

# Mesenchymal Stem Cells (MSCs) Therapy for Recovery of Fertility: a Systematic Review

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**Abstract** In recent years, the mesenchymal stem cells (MSCs) have provided the new opportunities to treat different disorders including infertility. Different studies have suggested that the MSCs have ability to differentiate into germ-like cells under specific induction conditions as well as transplantation to gonadal tissues. The aim of this systematic review was to evaluate the results obtained from different studies on MSCs therapy for promoting fertility. This search was done in PubMed and Science Direct databases using key words MSCs, infertility, therapy, germ cell, azoospermia, ovarian failure and mesenchymal stem cell. Among the more than 11,400 papers, 53 studies were considered eligible for more evaluations. The obtained results indicated that the most studies were performed on MSCs derived from bone marrow and umbilical cord as compared with the other types of MSCs. Different evaluations on animal models as well as in vitro studies supported from their role in the recovery of spermatogenesis and folliculogenesis. Although the data obtained from this systematic review are promising, but the

further studies need to assess the efficiency and safety of transplantation of these cells in fertility recovery.

**Keywords** Stem cell therapy · Mesenchymal stem cell · Infertility · Treatment

## Introduction

Infertility is known as an important health and social problem, affecting both men and women. This condition was defined as the inability to get pregnant after more than 1 year of unprotected intercourse. The studies indicated that this disorder affects 1 in 6 couples [1]. Different factors have been demonstrated to play a role in the fertility ability of individuals. They included anatomical defects, environmental and genetics factors [2, 3].

Although the assisted reproductive technique (ART) is considered as the most effective way for treatment of infertility in humans, but the use of this method was accompanied with some limitations. This technique cannot be used for patients with no sperm [4]. The recent studies have been focused on the derivation of germ like cells from stem cells or the evaluation of stem cell transplantation in treatment of infertility [5–9].

Numerous researches indicated that embryonic stem cells (ESCs) had the ability to generate the differentiated cells expressing some germ cell markers [5, 6, 10–12]. However, the use of these stem cells has disadvantages including the tumor formation and ethical limitations. Furthermore, the isolation of these cells needs the destruction of human embryos [13–15].

Recently, mesenchymal stem cells (MSCs) therapy has been considered as the new option to treat infertility [16]. These spindle-shaped cells could be proliferated and formed

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colony forming unit-fibroblasts (CFU-Fs). These cells were generally isolated by the adherent ability to the plastic surface and their identity was confirmed by the positive expression of CD105, CD73 and CD90 as well as the negative expression of CD45, CD34, CD14, CD19 and HLA-DR [17]. Mesenchymal stem cells (MSCs) were reported to have the differentiation ability into cells of all three germ layers including osteocytes, chondrocytes, adipocytes, cardiac cells, neuronal cells and germ cells [18, 19]. However, the studies have been demonstrated that the differentiation ability of bone marrow-derived mesenchymal stem cells (BM-MSCs) decreased with increasing age [20, 21]. The growth factors secreted by MSCs have been revealed to involve in cell survival, proliferation, migration as well as angiogenesis and immune modulation. Hence, these cells were suggested as ideal candidates for regenerative medicine [22].

These cells can be obtained from different tissues such as bone marrow, lung, liver, adipose tissue, umbilical blood, amniotic fluid, umbilical cord and peripheral blood as well as reproductive tissues [23–32]. Some reports indicated that there was very small embryonic like stem cells (VSELs) in testis and ovary. They showed the characteristics resembling MSCs [33]. These cells have been known to be in the basal layer of seminiferous epithelium of the testis and surface epithelium of the ovary. They could undergo asymmetric division and give rise to the progenitor cells. Different evidence presented that these cells survive following chemotherapy exposure and can undergo germ cell differentiation. It appears that these cells function as a backup population of stem cells [34, 35]. It is likely that the interaction between these cells and the transplanted MSCs play a role in recovery of fertility. Currently, a small subpopulation of MSCs has been identified to have the pluripotency ability. These cells could migrate into damaged tissue via bloodstream and undergo differentiation into the cells identical with the cells of target tissue. These cells were known as the multilineage-differentiating stress-enduring (Muse) cells [36].

The aim of this systematic review is to investigate the effects of mesenchymal stem cells derived from different sources in the treatment of infertility. The results obtained from the present review could play an important role in the assessment of translation of MSCs therapy in clinical trials.

## Materials and Methods

### Focused Question

This systematic review was performed to address the following question: “Could mesenchymal stem cells be used as a suitable option in the treatment of infertility?”

