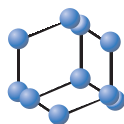


REVIEW ARTICLE


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Stem Cells as a Resource for Treatment of Infertility-related Diseases



Jing Wang^{1,2,#}, Chi Liu^{2,4,#}, Masayuki Fujino^{2,3}, Guoqing Tong¹, Qinxiu Zhang⁴, Xiao-Kang Li^{2,*} and Hua Yan^{1,*}

¹Reproductive Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China; ²Division of Transplantation Immunology, National Research Institute for Child Health and Development, Tokyo, Japan; ³AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; ⁴Department of Medical and Life Sciences, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Abstract: Worldwide, infertility affects 8-12% of couples of reproductive age and has become a common problem. There are many ways to treat infertility, including medication, intrauterine insemination, and *in vitro* fertilization. In recent years, stem-cell therapy has raised new hope in the field of reproductive disability management. Stem cells are self-renewing, self-replicating undifferentiated cells that are capable of producing specialized cells under appropriate conditions. They exist throughout a human's embryo, fetal, and adult stages and can proliferate into different cells. While many issues remain to be addressed concerning stem cells, stem cells have undeniably opened up new ways to treat infertility. In this review, we describe past, present, and future strategies for the use of stem cells in reproductive medicine.

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1. INTRODUCTION

Infertility is defined as being unable to achieve pregnancy despite having regular intercourse without birth control for 12 consecutive months. Even among young couples, it is rapidly becoming a common problem [1, 2]. For this reason, the International Commission's Monitoring of Assisted Reproductive Technology (ICMART) and World Health Organization (WHO) jointly issued a revised version of the terminology defining infertility as a systemic disease in 2009 [3].

Worldwide, 8-12% of couples of childbearing age (up to 186 million) suffer from infertility [4]. It is considered to be among the major reasons underlying the globally decreasing number of children and can be categorized into primary and secondary infertility. Every step in the process of ovulation and fertilization needs to occur correctly in order for pregnancy to take place. Male factors are the cause of one-third of infertility

cases, mainly due to sperm morphological and functional disorders, including precocious puberty, hereditary diseases, and structural problems such as testicular blockage; genital damage or injury leading to sperm dysfunction, and environmental and psychological factors [5]. Female factors include ovulation dysfunction, abnormal uterus or fallopian tube, endometritis, primary ovarian insufficiency, and pelvic adhesions [6].

Treatment for infertility varies from pharmacologic treatment to assisted reproductive technology (ART), depending on the cause and patient characteristics. For the diagnosis of male fertility, a sperm analysis, hormonal examination, genetic testing, and testicular biopsy are the primary examination options. Therapy for male infertility includes lifestyle improvements, medications, surgery, and sperm regeneration. The diagnosis of female infertility is made through hormone testing and ovulation testing, and the primary therapy is medication or an ART procedure, including *in vitro* fertilization-embryo transfer (IVF), intrauterine insemination (IUI), and intracytoplasmic sperm injection (ICSI) [7-9]. Other treatment options include supplements or antioxidants, such as zinc, vitamin E, and L-carnitine, which sometimes improve the patient's pregnancy rate.

ART artificially induces pregnancy using medication, surgery, or microscopic fertilization technologies [10] and includes all forms of infertility treatment along with

*Address correspondence to these authors at the Reproductive Center, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, China; Tel: +862120256688; E-mail: 13524658288@163.com
Division of Transplantation Immunology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo, 157-8535 Japan; Tel: +81-3-3416-0181; Fax: +81-3-3417-2864; E-mail: ri-k@ncchd.go.jp

#These authors contributed equally.

the administration of hormones such as estrogen, testosterone, and glucocorticoid. ART can be applied to manage infertility for both men and women and has been shown to benefit many infertile patients [11]. However, ART has caused ethical disputes and disagreements. Furthermore, it is invasive, expensive, and unpredictable and often leads to side effects or symptoms.

