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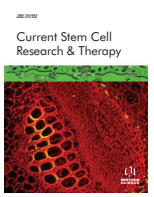
REVIEW ARTICLE

Functional Germ Cells From Non-Testicular Adult Stem Cells: A Dream or Reality?



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Abstract: ***Background:*** Some research studies provided evidence for the differentiation capacity of adult stem cells (ASCs) into germ cells (GCs). Since the generation of GCs from stem cells (SCs) has been proposed as a potential way for treatment of infertility, many research groups have begun their creative studies on generation of new GCs both *in vitro* and *in vivo*, and utilized different ASC types such as bone marrow mesenchymal stem cells (BM-MSCs), skin stem cells, pancreatic stem cells, and adipose tissue MSCs. Despite many interesting reports with promising results, an obvious problem in the research projects was the functionality of the produced GCs.

Objective: In this paper, we have reviewed the results of almost all previously published reports on derivation of male and female GCs from ASCs to provide a better insight into this field of research.

Results: The most evaluated papers have shown that ASCs from various tissues can differentiate into GCs but rarely were the produced GCs functional and could form fertile gametes neither *in vitro*, nor *in vivo* (after transplantation into the gonads).

Conclusion: There are still so many unknown issues about gametogenesis. Perhaps making alterations in treatment methods and utilizing creative techniques like tissue engineering and gene targeting help to achieve a standard method of *in vitro* GC production from ASCs.

Keywords: Adult stem cells, differentiation, germ cells, infertility treatment, skin stem cells, pancreatic stem cells.

1. INTRODUCTION

Stem cells (SCs) were characterized in the late 20th century and their extraordinary ability to differentiate into various cell types was recognized; researchers in the field of infertility thought that they would find their key to solve the infertility problem. Several researchers all around the world began to investigate the probable applications of SCs for infertility treatment. Various reports have indicated that infertility affects up to 15% of couples, where the vast majority have disturbed gametogenesis [1] and need to have new gametogenic or germ cells (GCs). In this regard, during the past couple of decades, a number of research groups have focused on the generation of GCs from different types of SCs. The findings indicated that different kinds of SCs including embryonic stem cells (ESCs) [1-8], induced pluripotent stem cells (iPSCs) [9-12] and adult stem cells (ASCs) [13-26] can differentiate into GCs *in vitro* and/or *in vivo*. Due to both ethical and immunological issues related to clinical studies and applications of ESCs, and safety problems in using iPSCs for therapy purposes [27-29], some research teams tend to use ASCs for their studies on generation of GCs, though most of them utilize mesenchymal stem cells (MSCs) in their projects.

Therefore, the purpose of this article is to review all of the published work in the field of derivation of GCs from ASCs, and to discuss their results to achieve a clear insight into this issue for more efficient studies in the future.

For a better understanding, details of the studies discussed here are presented separately on derivation of male (m) and female (f) primordial germ cells (PGCs) as well as adult GCs. In addition, each section is divided into subsections based on the cells from which GCs are generated.

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2. DEVELOPMENTAL BIOLOGY OF GERM CELLs (GCs)

GCs are of the most important types of cells in the body since they are the only cells, which can go through meiotic divisions and form gametes to transfer genetic information into the next generation. Primary germline cells or primordial germ cells (PGCs) are established in post-implantation mammalian embryos from the pluripotent cells of proximal epiblast under the influence of some signals from adjacent extraembryonic ectoderm-like BMP4 and BMP8 [30-32]. The produced PGCs then begin to migrate through the hindgut into genital ridge (which will form the primitive gonad), while they proliferate by mitotic divisions [33, 34]. Two important genes, which are activated by BMP signaling and play an important role in specification of PGCs are Blimp1 and Prdm14 [33, 35]. Moreover, PGCs at this stage express some other markers like POU5F1/OCT4, GAGE, MAGE-A4, KIT, Dppa3/Stella, Ifitm3/Fragilis and Tnap/Alpl/Ap [34, 36].