## Search and Study Selection

Key words and subject terms included (“MSCs” AND “infertility”) OR (“MSCs” AND “infertility” AND “therapy”) OR (“mesenchymal stem cell” AND “infertility”) OR (“MSC” AND “germ cell”) OR (“MSC” AND “ovarian failure”) OR (“MSC” AND “azoospermia”) OR (“mesenchymal stem cell” AND “ovarian failure”) OR (“mesenchymal stem cell” AND “azoospermia”). The search strategy was applied to PubMed database and ScienceDirect, being focused on the animal models and in vitro studies. The filters included the publications in the English language. The abstracts not published as full manuscripts, reviews or the articles about MSCs therapy for diseases except infertility were excluded. Stem cell sources other than mesenchymal stem cells were also rejected. Data were collected from the full text of the articles as follows: (i) the source of MSCs (ii) type of the study (in vitro or in vivo) (iii) the method used for the evaluation of the germ cell differentiation ability of MSCs and (iv) the obtained results.

## Results

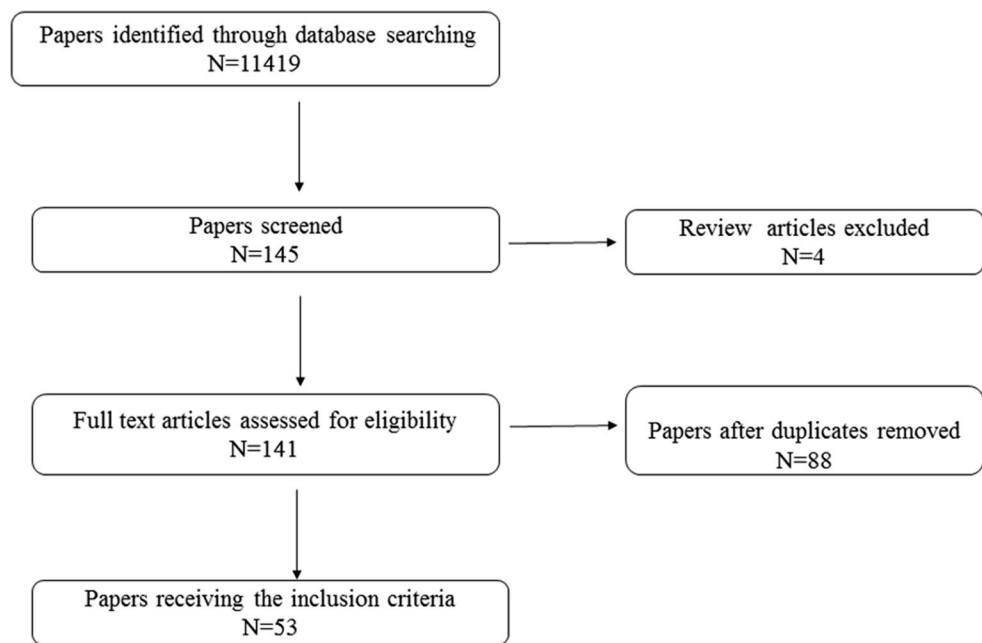
### Search Results and Characteristics of Included Studies

The electronic search process yielded 11,419 studies. Among them, 145 papers received all inclusion criteria (Fig. 1). A total 53 articles were selected after removing duplicates and reviews [6, 8, 11, 37–86]. From these articles, 16 articles included the results obtained from the MSCs transplantation in male animal models (Table 1), whereas 14 articles showed the data of the MSCs transplantation assay in female animal models (Table 2).

### Cell Sources of MSCs Used to Induce Germ Cell Differentiation

MSCs derived from bone marrow (BM-MSCs) were used in 23 studies, whereas 12 papers used umbilical cord-derived MSCs (UCMSCs) for treatment of infertility. Only one study has examined the microvesicles (MVs) generated from MSCs. In other studies, the MSCs derived from adipose tissue, amniotic membrane, chorionic membrane, menstrual blood, skin and lung were evaluated. In two papers, the researchers used mesenchymal stem cells of first trimester human umbilical cord-derived perivascular cells (FTM HUCPVCs) and term human umbilical cord-derived perivascular cells (HUCPVCs). One study also investigated the effect of mesenchymal stem cells derived from MSCs isolated from human orbital fat tissue (OFSCs).

The effects of MSCs transplantation into ovarian and testis tissues were studied in 23 studies, whereas in vitro study

**Fig. 1** The PRISMA chart used in the study process

has been used to evaluate the MSCs differentiation into germ cells in 22 papers. To investigate the role of MSCs on infertility treatment, in vitro assay was followed by transplantation in five studies. Most of these studies were performed with the use of MSCs derived from the human species.

#### Use of Transfection/Transduction for Differentiation of MSCs into Germ Cells

Li et al. (2016) transduced UCMSCs using plasmid including CD61. The expression analysis of markers indicated the differentiation of these cells into germ like cells [78]. In another study, Fang et al. (2017) increased the CD61 expression by the transfection and the expression of germ cell markers were evaluated by qRT-PCR, immunocytochemistry and western blot. Their results revealed that CD61 overexpression could induce the differentiation of MSCs into primordial germ cell (PGC)-like cells through integrin- $\beta$ 3-transforming growth factor beta (TGF- $\beta$ ) pathway [37]. In two other studies, the researchers demonstrated that the overexpression of STRA8, BOULE, and DAZL genes by plasmid transfection could contribute to the differentiation of BM-MSCs into germ like cells. These observations were also confirmed by qRT-PCR, immunocytochemistry and western blot [71, 86].

