y.; Zhang, L.-L.; Guo, X.-Z. Generation of male germ cells from induced pluripotent stem cells (iPS cells): An in vitro and in vivo study. Asian J. Androl. 2012, 14, 574.

81. Easley IV, C.A.; Simerly, C.R.; Schatten, G. Stem cell therapeutic possibilities: Future therapeutic options for male-factor and female-factor infertility? Reprod. Biomed. Online 2013, 27, 75–80.

82. Lindsay, T.J.; Vitrikas, K. Evaluation and treatment of infertility. Am. Fam. Physician 2015, 91, 308–314.

83. Valle, R.F.; Sciarra, J.J. Intrauterine adhesions: Hysteroscopic diagnosis, classification, treatment, and reproductive outcome. Am. J. Obstet. Gynecol. 1988, 158, 1459–1470.

84. Sugimoto, O. Diagnostic and therapeutic hysteroscopy for traumatic intrauterine adhesions. Am. J. Obstet. Gynecol. 1978, 131, 539–547.

85. Zikopoulos, K.A.; Kolibianakis, E.M.; Platteau, P.; de Munck, L.; Tournaye, H.; Devroey, P.; Camus, M. Live delivery rates in subfertile women with Asherman's syndrome after hysteroscopic adhesiolysis using the resectoscope or the Versapoint system. Reprod. Biomed. Online 2004, 8, 720–725.

86. Alawadhi, F.; Du, H.; Cakmak, H.; Taylor, H.S. Bone marrow-derived stem cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome. PLoS ONE 2014, 9, e96662.

87. Gan, L.; Duan, H.; Xu, Q.; Tang, Y.-Q.; Li, J.-J.; Sun, F.-Q.; Wang, S. Human amniotic mesenchymal stromal cell transplantation improves endometrial regeneration in rodent models of intrauterine adhesions. Cytotherapy 2017, 19, 603–616.

88. Kilic, S.; Yuksel, B.; Pinarli, F.; Albayrak, A.; Boztok, B.; Delibasi, T. Effect of stem cell application on Asherman syndrome, an experimental rat model. J. Assist. Reprod. Genet. 2014, 31, 975–982.

89. Nagori, C.B.; Panchal, S.Y.; Patel, H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. J. Hum. Reprod. Sci. 2011, 4, 43.
90. Singh, N.; Mohanty, S.; Seth, T.; Shankar, M.; Bhaskaran, S.; Dharmendra, S. Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy. J. Hum. Reprod. Sci. 2014, 7, 93.
91. Kawamura, K.; Cheng, Y.; Suzuki, N.; Deguchi, M.; Sato, Y.; Takae, S.; Ho, C.-h.; Kawamura, N.; Tamura, M.; Hashimoto, S. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc. Natl. Acad. Sci. USA 2013, 110, 17474–17479.
92. Suzuki, N.; Yoshioka, N.; Takae, S.; Sugishita, Y.; Tamura, M.; Hashimoto, S.; Morimoto, Y.; Kawamura, K. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. Hum. Reprod. 2015, 30, 608–615.
93. Zhai, J.; Yao, G.; Dong, F.; Bu, Z.; Cheng, Y.; Sato, Y.; Hu, L.; Zhang, Y.; Wang, J.; Dai, S. In Vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. J. Clin. Endocrinol. Metab. 2016, 101, 4405–4412.
94. Johnson, J.; Canning, J.; Kaneko, T.; Pru, J.K.; Tilly, J.L. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004, 428, 145–150.
95. Legro, R.S.; Arslanian, S.A.; Ehrmann, D.A.; Hoeger, K.M.; Murad, M.H.; Pasquali, R.; Welt, C.K. Diagnosis and treatment of polycystic ovary syndrome: An Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab. 2013, 98, 4565–4592.
96. Xie, Q.; Xiong, X.; Xiao, N.; He, K.; Chen, M.; Peng, J.; Su, X.; Mei, H.; Dai, Y.; Wei, D. Mesenchymal stem cells alleviate DHEA-Induced polycystic ovary syndrome (PCOS) by inhibiting inflammation in mice. Stem Cells Int. 2019.
97. Chugh, R.; Ashour, D.; Garcia, N.; Park, H.; Takala, H.; Ismail, N.; McAllister, J.; Al-Hendy, A.; El Andaloussi, A. Towards cell therapy of polycystic ovary syndrome (PCOS): Human mesenchymal stem cells secretome inhibits androgen production by pcos theca cells. Cytotherapy 2019, 21, S81.
98. Verkauf, B. Incidence, symptoms, and signs of endometriosis in fertile and infertile women. J. Fla. Med Assoc. 1987, 74, 671–675.
99. The Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: A committee opinion. Fertil. Steril. 2012, 98, 591–598.
100. Macer, M.L.; Taylor, H.S. Endometriosis and infertility: A review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet. Gynecol. Clin. 2012, 39, 535–549.