After arrival and colonization of PGCs into the primary gonad, they are called gonocytes. These cells express some specific markers like Ddx4/Vasa, MAGE-A4, DAZ, KIT, PLAP, POU5F1, TFAP2C/AP-2γ and UTF1. Determination of whether GCs enter into male or female gamete developmental processes (sex determination) happens at this stage [34, 37]. This decision is completely under the influence of signals from the surrounding somatic compartments rather than their own XX or XY chromosomal contents [34, 38, 39]. Gonocytes in females form oogonia while in males they form spermatogonia. In female gonads, oogonia normally stop their proliferation and begin the chromosomal condensation to enter into meiosis. This stage is mediated by retinoic acid (RA) and activation of Stra8 gene. Finally, these cells arrest at the first meiotic prophase until puberty and ovulation. In contrast, in male gonads spermatogonia do not enter meiosis and arrest at G0/G1 stage until puberty. Meiosis in fetal spermatogonia is prohibited by the activity of Cyp26b1 gene and therefore, degradation of RA [33, 37, 40]. In addition, Stra8, Scp3, Dmc1, Rec8, Dazl and Boule are the genes, which play important roles in the meiosis of oogonia. Moreover, some of the genes, which play undeniable roles in the initiation of spermatogonia development, are Dhh, Fgf9, M33, Dmrt1, Amh, Sry and Sox9 [33, 34]. Primary oocytes, which are surrounded by somatic granolosa cells, grow under the influence of factors like hormones and eventually change to antral follicles with the ability to ovulate. In this transition, mollecular markers like Figla, Nobox and Tbp2, Gdf9 and Zp3 play crutial roles [33]. In adult males, renewal and differentiation of spermatogonial stem cells (SSCs) are regulated by the function of sertoli cells and genes such as Gfrα1, Thy1/CD90, Plzf , Mage-A4, Dazl, Gpr125, Rbm, c-kit, Hsp90α, Tex18, Dazl, Piwil2, stra8 as well as β1 and α6 integrins [25, 34]. Evantually, the product of male and female gametogenesis process is sperm and oocyte, respectievly, which possess an intact genomic pack to transfer into the next generation. A schematic summary of spermato- and oogenesis is illustrated in Fig. (**1**).

3. MALE PGCs FROM ASCs

3.1. Bone Marrow (BM)-MSCs

In 2011, Mazaheri and colleagues conducted a study on measuring the effects of different doses of BMP4 on viability and proliferation of murine BM-derived MSCs (BM-MSCs), and also the expression of Vasa, which is necessary for the development of GCs. In this study, researchers treated mouse BM-MSCs with various concentrations of BMP4 (0.01, 0.1, 1, 5, 25, 50, and 100 ng/ml) for 4 days. Their results indicated that both cell viability and proliferation rates were increased in 1, 5 and 25 ng/ml groups, while they were decreased in 50 and 100 ng/ml groups. On the other hand, the expression of mouse vasa homolog (Mvh) significantly increased in the cells treated with 25, 50 and 100 ng/ml compared to the three other treatment groups. Based on these results, the authors suggest that 25 ng/ml BMP4 was the most effective concentration among the tested concentrations for generation of PGC features in BM-MSCs [41].

In 2012, a study by Shirazi and his colleagues reported generation of PGCs from mouse BM pluripotent (SSEA-1^+) cells. In this research, BM SSEA-1^+ cells were isolated using magnetic-activated cell sorting (MACS) and after confirmation of their pluripotency, the isolated cells were treated with 20 ng/ml BMP4 for 4 days in order to induce differentiation into PGCs. The results showed that BM SSEA-1^+ cells expressed a number of pluripotency- and also GC-specific markers like Oct4, Nanog, SSEA-1, ALP, Stella and Fragilis, without any treatment, and BMP4 treatment induced the expression of c-Kit, Dazl and Mvh in the cells. Further findings indicated that the produced PGC-like cells did not further differentiated along the germ cell lineage and never expressed markers like Stra8 and Piwil2, SYCP3 and Pgk2, TP2, GDF9 and ZP3 (male and female meiotic and post-meiotic GC-specific markers) in the culture. Therefore, the researchers concluded that 4 days of treatment with 20 ng/ml BMP4 induced differentiation in BM SSEA-1^+ cells only up to the PGC stage [42].