#### Treatment Approaches Used for Differentiation of MSCs into Germ Like Cells

Different combinations of growth factors were used in 13 studies to induce the differentiation of MSCs into germ like cells. Hua et al. (2009) treated the human BM-MSCs by

retinoic acid (RA) and testicular extracts. Although the low number of cells differentiated into germ cells, the expression pattern of germ cell markers showed that these cells could be used for treatment of infertility [11]. These researchers (2009) in another study also showed that a small proportion of lung-derived MSCs (L-MSCs) can differentiate into germ like cells after treatment with RA. However, it was not clear that whether these cells had the ability to function as spermatozoa [48]. Hosseinzadeh Shirzeily et al. (2013) induced two types of MSCs by treatment with RA. Their obtained results revealed that BM-MSCs had the germ cell differentiation ability more than adipose-derived MSCs (ADM-SCs) [56]. In another study, Ghasemzadeh-Hasankolaei et al. (2014) evaluated the effect of BMP4, BMP8b and TGF $\beta$ 1 on the differentiation ability of BM-MSCs into germ like cells in vitro conditions. They found that TGF $\beta$ 1 treatment could be led to more effective differentiation of BM-MSCs into primordial cells, as compared with BMP4 and BMP8b treatment [6]. Jouni et al. (2014) also demonstrated that BM-MSCs can differentiate into germ cells through activation of the MAPK pathway. They used SMF and BMP4 for induction of differentiation [62].

Li et al. (2014) and Wei et al. (2016) assessed the effect of BMP4 treatment on UCMSCs and ADMSCs, respectively. Their observations indicated that these cells can differentiate into germ like cells under medium including BMP4 [77, 84]. In other research, Kaviani et al. (2014) investigated the expression of germ cell markers in vitrified-UCMSCs and non-vitrified-UCMSCs treated with RA, testosterone and conditioned medium prepared from testicular cell cultures (TCC). They found that vitrification did not have any effect on the expression of germ cell markers [57]. Amidi et al.

**Table 1** Data of the animal studies in which the spermatogenesis was supported by MSCs transplantation

| Author (year)                              | Cell source  | Intervention (s)   | Statistical tests   | Outcome  |
|--|--|--|---|--|
| Abd Allah et al. (2017) [39]               | Human UCB-MSCs                                     | The injection of these cells into testis   | Analysis of variance (ANOVA)  | The injection of UCB-MSCs into busulfan-induced azoospermia mice restored the spermatogenic ability  |
| Ghasemzadeh-Hasankolaie et al. (2016) [43] | BM-MSCs isolated from male rats' tibias            | Transplantation into the testes of infertile rats  | One-way ANOVA, Tukey's HSD  | Data demonstrated that BM-MSCs had the capacity to differentiate into spermatogonia in the testes  |
| Hassan and Alam (2014) [46]                | Rat BM-MSCs  | Treatment of rats with BM-MSCs after injection of lead nitrate (LN)  | ANOVA, Duncan's test  | Treatment with MSCs can decrease the toxic effects induced by LN and it can be used as an effective way for treatment of infertility   |
| Yang et al. (2014) [47]                    | Human-UCMSCs                                       | The injection of UCMSCs into the interstitial space of one testis from busulfan-treated mice   | Student's <i>t</i> -test  | The results demonstrated that UCMSCs could increase the expression of germ cell markers in the testis of busulfantreated mice  |
| Cakici et al. (2014) [8]                   | rat ADMSCs   | Transplantation of ADMSCs into testes of busulfan-treated mice   | Two-tailed paired <i>t</i> -test  | Transplantation of MSCs derived from adipose tissue could be used for recovery of spermatogenesis and treatment of infertility   |
| Hsiao et al. (2015) [49]                   | MSCs derived from human orbital fat tissue (OFSCs) | The injection into torsion-induced Sprague–Dawley rats   | Tukey–Kramer <i>t</i> test  | The results showed that MSCs reserved spermatogenesis in torsion-induced rats  |
| Chen et al. (2015) [52]                    | Human UCMSCs                                       | The injection of UCMSCs into the seminiferous tubules of busulfan-treated mice   | Paired <i>t</i> -test   | The expression of germ cell markers in transplanted MSCs indicated their differentiation into germ cells   |
| Sabbaghi et al. (2012) [58]                | Rat BM-MSCs  | Transplantation into torsion azoospermia testes  | –   | Although the expression of germ cell markers indicated the differentiation of the MSCs into germ cells, but no regeneration of mature sperm was found after 95 days post-transplantation |
| Mehrabani et al. (2015) [66]               | Rat ADMSCs   | The injection of ADMSC into the efferent duct of right testes  | One-way ANOVA, Mann–Whitney <i>U</i> test                                     | The histological assay showed the presence of spermatogenesis in treated seminiferous tubules  |
| Aghamir et al. (2014) [67]                 | Mouse BM-MSCs                                      | The injection of BM-MSCs into ventral vein of tail   | Fisher exact test   | The transfusion of MSCs showed the immunomodulatory properties on production of antisperm antibody (ASA)   |
| Maghen et al. (2016) [41]                  | FTM HUCPVCs  | 1- Culturing murine germ cells on mitomycin-inactivated FTM HUCPVC feeders<br>2- The injection of FTM HUCPVCs in an immunocompromised mouse model of mono-2- ethylhexyl phthalate (MEHP)-induced testicular damage | Student's <i>t</i> -test, ANOVA followed by Tukey's multiple comparisons test | The results indicated that FTM HUCPVCs could support spermatogonial stem cell proliferation and the regeneration of seminiferous tubules   |
| Ghasemzadeh-Hasankolaie et al. (2016) [44] | Ram BM-MSCs  | Three different treatments including RA for 14 days, RA for 21 days and TGF- $\beta$ 1 for 21 days and then transplantation of the treated cells into the testes of ram lambs                                      | One-way ANOVA, Tukey's <i>b</i> , Kruskal–Wallis test                         | TGF- $\beta$ 1 Treatment could be used as an efficient way for differentiation of BM-MSCs into spermatogonia-like cells  |