While some degree of success has been achieved with the above strategies, they all have their own shortcomings [12]. In recent years, stem cells have garnered significant attention in the field of infertility [13]. Stem cells are multipotent original cells that can divide into various other cells for repair, development, and regeneration. Studies of experimental models have shown that treating infertility with stem cell therapy is gaining acceptance [14]. A series of female infertility studies using stem cells from different sources was recently launched. Pre-clinical studies on sexual infertility-related diseases have suggested new directions to consider for the treatment of infertility [15]. Studies using experimental models have revealed the power of stem cell therapy for treating infertility and verified these results [16]. We herein review the research progress concerning the application of stem cells for treating infertility.

2. CURRENT TREATMENT FOR INFERTILITY THERAPY

Although some progress has been made in ART treatment in recent years, more than 80% of couples are still faced with intractable infertility [17]. Infertility is a global issue affecting more than 15% of all couples, with male infertility accounting for about 30% of cases and female factors accounting for approximately 40% of cases [18]. Infertility is a complex disease, and its treatment depends on the patients' ages, etiology, and other factors. Treatment needs to withstand physical, psychological, economic, and time-related stress.

Depending on the patients' conditions, multiple treatments, such as surgery and medication, may be required at the same time. For women afflicted with ovulation disorders, fertility drugs are the main methods of stimulating ovulation. The sudden elevation in levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) causes the dominant follicle to rupture and expel the egg. For example, Clomid causes the pituitary gland to release more FSH and LH in order to stimulate ovulation and promote follicular growth [19]. These injected gonadotropins directly stimulate the ovaries to produce multiple eggs. However, the use of fertility drugs does pose some risks, including multiple births, preterm birth, ovarian hyperstimulation syndrome (OHSS), and ovarian tumor [20]. The restoration of female fertility through surgery may involve laparoscopy, hysteroscopic, and fallopian tube surgeries. Other ARTs in women include IUI and IVF. Treatments for male infertility include surgery, hormone therapy, and drug therapy along with ART [21]. For example, in patients with azoospermia, sperm can be surgically removed from the testis or epididymis. In

addition, varicocele can usually be corrected by surgery. However, in some cases, male fertility problems cannot be treated, and men are ultimately unable to father children except through sperm donation, or else they must consider adoption. These drawbacks have resulted in an ongoing desire for better solutions to the issue of infertility.

3. STEM CELLS REPRESENT A NEW HOPE IN CELL THERAPY

In recent years, significant progress has been made in the *in vitro* differentiation of male germ cells from pluripotent stem cells *in vitro*. For female infertility, stem cells can be used for ovarian regeneration and oocyte generation [22]. Stem cells are undifferentiated cells that exist in embryos, fetuses, and adults to produce differentiated cells (Table 1). They typically come from two sources: early embryonic cells and adult tissues. Tissue-specific stem cells are found in differentiated organs during the postnatal and adult stages of life and play an important role in the repair of organ damage. The main types of stem cells are embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), spermatogonial stem cells (SSCs), and induced-pluripotent stem cells (iPSCs) [23]. Totipotent cells are the most undifferentiated cells and are found only in early development. The fertilized egg and the first two dividing cells are totipotent cells, as they differentiate into embryonic and extraembryonic tissues, which can form embryos and placenta. iPSCs can differentiate into all three embryonic stem cell lines, including mesoderm, ectoderm, and endoderm, and all tissues and organs develop from the mesoderm [24].

