Preservation of ovarian function using human pluripotent stem cell-derived mesenchymal progenitor cells

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Ovarian reserve diminishes with age, and older women experience a corresponding shift in sex hormone levels. These changes contribute to an age-dependent decrease in fertility and a decline in overall health. Furthermore, while survival rates following cancer treatment have improved for young female patients, a reduction in ovarian function due to the side effects of such treatments can be difficult to avoid. To date, no effective therapy has been recommended to preserve ovarian health in these patients. Mesenchymal progenitor cells (MPCs) are considered a promising option for cell therapy aimed at maintaining fertility and fecundity. Although MPCs derived from human adult tissues are recognized for their various protective effects against ovarian senescence, they are limited in quantity. Consequently, human pluripotent stem cell-derived MPCs (hPSC-MPCs), which exhibit high proliferative capacity and retain genetic stability during growth, have been utilized to delay reproductive aging. This review highlights the impact of hPSC-MPCs on preserving the functionality of damaged ovaries in female mouse models subjected to chemotherapy and natural aging. It also proposes their potential as a valuable cell source for fertility preservation in women with a variety of diseases.

Keywords: Fertility preservation; Human embryonic stem cell-derived mesenchymal progenitor cells; Human pluripotent stem cell-derived mesenchymal progenitor cells; Ovarian aging; Reproductive senescence
pectancy has increased [6]. Furthermore, advancements in chemotherapy have markedly improved the survival rates of young women with cancer, heightening the importance of restoring ovarian function post-treatment. Consequently, over the past two decades, various strategies have been explored to maintain and extend the reproductive potential of women undergoing anticancer therapy or those of advanced age. Initially, direct transplantation of ovarian tissues was employed to preserve ovarian function, with clinical reports of successful fertility restoration in humans using frozen/thawed ovarian tissues [7,8]. However, this approach is more suitable for patients at risk of premature ovarian failure than for women seeking to extend fecundity or achieve healthy aging. Indeed, early preservation of autologous ovarian tissues for future transplantation is not a practical option for most women. As a result, stem cell-based strategies have emerged as an alternative for fertility preservation and anti-aging therapy, aiming to replace functional ovarian cells or restore endocrine function. Numerous studies have investigated the administration of various types of stem cells to address these challenges [9]. This review focuses on recent research involving the application of human pluripotent stem cell-derived mesenchymal progenitor cells (hPSC-MPCs) for preserving ovarian function in female mouse models of chemotherapy-induced premature ovarian insufficiency (POI) or reproductive aging. We focused on the potential usefulness of these treatments.

Adult tissue-derived MPC therapy for recovery of ovarian functions

With the advancement of regenerative medicine, a variety of stem cells are being explored for the treatment of both artificial and natural reproductive aging. Initially, research was focused on chemotherapy-induced POI [10,11] and permanent infertility. As anticancer treatments, particularly chemotherapy, prolong the lives of young female patients with cancer, they also pose a risk to their quality of life by inducing POI. Originally defined as the loss of ovarian function before the age of 40 years, POI is characterized by the presence of amenorrhea or oligomenorrhea for at least 3 months, significantly diminished ovarian reserve, hypergonadotropism, abnormally low estradiol levels, and poor ovarian response to circulating follicle-stimulating hormone [12]. Consequently, the effects of chemotherapy-induced POI may extend beyond impaired fertility, potentially including bone loss, memory issues, mood fluctuations, increased risks of cardiovascular and neurological diseases, and reduced life expectancy [13-15].