101. Liu, Y.; Kodithuwakku, S.P.; Ng, P.-Y.; Chai, J.; Ng, E.H.; Yeung, W.S.; Ho, P.-C.; Lee, K.-F. Excessive ovarian stimulation up-regulates the Wnt-signaling molecule DKK1 in human endometrium and may affect implantation: An In Vitro co-culture study. Hum. Reprod. 2010, 25, 479–490.

102. Sallam, H.N.; Garcia-Velasco, J.A.; Dias, S.; Arici, A.; Abou-Setta, A.M. Long-Term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. Cochrane Database Syst. Rev. 2006.

103. Benschop, L.; Farquhar, C.; van der Poel, N.; Heineman, M.J. Interventions for women with endometrioma prior to assisted reproductive technology. Cochrane Database Syst. Rev. 2010.

104. Cocuzza, M.; Alvarenga, C.; Pagani, R. The epidemiology and etiology of azoospermia. Clinics 2013, 68, 15–26.

105. Kanatsu-Shinohara, M.; Shinohara, T. Spermatogonial stem cell self-renewal and development. Annu. Rev. Cell Dev. Biol. 2013, 29, 163–187.

106. Hermann, B.P.; Sukhwani, M.; Winkler, F.; Pascarella, J.N.; Peters, K.A.; Sheng, Y.; Valli, H.; Rodriguez, M.; Ezzelarab, M.; Dargo, G. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. Cell Stem Cell 2012, 11, 715–726.

107. Vij, S.C.; Sabanegh, E., Jr.; Agarwal, A. Biological therapy for non-obstructive azoospermia. Expert Opin. Biol. Ther. 2018, 18, 19–23.

108. Hou, J.; Yang, S.; Yang, H.; Liu, Y.; Liu, Y.; Hai, Y.; Chen, Z.; Guo, Y.; Gong, Y.; Gao, W.-Q. Generation of male differentiated germ cells from various types of stem cells. Reproduction 2014, 147, R179–R188.

109. Nayernia, K.; Nolte, J.; Michelmann, H.W.; Lee, J.H.; Rathsack, K.; Drusenheimer, N.; Dev, A.; Wulf, G.; Ehrmann, I.E.; Elliott, D.J. In Vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev. Cell 2006, 11, 125–132.

110. Hayashi, K.; Ohta, H.; Kurimoto, K.; Aramaki, S.; Saitou, M. Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 2011, 146, 519–532.

111. Igboeli, P.; El Andaloussi, A.; Sheikh, U.; Takala, H.; ElSharoud, A.; McHugh, A.; Gavrilova-Jordan, L.; Levy, S.; Al-Hendy, A. Intraovarian injection of autologous human mesenchymal stem cells increases estrogen production and reduces menopausal symptoms in women with premature ovarian failure: Two case reports and a review of the literature. J. Med. Case Rep. 2020, 14, 108.

112. Cao, Y.; Sun, H.; Zhu, H.; Zhu, X.; Tang, X.; Yan, G.; Wang, J.; Bai, D.; Wang, J.; Wang, L. Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: A phase I clinical trial. Stem Cell Res. Ther. 2018, 9, 192.

113. Mashayekhi, M.; Mirzadeh, E.; Chekini, Z.; Ahmadi, F.; Eftekhari-Yazdi, P.; Vesali, S.; Madani, T.; Aghdami, N. Evaluation of safety, feasibility and efficacy of intra-ovarian transplantation of autologous adipose derived mesenchymal stromal cells in idiopathic premature ovarian failure patients: Non-randomized clinical trial, phase I, first in human. J. Ovarian Res. 2021, 14, 5.
114. Cheng, Y.C.; Takagi, M.; Milbourne, A.; Champlin, R.E.; Ueno, N.T. Phase II study of gonadotropin-releasing hormone analog for ovarian function preservation in hematopoietic stem cell transplantation patients. Oncologist 2012, 17, 233.
115. Haahr, M.K.; Jensen, C.H.; Toyserkani, N.M.; Andersen, D.C.; Damkier, P.; Sørensen, J.A.; Lund, L.; Sheikh, S.P. Safety and potential effect of a single intracavernous injection of autologous adipose-derived regenerative cells in patients with erectile dysfunction following radical prostatectomy: An open-label phase I clinical trial. EBioMedicine 2016, 5, 204–210.