The stem cell function can be divided into five major types. The first type involves the replacement and repair of dead and damaged cells. Stem cells are self-homing, and when injected into the human body, they will accumulate in damaged organs and corresponding parts and differentiate into the cells types native to these organs and parts. For example, the homing ability of SSCs directs them to their niches upon transplantation into sterile testes. The transplanted SSCs then attach to the Sertoli cells and closely connect the blood-testicular barrier (BTB) to migrate to their niche on the basement membrane [25]. The second type involves the activation of dormant and suppressing cells. The growth and development of the human body is accomplished through cell division. With age, some cells stop undergoing normal cell cycles after division and show a state of functional dormancy. Stem cells can activate dormant cells and suppressor cells and encourage them to re-enter the cell cycle, proliferating by division. This increases the number of new cells in the body and restores the body's metabolic process to normal or even reverses it. Chemotherapy-induced premature ovarian failure (POF) preclinical mouse models have shown that these transplanted stem cells can reside in ovarian tissues and save the ovarian function; however, these mechanisms need to be studied further [26]. The third type involves the paracrine secretion of various enzymes, proteins, and

Table 1. Characteristics of stem cells in the treatment of infertility by stem cells

	ESCs	MSCs	SSCs	iPSCs
Source of generation	inner cell mass cells of blastocysts	cord blood, bone marrow, and adipose tissue	testicular tissues	human somatic cells
Self-renewal capacity	differentiate into derivatives of three major germ layers	mesodermal-derived tissue, such as bone, cartilage, adipose tissue, and muscle	differentiate into the derivatives of all three primary germ layers	differentiate into the derivatives of all three primary germ layers
Nature of cells	pluripotent	multipotent	pluripotent	pluripotent
Ethical concerns	Ethical and moral concerns present	No ethical or moral concerns	No ethical or moral concerns	No ethical concerns
Clinical applications	Restricted	Widely used	Widely used	Widely used
Immuno-rejection	Yes	No	Yes	Yes

ESCs: embryonic stem cells; MSCs: mesenchymal stem cells; SSCs: spermatogonial stem cells; iPSCs: induced pluripotent stem cell.

cytokines to promote cell proliferation, inhibit apoptosis of functional cells, and differentiate existing tissue progenitor cells into tissue cells in order to repair damaged tissues and grow new tissues. Spermatogenesis is a process regulated by testosterone, endocrine, and paracrine secretion/autocrine factors, such as the IL-1 family [27]. Under pathological conditions, the level of pro-inflammatory cytokines is increased, which has a negative effect on spermatogenesis. Therefore, the expression of testicular paracrine/autocrine factor and its regulation mechanism should be considered in the future treatment strategy of male infertility [28]. The fourth type involves the exertion of an immunosuppressive function through cell-cell contact and secretion of soluble factors, inhibiting the proliferation of natural killer cells. The fifth type involves the promotion of the recovery of inter-cellular signaling. The signal molecule of the cell interacts with the receptor protein on the cell membrane, causing a conformational change in the receptor and the subsequent production of a new signal substance inside the cell. This triggers a response, such as ion permeability, cell shape change, or some other cellular function change [29].

4. ESCs

ESCs are derived from pre-implantation inner cell mass (ICM) and can self-renew indefinitely to maintain their undifferentiated state and normal karyotype during growth. Human ESC (hESC) lines were first developed in 1998 [30]. Hübner *et al.* showed that embryonic stem cells can differentiate into oocytes by germ cell-specific Oct4 markers [32]. These markers are derived from excess *in vitro* fertilized embryos and should be used only for research due to ethical concerns. hESCs are therefore a potential cell replacement therapy [31].

Reports of uterine reconstruction are rare. Due to the physical and functional characteristics of the uterus and the complex mechanism of hormones, the effects of medical treatments are uncertain. Advances in ART therapy have helped physicians overcome many of the barriers in male and female infertility. However, for patients with severe intrauterine adhesions (IUAs),