Human MPCs (also known as mesenchymal stem cells or mesenchymal stromal cells) derived from fetal or adult tissues are being explored as a potential therapeutic cell source for a variety of diseases. Broadly, MPCs can be administered through two approaches: systemic administration via intravenous (IV) injection and direct local administration into the ovaries. These approaches have been extensively developed for the purpose of restoring ovarian function [16]. MPCs can secrete various cytokines, growth factors, and exosomes that contain microRNAs and other molecules. These secretions are involved in immune modulation, angiogenesis, apoptosis, cell survival, and proliferation [17,18]. Recent studies have focused on MPCs from various tissues to improve clinical outcomes and to address POI in animal models [9,19-23]. In models of chemotherapy-induced POI, transplanted MPCs have been shown to secrete specific cytokines, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor 1. These cytokines exhibit a protective effect against apoptosis in both stromal cells and granulosa cells [24]. Additionally, MPCs may inhibit fibroblast proliferation and reduce extracellular matrix deposition, thereby playing a beneficial role in maintaining ovarian function. VEGF, fibroblast growth factor, and angiogenin derived from MPCs induce neovascularization and improve blood perfusion in damaged ovarian tissues [24,25]. Beyond chemotherapy-induced POI models, transplanted MPCs have also been reported to play a key role in the inhibition of ovarian aging in naturally aged mice by secreting epidermal growth factor and HGF [26].

The harvesting of adult MPCs can be invasive, leading to severe side effects, and the protocols for their long-term culture are challenging to standardize. Consequently, several researchers have proposed the use of human embryonic stem cell-derived MPCs (hESC-MPCs) as an alternative (Figure 1). These cells offer advantages due to their high proliferative capacity and the relative ease of standardization [9,27].

hPSC-MPC therapy for functional recovery of chemotherapy-damaged ovaries

hESCs are derived from the inner cell mass of human blastocysts and possess pluripotency, enabling them to differentiate into any of the three germ layers [28]. Recently, to address the challenge of immunological rejection, researchers have established two types of hESC-like cells that are isogenic: somatic cell nuclear transfer-derived PSCs and induced PSCs [29-31]. These three types of PSCs share similar gene expression patterns, the potential for unlimited proliferation, and the capacity to differentiate into functional cells from all three germ layers. Consequently, PSCs have become invaluable resources for stem cell therapy.

hPSC-MPCs can be generated through a straightforward method involving a differentiation medium and have demonstrated high genomic stability during differentiation and cultivation [32,33]. In our
previous researches, hPSC-MPCs exhibited a restorative capacity comparable to that of adult tissue-derived MPCs in restoring ovarian functions and uterine functions. Specifically, the direct injection of hESC-MPCs into mice with cisplatin-induced ovarian damage (the hESC-MPC group) resulted in a relative restoration of ovary size and body weight. The numbers of primary and primordial follicles were significantly higher in the hESC-MPC group compared to the control animals, and apoptosis, which leads to the loss of ovarian stromal cells, was markedly reduced. Additionally, the hESC-MPC group yielded a significantly greater number of ovulated oocytes than the control group. Furthermore, the rates of blastocyst formation from these oocytes and live births per mouse were also significantly higher in the hESC-MPC group. Therefore, all hPSC-MPCs may serve as valuable cell sources for the restoration of ovarian function in women at risk of chemotherapy-induced premature menopause.

Impact of hPSC-MPC delivery method on functional recovery of chemotherapy-damaged ovaries

The functional potentials of all hPSC-MPCs are comparable to those of tissue-derived MPCs. Notably, hPSC-MPCs exhibit a very high proliferative capacity in vitro and can be produced in large quantities, suitable for use in cell therapy, through mass culture. However, a major challenge in PSC-based cell therapy is the risk of tumor development if undifferentiated hESCs are not completely removed. This concern has spurred considerable research on the unique molecular properties of hPSCs, which may have clinical applications. Our group has developed an alternative approach involving the clonal expansion of MPCs from single hESC-MPCs, leveraging their high proliferative potential. We have successfully generated multiple cell lines through the clonal expansion of single-cell-derived hESC-MPCs, while confirming their safety via teratoma formation assay. Nevertheless, cells prepared for cell therapy using these methods face a secondary issue: genetic instability that can arise during long-term culture. Consequently, concretely demonstrating the stability of hPSC-derived cells remains a challenge.