**Table 1** (continued)

| Author (year)                 | Cell source  | Intervention (s)  | Statistical tests  | Outcome   |
|-------------------------------|--------------|---|--|---|
| Zhang et al. (2014) [55]      | rat BM-MSCs  | Co-culturing with testicular sertoli cells in vitro and then, transplantation into the seminiferous tubules of the busulfan-induced azoospermia rat | Student–Newman–Keuls test  | The results obtained from expression analysis indicated that these cells were able to differentiate into spermatogenic cells in the azoospermic rat model   |
| Wang et al. (2013) [60]       | Goat BM-MSCs | 1- Treatment with RA<br>2- Transplantation of MSCs into seminiferous tubules of busulfan treated mice   | Student's t test   | The results showed that these MSCs can differentiate into germ cells after treatment with RA, but the number of differentiated cells is low. The results obtained from transplantation also indicated the differentiation of some MSCs into germ cells in the mouse model |
| Vahdati et al. (2017) [85]    | BM-MSCs      | BM-MSCs transplantation to busulfan-induced azoospermia hamster model   | One-way ANOVA, followed by LSD post-hoc Tests, Mann–Whitney U test | The results indicated that BM-MSCs transplantation improved spermatogenesis in busulfan-induced azoospermia model   |
| Rahmanifar et al. (2016) [74] | BM-MSCs      | Transplantation of BM-MSCs into efferent duct of testis in azoospermia rat models   | One-way ANOVA, Tukey post hoc test and Mann–Whitney U test         | MSCs transplantation led to induction of spermatogenesis in azoospermia rats  |

*ADMSCs* Adipose-derived mesenchymal stem cells, *UCB-MSCs* Umbilical cord blood-derived mesenchymal stem cells, *BM-MSCs* Bone marrow- mesenchymal stem cells, *UCMSCs* Umbilical cord derived mesenchymal stem cells, *FTM HUCPVCs* First trimester human umbilical cord-derived perivascular cells, *term HUCPVCs* Term human umbilical cord-derived perivascular cells

(2015) also demonstrated that UCMSCs in the presence of RA and TCC had the ability to differentiate into germ like cells [51]. Other study performed by Yan et al. (2015) also confirmed that RA can have the more ability than BMP4 to induce the expression of germ cell markers [86].

In 2016, Afsartala et al. was evaluated the ability differentiation of amniotic-derived MSCs (AMSCs). They found that these AMSCs can differentiate into germ like cells through a two-step treatment method with BMP4 and RA [42]. Hou et al. (2016) also tried to establish Leydig cells from BM-MSCs by treatment with gonadotropins. These cells can be suggested as a suitable option in infertile patients with low level of androgen [61].

In one study, Shlush et al. (2017) also showed that mesenchymal stem cells derived from HUCPVCs had the ability to differentiate into germ like cells by using the cocktail of growth factors containing retinoic acid (RA), leukemia inhibitory factor (LIF), glial cell-derived neurotrophic factor (GDNF), putrescine, testosterone and follicle-stimulating hormone (FSH). The differentiation of HUCPVCs was confirmed by xenotransplantation assay as well as expression analysis. Fluorescence in situ hybridization (FISH) also showed that 10–30% of cells were given rise to haploid cells after treatment for 5 weeks. Also, the results obtained from expression analysis demonstrated that these cells can generate the Sertoli like cells under the similar conditions [38]. These observations suggested that the similar signal pathways function in the development of germ cells and Sertoli cells.

### Co-culture Method Used for Differentiation of MSCs into Germ Cells

Co-culture of MSCs for induction of germ cell differentiation has been reported only in three studies. Asgari et al. (2015) cultured UCMSCs in the presence of placental cell. They found that the factors secreted from these cells could lead to differentiation of MSCs into primordial germ cells (PGCs) [53]. The study performed by Xie et al. (2015) revealed that the expression pattern and morphology of MSCs co-cultured with Sertoli cells (SCs) was similar to germ cells, confirming their differentiation into male germ cells [54]. The results obtained by Liu et al. (2014) also indicated that co-culturing granulosa cells (GCs) with BM-MSCs contributed to reduce apoptosis induced by cisplatin [81].