3.2. Umbilical Cord (UC)-MSCs

In 2014, Latifpour and co-workers performed a study on human UC-MSCs. In this study, researchers isolated and characterized human UC-MSCs, then treated them with 20 ng/ml BMP4 for 4 days and after that with 10 μM RA for 14 days. Their results showed that the treatment with BMP4 and RA resulted in morphological changes in the cells. Moreover, gene expression analysis and immunocytochemistry showed that the expression of a number of GC-specific markers like Vasa (DDX4), SSEA4 and Oct4 were increased in the treated cells. Therefore, the researchers concluded that they had some PGCs in the culture after treating Human UC-MSCs for 18 days with BMP4 and RA [43].

Li *et al.* recently reported that the overexpression of CD61 (Itgb3) in human UC-MSCs can prone these cells to differentiate into PGC-like cells under the influence of 12.5 ng/ml BMP4 treatment for 7 days. They showed that transfected CD61- human UC-MSCs after two days of treatment changed into spheroid-shaped cells resembling PGCs. Molecular evaluation on day 7 post-treatment with BMP4

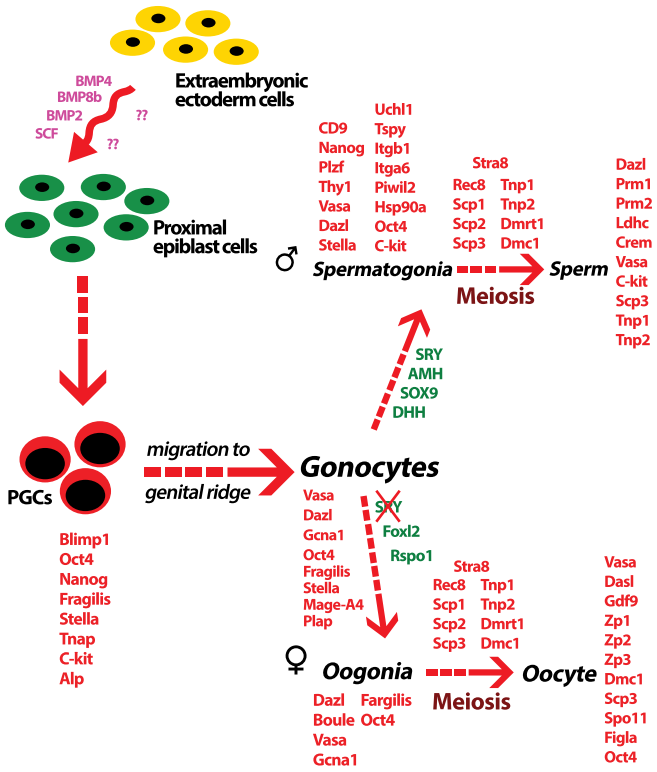

Fig. (1). Primordial germ cells (PGCs) are progenitors of gametes and arise from proximal epibalsts of early embryos (around day 6.5 of gestation in the mouse and third week of gestation in humans), under the influence of some inductive factors from adjacent visceral endoderm and extraembryonic ectoderm. Although a number of these factors have been characterized like BMP4, BMP8b, BMP2 and SCF, there are surely some other factors, which have not been identified yet. The formed PGCs migrate to two genital ridges, which will form gonads. During migration, the cells highly proliferate. After the cells reside in genital ridge and are surrounded by somatic cells, they are called gonocytes. In males, gonoctes start to change into spermatogonia following activation of proteins like SRY, SOX9, WT1, and AMH. These cells stop proliferating until birth and migrate to basal membrane of primary seminiferous tubules during the first 6-month after birth to become spermatogonia. In contrast, in female fetus, SRY is not expressed and gonocytes immediately enter into first meiotic division and form primary oocyets under the influence of retinoic acid (RA) from the nearby somatic cells. In males, an enzyme called CYP26B1 destroys RA. Both primary oocytes and spermatogonia become quiescent until puberty, and they begin gametogenesis because of changes in their microenvironment (niche). In adult males, the production of CYP26B1 enzyme is stopped and meiosis occurs. Each developmental stage from PGCs into gametes is controlled by a number of genes and/or protein markers and each cell type in this process expresses its specific genes as well. Some of the markers are shown here.

revealed that the expression of some GC-specific markers like SSEA-1, Prdm1, Prdm14, Ap2c, Stella, Sox2, C-kit, Stra8, Scp3 and Prm1 were upregulated in the transfected cells far more than in the non-transfected BMP4 treated human UC-MSCs. Although the researchers claimed that they saw a number of spermatid-like cells, which expressed post meiotic marker, Acr, they did not show further evidence such as the chromosomal content and fertilizing capacity of the cells [44].