which can be induced by inappropriate uterine treatment, pregnancy is still difficult [32]. Songkran *et al.* found that hESC-derived endometrial cells can support endometrial repair and functional recovery [33]. ESCs were obtained from cloned blastocysts, themselves obtained from somatic cell nuclear transfers (SCNTs) (the resulting embryonic stem cells were called Kitw/Kitwv, ntESCs) [34]. The corrected ntESCs can differentiate into primordial germ cell-like cells (PGCLC) that can be transplanted into mouse testes for spermatogenesis reconstruction. Sperm deficiency caused by genetic mutations may lead to male infertility. Yan Yuan *et al.* used the Kitw/Kitwv mouse model to study the feasibility of producing functional sperm in Azoospermia mice by gene repair techniques [35]. Cancer survivors may face secondary infertility due to the treatment of cancer. ESCs can produce germ cells and thereby treat infertility. ESC-derived PGCs are susceptible to ethical controversy because the process involves destroying human embryos. Sperm/oocyte-like cells can now be produced from embryonic stem cells [36]. However, deriving germ cells from embryonic stem cells still suffers from very low efficiency. Furthermore, the desired gamete function has been achieved in only one case so far. Little research exists on the derivative mechanism, and the process of epigenetic signature establishment remains unclear. For humans, only the generation of PGCs has been described, and many problems remain unsolved [37].

5. MSCs

MSCs can be acquired from adipose tissue, umbilical cord blood, amniotic fluid, and endometrium, as well as other sources. MSCs have special functions such as a self-renewal ability, differentiation potential, and colony formation. MSCs are derived from placental tissue, amniotic membrane/amniotic fluid, cord blood, umbilical cord vein, and Wharton's jelly, which are contained in the umbilical cord, sometimes referred to as umbilical cord tissue. In addition, MSCs are also capable of secreting some trophic factors, such as growth factors and cytokines [38].

Treatment using MSCs regulates the production of systemic and local Th1/Th2 cytokines while protecting the fetus in abortion-prone mice [39]. The application of MSCs for the treatment of various disease cell types is under intensive research. On the other hand, another cause of female infertility is premature ovarian failure. Studies using cyclophosphamide in rat models have shown the reversal of experimentally induced premature ovarian failure [40]. The intravenous injection of bone marrow (BM)-MSCs from male rats reversed the low estrogen and high nutritional status of experimental rats in the treated group according to the serum FSH and E2 levels and follicular and corpus luteum production responses. Johnson *et al.* showed that BM transplanted into female mice was able to produce new follicles and oocytes in the recipient's ovaries [41]. Another major cause of female infertility is endometriosis. Ding *et al.* constructed BM-MSCs on a degradable collagen membrane. BM-MSC-collagen construct transplantation was used to treat severe endometrial injury in rats [42]. MSCs migrated mainly to the damaged areas, such as the endometrial basement. The possible mechanism involved MSCs secreting fibroblast growth factors to induce local endometrial fineness. Some reports showed that human umbilical cord MSCs inhibited the proliferation of endometriotic cells *in vitro* and promoted their apoptosis. Furthermore, some studies have demonstrated the inhibitory effects of smoking on both the recruitment and differentiation of BM-MSCs into uterus cells [43]. Other studies have shown the existence of "endometrial stem cells": resident stem cells that are structurally cloned endometrium and indeed function as MSCs [44]. Endometrial stem cell transplantation has potential applications in the recovery of endometriosis as an autologous source.

In order to explore the complex mechanism between MSCs and various related diseases that cause female infertility, further clinical research on MSCs will need to be conducted, as currently available results of MSC research in female infertility-related diseases are mostly limited to findings in animal models [45].

6. SSCs

Healthy SSCs can lead to sperm regeneration. In 1971, the utility of SSCs for rat spermatogenesis and maintenance of male fertility was recognized [46]. SSCs are derived from primordial germ cells (PGCs) during embryonic development. SSC transplantation is presented as a novel and promising strategy, based on the premise of spermatogenesis and stem cell self-renewal [47]. In 1994, Brinster *et al.* first injected spermatogonial stem cells from fertile donor male mice into the seminiferous tubules of sterile mice. As a result, the recipient mice produced sperm with fertilization ability and produced normal offspring [48]. SSCs are self-renewing and can produce numerous committed progenitor cells that can differentiate into sperm throughout life. In mice, the DBA/2J mouse strain can form a densely packed cell mass and