### Use of MSCs as a Feeder Layer to Expand Germ Cells

Cui et al. (2009) demonstrated that BM-MSCs feeder layer could play a role in supporting embryonic stem cell differentiation into germ-like cells [68]. In only one study, the MSCs used as the feeder cells to expand spermatogonial stem cells (SSCs). The study of FTM HUCPVCs by Maghen et al. (2016) revealed that these MSCs secreted factors

**Table 2** Data of the animal studies in which the oogenesis was supported by MSCs transplantation

| Author (year)                | Cell source                             | Intervention (s)   | Statistical tests   | Outcome   |
|------------------------------|---|--|---|---|
| Song et al. (2016) [50]      | Human UCMSCs                            | The injection into POF rat model induced by cyclophosphamide (CTX)   | Student's t-test, one-way ANOVA   | The obtained data showed that human UCMSCs transplantation had the ability to reverse the normal hormone secretion and folliculogenesis in POF model rats           |
| Abd-Allah et al. (2013) [59] | Rabbit BM-MSCs                          | Injection into cyclophosphamide (CTX) induced rabbits with ovarian failure   | Analysis of variance (ANOVA)  | The results indicated that the injection of MSCs improved the ovarian function in CTX-induced rabbits   |
| Elfayomy et al. (2016) [65]  | Human UCB-MSCs                          | The injection of UCB-MSC into Paclitaxel-treated ovarian failure   | One-way analysis of variance (ANOVA), LSD multiple comparison test                              | The results indicated that UCB-MSCs can improve the ovarian function in Paclitaxel-treated rats   |
| Wang et al. (2017) [70]      | MenSCs                                  | The infusion of MenSCs into the tail vein of POF mouse model   | One-way analysis of variance (ANOVA)  | MenSCs could play a role in POF treatment through repair oocytes more than direct differentiation into oocytes  |
| Wang et al. (2013) [72]      | UCMSCs                                  | Transplantation of UCMSCs into POF model mice  | –   | UCMSCs may contribute to the restoration of ovarian function in POF model mice  |
| Gan et al., (2017) [73]      | AMSCs                                   | Transplantation of AMSCs into rat intrauterine adhesions (IUA) models  | One-way analysis of variance tests, Tukey's honestly significant difference (HSD) post hoc test | Transplantation of AMSCs play a role in regeneration of endometrial epithelial cells in IUA models  |
| Liu et al. (2012) [75]       | AMSCs                                   | The injection of AMSCs into a POF model  | Student's t-tests   | The observations demonstrated that MSCs has ability to survive and proliferate in the ovarian tissues long term   |
| Kilic et al. (2014) [76]     | BM-MSCs                                 | The administration of cyclophosphamide and BM-MSCs to Wistar albino female rats  | Kruskall–Wallis and Mann–Whitney U tests  | Treatment with MSCs could play a protective role from germ cells against toxicity of chemotherapy   |
| Fouad et al. (2016) [79]     | AMSCs and ADMSCs                        | The injection of MSCs (AMSCs or ADMSCs) to cyclophosphamide (CTX) induced POF model  | Single-factor ANOVA, Tukey–Kramer test post hoc tests   | Administration of MSCs (ADMSCs or AMSCs) could increase follicles in ovarian failure model  |
| Mohamed et al. (2017) [80]   | Human BM-MSCs                           | Intraovarian injection of MSCs in a chemotherapy-induced ovarian failure mouse model   | Bonferroni method   | Intraovarian administration of MSCs restored folliculogenesis in the chemotherapy-induced ovarian damage model  |
| Lai et al. (2014) [82]       | SMSCs                                   | Intravenous injection of SMSCs to busulfan and cyclophosphamide-treated female mice  | ANOVA, Kruskal–Wallis test  | Transplanted SMSCs migrated to the ovaries. They decreased the expression level of pro-inflammatory cytokines and increased the expression level of oogenesis genes |
| Liu et al. (2014) [83]       | Human MenSCs                            | The injection of MenSCs into a cyclophosphamide-induced mouse POF model  | Student's t-test  | Transplantation of MenSCs increased the expression of ovarian markers in the ovaries of POF model   |
| Fu et al. (2008) [64]        | BM-MSCs derived from female Wistar rats | 1- Co-culturing of granulosa cells (GCs) with MSCs<br>2- Injection of MSCs into bilateral ovaries                                      | Student-Newman-Keuls (SNK) test, unpaired Student's t-test                                      | The results indicated that MSCs can diminish apoptosis of granulosa cells   |
| Liu et al. (2014) [81]       | BM-MSCs                                 | a) Co-culturing granulosa cells (GCs) with BM-MSCs and cisplatin<br>b) Intravenous injection of BM-MSCs to cisplatin-induced POF model | One-way ANOVA   | The migration and homing BM-MSCs could play a role in restoration function of ovarian in POF model  |