The third challenge associated with PSC-based cell therapy is the method of delivery. In our prior research, we found that systemic administration of ESC-MPCs via IV injection can restore ovarian function in mice with ovaries damaged by cisplatin. However, this approach has several disadvantages, including the risk of pulmonary embolism and the low percentage of injected cells that reach the target site. Instead, many cells become sequestered in the spleen.
hESC-MPCs, which ensures a homogeneous distribution of cells, and increases the proportion of cells that remain viable in vivo. Scaffolds are three-dimensional polymeric biomaterials that provide structural support for tissue regeneration and facilitate cell attachment. They can also serve as delivery vehicles for growth factors, cytokines, and cells, aiding in the formation and remodeling of new tissue. These scaffolds are typically categorized into two transplantation methods: implantable scaffolds and injectable scaffolds. In a cisplatin-induced POI mouse model, locally delivered ESC-MPCs using scaffolds—either an injectable crosslinked hyaluronic acid hydrogel (HA gel) or a porous poly(D, L-lactide-co-glycolide) sponge containing magnesium hydroxide (PLGA/MH sponge)—were maintained for 4 weeks or more. This contributed to the preservation of ovarian function by mitigating chemotherapy-induced apoptotic processes. The injectable HA gel used in that study can be administered into the subcutaneous area of the back with minimal injury; in contrast, the implantable sponge scaffold requires invasive surgery for in vivo introduction. Moreover, the injectable HA gel is potentially more suitable for tissue integration, as it can readily fill the injured cavity, distribute homogeneously, and support the formation of normal tissue. To our knowledge, that study is the first to report a fertility restoration strategy using the simple introduction of hESC-MPCs onto the backs of mice with cisplatin-induced POI through subcutaneous transplantation using either implantable or injectable scaffolds. After transplantation, residual cells were detected in both types of retrieved scaffolds from the implantation site for 4 weeks after introduction.

Our previous report showed that IV-delivered hESC-MPCs could be detected only between 3 days and 2 weeks after introduction; thus, the survival rate of cells in the study using subcutaneous transplantation with injectable scaffolds was twice as high as that in previous research, with a significant increase in the functional recovery of damaged ovaries. Additionally, the HA gel group, which received an injectable HA gel with ESC-MPCs, exhibited a more regular estrous cycle than the POI group 2 months after transplantation. This finding suggests that the introduction of hESC-MPCs using the HA gel method could be employed for fertility preservation and as long-term protection against POI. This study also confirms that the secretome of hESC-MPCs plays a pivotal role in directly preventing ovarian degeneration in cell therapy.

hPSC-MPC therapy for functional recovery of naturally aged ovaries

Our primary approach was intended as a novel, straightforward, and effective method for preserving ovarian function in patients with POI or cancer survivors. Additionally, however, therapy based on hPSC-MPCs has been expanded to support the long-term maintenance of reproductive capacity in middle-aged women experiencing natural aging. This issue has gained importance as more women opt to postpone childbearing and as their life expectancy rises. Moreover, menopause typically begins in the mid-50s and represents not only the cessation of reproductive capabilities in the ovaries but also an inevitable aspect of female aging that entails numerous health concerns.

Several recent studies have reported that a variety of adverse factors, including inflammation, reactive oxygen species, DNA damage, and apoptosis, can diminish follicle quality and shorten ovarian lifespan in older women. Furthermore, tissue fibrosis due to increased collagen deposition has been observed in the ovaries of postmenopausal women and in animal models of reproductive aging. The targeted removal of fibrotic collagen from the mouse ovary has been shown to potentially extend the female reproductive lifespan. In our recent study, we found that repeated administration of hESC-MPCs may delay ovarian senescence and preserve ovarian reserve, thereby producing good-quality embryos in perimenopausal female mice. This effect is achieved through the reduction of inflammation and fibrosis. Notably, long-term treatment with hESC-MPCs decreased the population of myeloid-derived suppressor cells (MDSCs), which increase with age and play a pivotal role in tissue fibrosis. This inhibitory effect on MDSC proliferation was further confirmed through in vitro co-culture of MDSCs with hESC-MPCs. While the precise mechanisms by which hESC-MPCs inhibit MDSC proliferation and delay ovarian aging require further investigation, the results suggest that hESC-MPCs could play a key role in combating ovarian and biological aging.