continue to proliferate under the intervention of glial cell line-derived neurotrophic factor (GDNF) [49]. SSCs in culture have proliferated for more than six months. After their transplantation into a recipient's testis, the sperm can be reconstituted and restore fertility to an infertile recipient [50]. Indeed, the stem cells isolated from the testes of donor male mice were injected into the seminiferous tubules. Donor spermatogonial stem cells induce spermatogenesis with normal morphological characteristics in the testis and produce mature sperm. In humans, SSCs are responsible for the continuous production of male sperm. Spermatogonial stem cells are located in the basement membrane that maintains the seminiferous tubules of spermatogenesis. Transplantation of human SSCs may be an effective treatment for male infertility. SSCs are the only stem cell that can convey parental genetic information to offspring. Neonatal SSCs are used in tissue recombination techniques to produce prostate, uterus, and skin epithelium [51]. Mouse SSCs acquire the morphological and functional characteristics of hematopoietic cells *in vivo*. Furthermore, the technology is also applicable to other animal species such as pigs, goats, and monkeys [52]. This technology can also be used to treat human infertility in the context of solving technical problems.

7. iPSCs

In August 2006, the Yamanaka research team identified four transcription factors (Oct4, Klf4, Sox2, and c-Myc). These factors were transferred into mouse fibroblasts that were then reprogrammed into iPSCs [53]. iPSCs show similar morphology to ESCs, express ESC markers, have normal karyotypes, express telomerase activity, and maintain the differentiation potential of the three main embryonic layers. Therefore, iPSCs are adult cells that are genetically reprogrammed into a state resembling an ESC by expressing genes and factors essential for maintaining ESC characteristics.

Different types of somatic cells can be reprogrammed into iPSCs, mainly *via* gene introduction technology, and mouse iPSCs have been confirmed to possess the developmental pluripotency of ESCs. iPSCs have several advantages over human ESCs. For example, they do not cause ethical problems or immune rejection, and sperm can be obtained from iPSCs cells in azoospermia patients [54]. Male-specific development requires the early embryoid body (EB) expression of Stra-8 stimulated by retinoic acid (RA) [55]. Some studies have used RA to promote the differentiation of *in vitro* iPSCs into PGCs and SSCs [56]. After induction, iPSCs can be further differentiated into mature male germ cells by transplanting their derived germ cells into the seminiferous tubules of mice treated with busulfan [57].

One of the most promising treatments for many incurable diseases is the transplantation of stem cells or their derivatives into the corresponding tissues or organs. However, due to the specificity and complexity of the human immune system, it is difficult to obtain

immunocompetent cells from any particular patient. In this regard, iPSCs and gene-editing technologies offer potential solutions for obtaining healthy autologous cells. It should be emphasized, however, that despite many technological advances in reprogramming, iPSCs are not yet ready to be transplanted into patients, except for in a few ongoing clinical studies. There are few reports on the molecular and functional equivalence of iPSCs and human ESCs, and the genome and epigenomic integrity of human iPSCs need to be carefully evaluated before their clinical use [58].

8. STEM CELLS AND MALE INFERTILITY

Spermatogenesis is a complex process of SSC self-renewal and differentiation into haploid spermatozoa. SSCs reside in adult testes and maintain spermatogenesis throughout life. SSCs are adult stem cells, known as pluripotent adult germline stem cells (maGSC), which have a similar differentiation potential to ESCs. *In vitro*, maGSCs can spontaneously differentiate into derivatives of all embryonic germ layers and produce teratomas in immune-deficient mice [59]. When autologous and allogeneic SSCs were transplanted into the testes of adult and pre-adolescent rhesus monkeys that had been rendered infertile by alkylation chemotherapy, spermatogenesis was restarted, and functional sperm was produced [60]. These results strongly suggest that SSC transplantation can be a successful treatment for male infertility caused by premature chemotherapy. However, while SSCs appear to be a good candidate for stem cell-based treatment of male infertility, the challenges associated with the low concentrations of SSCs in mammalian testes and the protocol for the isolation, identification, and culture must be addressed before the clinical application of this therapy. Similarly, studies of human ESCs have revealed their ability to differentiate into advanced stages of spermatogenesis *in vitro*, including round sperm that cannot fertilize eggs in advanced mammals [61].