*ADMSCs*: Adipose-derived mesenchymal stem cells, *BM-MSCs*: Bone marrow- mesenchymal stem cells, *UCMSCs*: Umbilical cord derived mesenchymal stem cells, *MenSCs*: Menstrual- derived mesenchymal stem cells, *SMSCs*: Skin-MSCs, *AMSCs*: Amniotic MSCs

FGF2, BMP4 and LIF, supporting SSCs differentiation. The evaluation of culturing murine germ cells on mitomycin-inactivated FTM HUCPVC feeders also showed that these cells could be applied as a feeder system for proliferation and survival of germ cells [41].

### **The Application of Microvesicles (MVs) Derived from MSCs in Treatment of Infertility**

In only one study, Mekarizadeh et al. (2013) evaluated the effects of microvesicles (MVs) derived from MSCs on the quality parameters of sperms. They found that these MVs enhanced the quality of crypserved sperms, suggesting that the MVs derived from MSCs can play a protective role against the sperm damage [63].

### **Animal Studies Performed for the Treatment of Male Infertility by MSCs Transplantation**

The efficacy of MSCs therapy on male infertility was evaluated in 16 different animal studies. In 2012, Sabbaghi et al. transplanted BM-MSCs into the testicular torsion rats to evaluate the ability of these cells in the treatment of infertility. They observed no spermatogenesis in the torsion testis after BM-MSCs transplantation. However, the expression of germ cell markers indicated the initiation of differentiation into germ cells. It is likely that it needs to follow up the study for a longer time [58]. In another study, Wang et al. (2013) used the MSCs isolated from goat bone marrow in xenotransplantation, demonstrating that MSCs had potential to restore spermatogenesis into the seminiferous tubules of busulfan-treated mice [60]. Other studies performed by Rahmanifar et al. (2016) and Vahdati et al. (2017) also confirmed these observations [74, 85].

Cakici et al. (2014) also investigated the effect of ADM-SCs transplantation in the treatment of infertility in rats. They found that these cells reversed the spermatogenesis in some seminiferous tubules of busulfan treated rats [8]. The other study on lead nitrate (LN)-treated male rats indicated that the administration of MSCs improved the sperm characteristics and could play a role in the reestablishment of spermatogenesis [46]. Yang et al. (2014) also revealed that UCMSCs transplantation improved the expression of germ cell markers in the testis of busulfan-treated mice and these cells could be suggested as a suitable option for treatment of infertility [47]. In another study, Zhang et al. (2014) demonstrated that transplantation of BM-MSCs co-cultured with Sertoli cells can induce trans-differentiation of MSCs into spermatogenic cells in busulfan-induced azoospermic rats [55]. Furthermore, the other study (2014) on the evaluation of MSCs transplantation into a mouse model of autoimmune infertility indicated that MSCs may play a role in suppression of antisperm antibody (ASA) [67].

In 2015, Chen et al. observed that UCMSCs had the ability to differentiate into germ like cells and facilitated the improvement of damaged testicular tissue in busulfan-treated mice [52]. The study of torsion-induced Sprague–Dawley rats also demonstrated that MSCs could prevent torsion-induced infertility through diminish apoptosis and oxidative stress as well as increase testosterone secretion [49]. Ghasemzadeh-Hasankolaei et al. (2016) transplanted the BM-MSCs into the testes of infertile rats. They observed that the number of transplanted cells decreased in the testes as time progressed. However, their results indicated that transplanted BM-MSCs differentiated into spermatogonia in the testes of infertile rats [43], being consistent with the similar results obtained by Mehrabani et al. [66]. Anand et al. (2016) also demonstrated that MSCs derived from bone marrow had the ability to restore spermatogenesis following busulfan treatment [69]. Ghasemzadeh-Hasankolaei et al. (2016) in another study investigated the differentiation ability of ram BM-MSCs into germ cells. They observed that TGF- $\beta$ 1 was more effective than RA in differentiation of BM-MSCs into spermatogonia. Indeed, these treated cells could home at seminiferous tubules and form colonies [44]. Maghen et al. (2016) also demonstrated that FTM HUCPVCs could improve the regeneration of the seminiferous tubule in mono-2-ethylhexyl phthalate (MEHP)-treated male mice [41]. In another study, Abd Allah et al. (2017) compared the differentiation ability of MSCs, hematopoietic stem cells (HSCs) and mono cell layer derived from umbilical cord blood. Their results showed that the injection of MSCs restored the spermatogenesis ability to testes of busulfan-treated mice model. In contrast; these results did not observe for treatment with HSCs and mono cell layer [39].