Further consideration of hPSC-derived MPC therapy for recovery of ovarian function

Human MPCs derived from various sources, including adult tissues, fetal tissues, and PSCs, have been proposed as novel therapeutic agents for treating a range of diseases, such as reproductive senescence. In this review article, we discuss how hESC-MPCs may help preserve ovarian function and delay reproductive senescence in both chemotherapy-induced and naturally aging perimenopausal female mice. Furthermore, a previous study indicated that the secretome of hESC-MPCs could prevent ovarian degeneration in cell therapy.
Table 1. Studies utilizing PSC-MPCs in models of ovarian aging

<table>
<thead>
<tr>
<th>Study</th>
<th>Sources of PSCs</th>
<th>Animal model</th>
<th>Stem cell use</th>
<th>Time points</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoon et al. (2020) [34]</td>
<td>Human ESC-MPCs</td>
<td>Mice (2.0 mg/kg cisplatin for 10 days)</td>
<td>Intravenous injection (5 × 10^6 cells)</td>
<td>4 weeks after transplantation</td>
<td>↑ Follicle count, ↑ Hormone levels, ↑ Blastocyst formation rate, ↑ Offspring</td>
</tr>
<tr>
<td>Bahrehbar et al. (2022) [51]</td>
<td>Human ESC-MPCs</td>
<td>Mice (50 mg/kg cyclophosphamide for 10 days)</td>
<td>Intravenous injection (1 × 10^6 cells)</td>
<td>1 week after transplantation</td>
<td>↑ Homing into ovary, ↓ Apoptosis</td>
</tr>
<tr>
<td>Shin et al. (2021) [42]</td>
<td>Human ESC-MPCs</td>
<td>Mice (2.0 mg/kg cisplatin for 10 days)</td>
<td>Hydrogel subcutaneous injection (5 × 10^6 cells)</td>
<td>4 weeks after transplantation</td>
<td>↑ Follicle count, ↑ Hormone levels, ↑ Blastocyst formation rate, ↑ Offspring</td>
</tr>
<tr>
<td>Cao et al. (2023) [52]</td>
<td>Human iPSC-MPC-exosomes</td>
<td>Mice (120 mg/kg cyclophosphamide once a week for 2 weeks)</td>
<td>Intravenous injection (250-µg exosomes)</td>
<td>18 days after introduction</td>
<td>↑ Follicle count, ↓ Apoptosis</td>
</tr>
<tr>
<td>Zhang et al. (2023) [56]</td>
<td>Human iPSC-MPC-exosomes</td>
<td>Mice (50 mg/kg cyclophosphamide for 14 days)</td>
<td>Injected into ovarian bursa (25-µg exosomes)</td>
<td>NA</td>
<td>↑ Hormone levels, ↑ Follicle count, ↓ Apoptosis</td>
</tr>
<tr>
<td>Shin et al. (2024) [50]</td>
<td>Human ESC-MPCs</td>
<td>Naturally aged mice (10–15 months old)</td>
<td>Intravenous injection (5 × 10^6 cells)</td>
<td>4 weeks after 4 times transplantation every other month</td>
<td>↑ Hormone levels, ↑ Blastocyst formation rate, ↓ Offspring, ↓ Aging markers</td>
</tr>
</tbody>
</table>

PSC-MPC, pluripotent stem cell-derived mesenchymal progenitor cell; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; NA, not applicable.

Table 2. Selected recent studies utilizing MPCs derived from various human adult tissues in models of ovarian aging