3.3. Adipose Tissue MSCs

More recently, Wei *et al.* showed that the treatment of canine adipose MSCs with 12.5 ng/ml BMP4 for 7 days caused the formation of PGC-like cells in the culture. The produced germ-like cells showed increased levels of GC-specific markers Prdm1, Prdm14, Ap2γ, CD61, Plzf and Dmrt1 [45].

In addition, there are many studies that reported the generation of male adult germ-like cells from SCs, that are described in the next section.

4. MALE ADULT GERM CELLs (MGCs) FROM DIFFERENT ASCs

4.1. BM Stem Cells

Nayernia and colleagues using mouse BM-MSCs published the first report on generation of mGCs from ASCs in 2006 [25]. They treated the characterized BM-MSCs with 1 μM retinoic acid (RA) for various periods of time and checked the emergence of GC characteristics in the cells through molecular analysis. They indicated that treatment of the cells for 7, 14 and 21 days with RA caused formation of some GCs in the culture, which expressed a number of GC-specific markers like fragilis, stella, Rnf17, Mvh (mouse vasa homologue), Oct4, Rbm, c-Kit, Tex18, Stra8, Piwil2 and Dazl. In addition, on day14 post-treatment, they observed large colonies of germ-like cells in their cultures. Interestingly, they also showed that the GCs that were derived *in vitro*, which arose from MSCs after 21 days of treatment with RA, not only can survive in the testis of infertile male mice for 8-12 month, but also they can proliferate, migrate to the basement membrane of the seminiferous tubules and

colonize them in about 33% of the recipient mice. They stated that one of their study unresolved questions was the arrest of the differentiated GCs at pre-meiotic stage and failed to enter into meiosis. Thus, they did not observe any haploid mGCs neither *in vitro* nor *in vivo*. They suggested that providing more support for the produced GCs like addition of some other growth factors to the culture medium or co-culturing with ESC-derived embryoid bodies (EBs) could help to solve the problem of entering meiosis.

Nayernia and his team published their second report on treatment of hBM-MSCs with 1 or 10 μM RA for 15 days [26]. They showed that this treatment can induce the cells for differentiation into mGCs *in vitro*. They observed that untreated hBM-MSCs expressed Oct4, Fragilis, Stella, Vasa, c-kit, cyclin A2 and Piwil2, which were upregulated after treating with RA. They suggested that expression of GC-specific markers by untreated BM-MSCs would be an evidence of the existence of a population of germ-like cells in the BM. The results of this study indicated that, hBM-derived MSCs have the capacity of differentiation into mGCs; however, their ability to undergo meiosis and to form sperm cells was not verified.

The first *in vivo* study on the effects of ASCs on testicular germinal epithelium regeneration was published in 2007. Lue and colleagues isolated BM cells from femurs and tibias of transgenic mice and immediately injected them into both the seminiferous tubules and interstitial space of testes in recipient mice. After 10 to 12 weeks, the cell-injected testes were evaluated by immunocytochemistry to determine the differentiation of BM cells into germ or somatic cells. The results showed that donor cells survived in the testes and were observed both in the lumen of seminiferous tubules and testicular interstitium. Moreover, immunostaining revealed that transplanted cells not only differentiated into GCs (spermatogonia and spermatocytes), but also differentiated into somatic cell types of the testis (sertoli and leydig cells). The produced donor-derived GCs were stopped at early spermatocyte stage and did not further differentiate into spermatid and sperm. Although the authors did not determine which of the two kinds of BM stem cells (hematopoietic or mesenchymal) differentiated into GCs and somatic cells in the testis, findings of this study were important [46].

In 2008, two *in vivo* studies were performed to investigate the effects of BM-derived cells on regeneration of testis germinal epithelium.

In the first study, Horn *et al*. isolated rat BM mononuclear cells using Ficoll Paque, and then transplanted them into the testes of busulfan-treated rats after labeling with DAPI. After fifteen days, the rats were sacrificed and their testes were evaluated by histology under microscope. Their results showed that all transplanted cells had died and no donor cells were observed in the germinal epithelium of seminiferous tubules [47].