### **Animal Studies Performed for the Treatment of Female Infertility by MSCs Transplantation**

In 14 studies, the transplantation of MSCs was investigated in female animal models of infertility. Fu et al. (2008) presented that MSCs secreted growth factors including VEGF, IGF-1 and HGF in culture medium, reducing the germ cell (GC) apoptosis and enhancing the function of the ovary [64]. Furthermore, Kilic et al. (2014) investigated the effect of MSCs therapy in cyclophosphamide-treated rats. They demonstrated that administration of MSCs following chemotherapy could protect from germ cells against apoptosis [76]. Abd-Allah et al. (2013) also demonstrated that MSCs could increase ovarian follicle in rabbits with ovarian failure [59]. Fouad et al. (2016) also obtained the similar results following the injection of AMSCs and ADMSCs to rats with cyclophosphamide-induced ovarian failure [79]. Mohamed et al. (2017) used the intraovarian injection of MSCs in chemotherapy-treated mouse model. They found that transplanted MSCs could improve folliculogenesis and pregnancies number in chemotherapy-induced ovarian failure mouse model [80].

In 2016, Elfayomy et al. established the ovarian failure model through treatment of rats with paclitaxel. The results obtained from MSCs injection indicated that these stem cells can improve the ovarian failure and folliculogenesis through the establishment of epithelium or the effects on the ovarian microenvironment [65]. The data obtained by Wang et al. (2017) also confirmed these observations [70]. In two other studies, the researchers used a cyclophosphamide (CTX)-induced rat model to evaluate the role of MSCs transplantation in improvement of infertility. They found that the injection of UCMSCs could diminish cell apoptosis in the ovary and improve folliculogenesis [50, 72]. Liu et al. (2012, 2014) also observed long-term survival and proliferation of MSCs in the ovary of cisplatin or cyclophosphamide-induced POF model [75, 81]. Lai et al. (2014) transplanted MSCs derived from the skin to the chemotherapy-treated females. The obtained results indicated that the migration of these cells to damage ovaries could play a role in regulation of pro-inflammatory cytokines as well as oogenesis marker genes, restoring the function of damaged ovaries [82]. Liu et al. (2014) also found that menstrual blood-mesenchymal stem cells (MenSCs) transplantation increased the expression level of ovarian markers in the POF model. The higher number of normal follicles was observed in this transplanted group, making them ideal cells in the infertility treatment [83].

Gan et al. (2017) also established the Intrauterine adhesion (IUA) models and then, they studied the efficiency of MSCs transplantation on damaged endometria in these models. They found that immunomodulatory properties of MSCs could play a role in endometrial regeneration in the injured uteri [73].

## Discussion

This study is the first systematic review about the ability of mesenchymal stem cells (MSCs) in restoration of fertility. Although the clinical trial has planned for effect of mesenchymal stem cell transplantation in treatment of infertility (<https://clinicaltrials.gov/ct2/show/NCT02062931>), but its outcome is unknown at present. The results of the present systematic review were based on *in vitro* differentiation and animal studies. The various sources of MSCs used in the studies and different evaluation methods make it difficult to compare the obtained results with each other.

In the most studies, MSCs derived from bone marrow (BM-MSCs) was used for the assessment of the role of these cells in infertility therapy. The results of these studies indicated that growth factors including RA, BMP4, BMP8b and TGF $\beta$ 1 could support from the differentiation ability of BM-MSCs into germ like cells. However, there were some limitations. For example, the no expression of ACR meiotic

marker was observed in TGF $\beta$ 1 treated cells, indicating these cells cannot enter into meiosis [6] or fewer numbers of sperm like cells was observed after treatment of BM-MSCs with RA and the extracts obtained from goat testes, as reported by Hua et al. (2009) [11]. Hou et al. (2016) was also reported that the BM-MSCs could be considered as the treatment option for infertile patients with complete loss of Leydig cells [61]. The studies indicated that the Leydig cells play a role in the support of spermatogonial stem cell proliferation [87]. Furthermore, it appears that the treatment of BM-MSCs before injection play a key role in the improvement of transplantation outcome. The comparison of the obtained results indicated that the type of treatment of BM-MSCs before transplantation could influence on the fate of transplanted BM-MSCs. Some growth factors may inhibit the meiotic division in these cells, as demonstrated by Ghasemzadeh-Hasankolaei et al. (2016) [44]. Furthermore, the tracing of transplanted cells demonstrated their migration into the basement membrane of the seminiferous tubules. In the study performed by Wang et al. (2013), the BM-MSCs derived from goat were transplanted into the seminiferous tubules of busulfan-treated mice. The observation of spermatogenesis improvement indicated that it is less likely that the difference between donor and recipient species affected on the outcome of MSCs therapy of infertility [60]. Also in some studies, the effect of BM-MSCs on germ cells (GCs) survival was reported in damaged ovaries. The authors found that BM-MSCs play a role in improving follicles survival and reduction of GCs apoptosis [59, 64, 80]. In fact, it appears that cytokines secreted from MSCs could influence on proliferation and survival of neighboring cells, be consistent with the results of the study performed on microvesicles (MVs) derived from MSCs [61]. Very low number of ovarian stem cells (OSCs) have been identified in ovarian surface epithelium and cortical tissue. These cells have been known to be expressed ovarian germ line markers and had ability to differentiate into cells of all three embryonic germ layers. Some reports indicated that these cells can produce new granulosa cells and primary follicles [88, 89]. Furthermore, discovery of spermatogonial stem cells (SSCs) suggested the new resource for the restoration of spermatogenesis in the testis. These cells showed the unusual feature as they are unipotent cells [90, 91]. The existence of these stem cells (OSCs and SSCs) in the gonadal tissues suggested that the growth factors secreted by transplanted MSCs may influence on these cells and led to progress the differentiation of these cells into germ cells.