The isolation of human ESCs is ethically controversial. Although ESCs are genetically unrelated to patients, their collection involves the destruction of human embryonic tissue. Major breakthroughs in stem cell biology and the discovery of patient-specific iPSCs may overcome these problems. Some studies have recently reported that both human and murine iPSC cells can differentiate into male germ cells [62]. Mouse iPSC cells have been shown to form functional sperm [62]. Functional tests have shown that the sperm produced by iPSCs were able to fertilize eggs after intra-cytoplasmic injection and generate fertile offspring after embryo transfer. Thus far, however, functional male gametes have not been obtained from human iPSC cells.

Sato *et al.* showed that testicular tissue fragments of newborn mice contain only germ cells or primitive spermatogonia [63]. They can produce sperm *in vitro* and create healthy fertile offspring. Mouse oocyte-like cells were recently obtained from primordial germ cells

(PGCs) and somatic cells, which in turn were able to be collected from E12.5 fetal gonads. However, obtaining oocyte-like cells from fetal ovarian tissues may cause ethical problems [64]. Understanding the development of stem cell at different stages *in vitro* can help facilitate the production of germ cells from stem cells. However, while the *in vitro* production of germ cells from stem cells is promising, many problems remain to be resolved [65].

9. STEM CELLS AND FEMALE INFERTILITY

Ovarian failure is inevitable with age. In recent years, two germline stem cells: “female germ stem cells” (fGSC) or “ovarian stem cells” (OSCs) were reported to induce ovarian regeneration and a sustained ovarian function [66]. White *et al.* confirmed that mitotically active germ cells from human ovaries, which they named germ stem cells (GSCs), could be purified and cultured *in vitro* to form oocytes [67]. In addition, injecting stem cells from the BM can stimulate the ovarian function, restore normal ovarian and hormone levels, and possibly allow pregnancy. In 2017, Li *et al.* found that on days 14, 21, and 28 after transplantation of human umbilical cord MSCs (UCMSCs) into rats, the number of follicles improved, the FSH levels decreased, and the AMH and E2 levels increased, resulting in an increase in the ovarian reserve function [68]. Transplantation of human BMSCs to mice can increase the ovarian weight, promote ovarian hormone production, and stimulate follicular development [69]. In addition, studies have shown that the transplantation of menstrual stem cells (hMensSCs) increased the ovarian weight, plasma E2 levels, and follicle numbers in mice [70]. Amniotic fluid stem cells can differentiate into granulosa cells, which inhibit follicular atresia and maintain healthy follicles [71].

In summary, stem cells are seen as a new hope for improving the treatment of female infertility through their ability to regenerate. However, eggs have not yet developed into an oocyte-like cell stage *in vitro*, and whether or not eggs can indeed develop into mature oocytes and acquire fertilization functions has not yet been confirmed.

CONCLUSION

Currently, research has shown that stem cell therapy can treat degenerative diseases, cure malignant cancers, and repair damaged tissue. However, a number of aspects of stem cell therapy remain unexplored; thus, vast untapped potential still exists with regard to applications in treating diseases such as infertility. We are confident that science will be able to cure infertility once the right approach is found.

Ovarian-derived stem cells have broad clinical application prospects. The clinical application of stem cells should be in line with ethical requirements, including informed voluntary consent and other ethical principles of clinical research. In addition, whether or not healthy progeny can be produced from gametes

derived from pluripotent stem cells remains unclear. At present, stem cell-derived gametes can be used as an *in vitro* model to evaluate the effects of drugs. Overall, stem cell research has resulted in important new breakthroughs in the treatment of infertility. We will continue our attempts to untangle the complex web of ethical issues associated with this therapy.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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