<table>
<thead>
<tr>
<th>Study</th>
<th>Sources of ASCs</th>
<th>Animal model</th>
<th>Stem cell use</th>
<th>Time points</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang et al. [2020] [57]</td>
<td>Human amniotic fluid MPCs</td>
<td>Naturally aged mice (12–14 months old)</td>
<td>Intraovarian injection</td>
<td>4 weeks after transplantation</td>
<td>↑ Follicle count, ↑ AMH, E2, ↑ DNA repair mechanism, ↑ PI3K/AKT activation</td>
</tr>
<tr>
<td>Kim et al. (2020) [58]</td>
<td>Human placenta MPCs</td>
<td>Naturally aged rats (13–14 months old)</td>
<td>Intravenous injection (5 × 10^6 cells, 3 times)</td>
<td>5 weeks after transplantation</td>
<td>↑ AMH, E2 levels, ↑ Follicle growth via miRNA</td>
</tr>
<tr>
<td>Li et al. (2017) [59]</td>
<td>Human umbilical MPCs</td>
<td>Naturally aged rats (12–14 months old)</td>
<td>Intravenous injection (1 × 10^6 cells)</td>
<td>4 weeks after transplantation</td>
<td>↑ Follicle count, ↑ AMH, E2 levels, ↑ VEGF, HGF, IGF-1 levels</td>
</tr>
<tr>
<td>Yang et al. (2020) [53]</td>
<td>Human umbilical MPC exosomes</td>
<td>Naturally aged mice (10 months old)</td>
<td>Infracoccygeal injection</td>
<td>3 weeks after transplantation</td>
<td>↑ Follicle count, ↑ Follicle growth, ↑ PI3K/AKT activation, ↑ Offspring</td>
</tr>
<tr>
<td>Park et al. (2023) [54]</td>
<td>Human bone marrow/umbilical MPC exosomes</td>
<td>Mice (120 mg/kg cyclophosphamide and 30 mg/kg busulfan)</td>
<td>Intravenous injection (107 exosomes)</td>
<td>2 weeks after transplantation</td>
<td>↑ Follicle count, ↑ AMH, E2 levels, ↑ Offspring, ↓ Fibrosis</td>
</tr>
<tr>
<td>Li et al. (2023) [55]</td>
<td>Human umbilical MPC exosomes</td>
<td>Naturally aged mice (14 months old)</td>
<td>Intraperitoneal injection (150-µg exosomes, 2 times)</td>
<td>10 weeks after transplantation</td>
<td>↑ Follicle count, ↓ PTEN activity, ↓ Apoptosis</td>
</tr>
</tbody>
</table>

MPC, mesenchymal progenitor cell; ASC, adult tissue-derived stem cells; AMH, anti-Müllerian hormone; E2, estradiol; PI3K, phosphatidylinositol 3-kinase; AKT, disambiguation; miRNA, microRNA; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; PTEN, phosphatase and tensin homolog.
generation and help maintain female fecundity when these cells were locally administered in a chemotherapy-induced mouse model [42]. Therefore, the ongoing administration of secretome or extracellular vesicles from hPSC-MPCs and adult tissue-derived-MPCs may aid in the functional recovery or preservation of damaged or aging ovaries [42,52-56]. These studies also suggest that such interventions could play a pivotal role in overcoming challenges associated with the clinical application of hPSC-MPCs.

Recent studies have reported that various substances secreted by hPSC-MPCs and adult tissue-derived MPCs play key roles in suppressing ovarian and biological aging by preventing inflammation and fibrosis (Tables 1 and 2) [34,42,50-59]. Therefore, defining their functions and uncovering their precise mechanisms could greatly contribute to the advancement of regenerative medicine, which seeks to treat aging-related diseases in various organs, as well as to the field of reproductive medicine.

**Conclusion**

The application of hPSC-MPCs may facilitate the repair of damaged ovarian environments and reserves in female mice, whether the damage is artificial or natural. This is achieved by reducing apoptosis, inflammation, and fibrosis.

Consequently, cell therapy employing hPSC-MPCs could serve as a valuable tool for prolonging fertility and fecundity, as well as preserving ovarian function in women undergoing chemotherapy or those of advanced age. Prior to widespread clinical application, additional research into hPSC-MPC delivery systems is essential to ensure patient safety, and the functional mechanisms of these therapies must be clarified.

**Conflict of interest**

No potential conflict of interest relevant to this article was reported.

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Conceptualization: DRL, JEL. Data curation: JEL. Funding acquisition: DRL. Project administration: DRL. Visualization: DRL, JEL. Writing-original draft: DRL, JEL. Writing-review & editing: JEL. Approval of final manuscript: DRL, JEL.

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