The interesting results has obtained from the assessment of the ADMSCs in treatment of infertility [8, 37, 56]. Although the results obtained by Hosseinzadeh Shirzeily et al. (2013) indicated that ADMSCs had less differentiation ability than BM-MSCs following treatment with RA [56], the other study performed by Cakici et al. (2013) on the transplantation of



ADMSCs into busulfan-treated testes demonstrated that these MSCs could differentiate into functional sperms. The observation of birth of 9 live offspring after mating healthy females with males transplanted with ADMSCs was the direct evidence of the role of ADMSCs in the restoration of fertility ability [8]. However, the recent study about the effect of over expression of CD61 on the differentiation of MSCs into germ cells indicated that the fewer numbers of cells (almost 1%) showed the haploid status in vitro [37]. Indeed, the results obtained from the studies showed that MSCs derived from adipose tissue could be a suitable and available source for the establishment of spermatogenesis in azoospermia males.

In some other studies, the generation of germ cells from UCMSCs was assessed in vitro [40, 45, 51, 53, 54, 57]. In two of them, the co-culture technique was used to evaluate the differentiation ability of UCMSCs into germ cells [53, 54]. The co-culture method has been identified to provide a suitable microenvironment for UCMSCs differentiation. Although the results obtained from these two studies indicated the differentiation of UCMSCs into germ like cells under co-culture conditions with placental cells or Sertoli cells [53, 54], but the absence of evaluation of meiotic markers such as SCP1 was considered as the limitation of these studies. The effect of vitrification on germ cell differentiation ability of UCMSCs was assessed only in one study performed by Kaviani et al. (2014) [57], indicating that the vitrification did not influence on the differentiation ability of these mesenchymal stem cells. Although the results obtained by expression analysis confirmed the differentiation ability of UCMSCs into germ like cells after culturing in presence of testicular cell conditioned (TCC)- medium or RA followed by placental feeder cells or BMP4, these cells did not show the haploid status [40, 45, 51, 57]. It seems that these cells showed the characteristics like to primitive germ cells. The outcome obtained from the animal studies was also supported from the efficacy of UCMSCs in the recovery of seminiferous tubules morphology, improvement of spermatogenesis and folliculogenesis in azoospermia and premature ovarian failure (POF) models [47, 50, 52]. These observations were consistent with the results of UCB-MSCs transplantation performed by Elfayomy et al. (2016) and Abd Allah et al. (2017) [39, 65].

Two different research groups tried to examine the germ cell differentiation ability of MSCs derived from amniotic membrane [40, 42]. Although they found the contradictory results, the comparison of these two studies showed that the type of growth factors used for treatment of these MSCs could influence on their differentiation ability into germ cells (GCs), indicating the presence of RA had an important role in induction of germ cell differentiation.

The conflicting results have been obtained in two studies about the role of HUPVCs, a rich source of MSCs, in the restoration of testes functions [38, 41]. Although Maghen et al. (2016) reported that these cells did not show the ability to

differentiate into germ cells or Sertoli cells in the condition similar with testes [41], the results obtained by Shlush et al. (2017) indicated that these cells could differentiate into germ cells and Sertoli cells by mimicking physiological conditions of spermatogenesis [38]. It is likely that the use of RA for induction of differentiation by Shlush et al. (2017) play an important role in the observation of the haploid status of the differentiated cells. However, the studies on animal models demonstrated that the HUPVCs showed the supportive characteristics of testicular tissue [41].

Until now, the evaluation of germ cell differentiation ability of lung-MSCs has been only performed by Hua et al. (2009) [48]. Although, there was evidence of differentiation of small population of these cells into germ cells, but decision about the application of these cells in infertility therapy needs more studies. There are some problems with generation of germ cells from stem cells in vitro. At first, it needs to be done meiosis correctly. Furthermore, the epigenetic changes during male and female gametogenesis should be performed properly. These two points are necessary for the generation of functional gametes [92].

## Conclusion

In summary, the results obtained from this systematic review provide better understanding about the role of mesenchymal stem cells in improvement of infertility before translating the MSCs therapy into clinical trials. The obtained results showed that MSCs derived from bone marrow and umbilical cord showed the better ability than other types of MSCs to restore the fertility. The most studies indicated that MSCs could be used as a suitable option for treatment of azoospermia in males as well as premature ovarian failure in females. However, the obtained results indicated that it needs to do more evaluation about the efficacy of these cells for treatment of infertility.

## Compliance with Ethical Standards

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