In the second study, whole BM-derived cells (including hematopoietic SCs, blood cells and MSCs) were obtained from the BM of transgenic (EGFP+) mice and transplanted into the testes of busulfan-induced infertile mice. Recipient testes were evaluated 3, 6 and 8-9 months after transplantation. It was found that although a very small percentage of the donor cells could survive in the testes, they did not at all colonize the seminiferous tubules, differentiate into mGCs, or participate in spermatogenesis. The entire transplanted testes were evaluated under flourecense microscope in order to detect localization of donor cells in the tubules. However, no immunostaining was performed and the testes were not evaluated at cellular level. Therefore, it may be that a number of questions still need to be answered in their study [48].

In one of the two papers that Hua and colleagues published in 2009, they report that they isolated MSCs from BM of human fetus and treated them with an inductive mixture composed of 10 μM RA and adult goat testis extracts for 10-15 days. Reverse transcription polymerase chain reaction (RT-PCR) revealed that human fetal BM-MSCs expressed many GC-specific markers such as Oct4, c-Kit, Ddx4 (Vasa), Dazl, Stella, Itgb, FshR, Stra8, Scp3, Acr, Prm1 and H2b innately. Immunocytochemistry showed the expression of a number of GC markers like Oct4, Vasa, Itgb1 and SSEA1 in the treated cells at protein level. Moreover, Immunocytochemistry revealed an increase in the number of cells expressing Vasa and two spermatid markers, FE-J1 and Ema1, in the culture after treatment with 10 μM RA and the testis extract. Although the authors concluded that MSCs differentiated into mGCs and even spermatid-like cells, which expressed post meiotic markers, they stated that they were not sure if complete meiosis has happened in the culture. Furthermore, they didn't report the formation of fully competent and functional sperm cells [20].

Since zinc (Zn) is one of the most important trace elements in the body, and is essential for male gametogenesis [49] in our own laboratory, we sought to determine if it has any inductive effects on BM-MSCs for differentiation into GC lineage. After isolation and characterization of MSCs from male sheep (ram) BM samples, the cells were treated with 0.14 µg/ml $ZnSO_4$ for 21 days. Our evaluations revealed that Zn treatment changed the expression levels of some GC-specific genes. For instance, Vasa and Integrin b1 (Itgb1) were mildly upregulated and Piwil2 and Oct4 were severely downregulated. The amount of alkaline phosphatase (ALP) protein remained almost unchanged and the expression of GC-specific marker, Pgp9.5 was not induced in treated MSCs. Overall, it seemed that Zn ions did not induce CG characteristics in MSCs and they only altered the expression of some GC-specific markers [19].

In 2013, an *in vivo* research report was published on the potency of MSCs for reconstruction of testis germinal epithelium. In this study, rat BM-MSCs were transplanted into the testes of busulfan-induced sterile rats. The testes were evaluated after 8 weeks. It was reported that germinal epithelium of infertile rats was repaired after transplantation of donor cells. The researchers claimed that not only did BM-MSCs differentiate into SSCs, but also they further differentiated into spermatocytes, spematids and spermatozoids. Although their entire conclusion was based on the morphological data of the testicular cells and did not really evaluate molecular characteristics and gene expression patterns of the produced cells. Furthermore, fertility potency of the produced gametes was not assessed [50].

In an *in vitro* study in 2013, researchers compared the capability of mouse BM-MSCs and adipose-derived MSCs

for differentiation into GCs. After isolation and characterization of MSCs from the two sources, the cells were treated with 10 μM RA for 7 days and then, the expression of a number of GC-specific markers (Mvh, Dazl, Stra8 and Scp3) was evaluated with real time RT-PCR, immunocytochemistry and flow cytometric analysis. Their results showed that all of the tested markers were upregulated after the treatment with RA, and the amount of upregulation was significantly higher for BM-MSCs compared to adipose MSCs. Moreover, the researchers detected the expression of Vasa and Dazl proteins in both types of MSCs after the treatment. However, flow cytometry revealed that the percentage of Vasa and Dazl positive cells were significantly higher in RA-treated BM-MSCs than RA-treated adipose MSCs. The authors concluded that both types of MSCs had the capacity of differentiation into mGCs, though BM-MSCs showed higher potentials [51].

Since different concentrations of RA were tested in previous *in vitro* studies, our team designed a study in order to determine the most effective concentration of RA for induction of differentiation into GCs in MSCs. For this goal, we evaluated the inductive effects of three different concentrations of RA (1, 5 and 10 μM) on ram BM-MSCs. The cells were divided into separate groups and treated with distinct concentrations of RA for 21 days. At the end of the treatment period, the cells were evaluated with real time RT-PCR and immunocytochemistry in order to detect the expression level of some GC-specific markers at mRNA and protein levels, respectively. Our results showed that all three concentrations of RA downregulated the expression levels of Piwil2 and Oct4 and upregulated Vasa and Itgb1. Moreover, all tested concentrations significantly increased ALP levels and induced the expression of Pgp9.5 protein in treated cells. Although the percentage of the positive cells in the 10 μM group was significantly more than that in the other two concentration groups. Therefore, we concluded that among the three tested concentrations of RA, 10 μM was the most effective concentration for induction of differentiation into mGCs in ram BM-MSCs [16].

In our next study, we assessed the inductive effects of three members of transforming growth factor (TGF) superfamily, BMP4, BMP8b and TGFb1, for derivation of mGCs from ram BM-MSCs. Therefore, after isolation and characterization of BM-MSCs, we treated the cells with 100 ng/ml BMP4, 100 ng/ml BMP8b and 10 ng/ml TGFb1 in separate groups for 21 days. The results of molecular and protein analyses confirmed that all growth factors induced GC characteristics (upregulation of Vasa and Itgb1, high ALP activity, downregulation of Piwil2 and Oct4, and induction of Pgp9.5 and Dazl expression) in BM-MSCs. Although BMP4 and BMP8b treated cells mainly showed characteristics similar to PGCs, when TGFb1 treated cells expressed adult mGCs (SSCs or spermatogonia) features [18], none of the produced cells expressed meiotic markers.

Zhang and his team in 2014 tested the differentiation potential of rat BM-MSCs both *in vitro* and *in vivo*. They co-cultured BM-MSCs with sertoli cells and evaluated the changes in morphology and molecular characteristics of the cells. They reported that co-culturing with sertoli cells changed the morphology of BM-MSCs into round shaped cells and induced Itgb1 protein expression. In their *in vivo* phase, they treated rat BM-MSCs with 20 μM RA for 3 days and then labeled the cells with Hoechst 33342 and transplanted them into the testes of busulfan-induced infertile rats. They evaluated the transplanted testes after 1, 2, 3, 4 and 8 weeks. Their results showed that only a few of donor BM-MSCs survived in the testes until week 8 post-transplantation. The surviving cells homed in the basement membrane of the seminiferous tubules. The researchers showed that transplantation of RA-treated BM-MSCs improved the expression of spermatogonia-specific markers like Vasa, Dazl, GCNF, c-Kit and Stella, in the recipient testes. Although they concluded that BM-MSCs have the potency of differentiation into mGCs both *in vitro* and *in vivo*, they did not observe any evidence for meiotic division in the donor cells. Moreover, in their *in vitro* study, they evaluated the expression of just one GC-marker, which was perhaps not enough evidence for making any judgments on BM-MSC differentiation into GCs [52].

In an interesting study by Yan *et al.* in 2015, the differentiation of goat BM-MSCs into GCs was induced with two methods, using extrinsic and intrinsic factors. In the first method, researchers treated goat BM-MSCs with different inducers in three treatment groups and each in 14 days. In the first group, the cells were treated with 1 μM RA, the second group received 25 ng/ml BMP4 and the third group was treated with both RA and BMP4 collectively. Their molecular analysis evaluations revealed that the expression of examined GC-specific markers (Oct4, Nanog, c-Kit, Mvh, Dazl, Stella, Stra8, and Scp3) increased in all three groups, though RA alone was more efficient than BMP4 alone and RA+BMP4. As an intrinsic factor, the authors overexpressed Dazl in goat BM-MSCs for GC development, and showed the importance of Dazl gene for induction of differentiation into GCs. They overexpressed Dazl at two different DNA and mRNA levels. Their results showed that the overexpression of Dazl increased the expression levels of Mvh and Scp3 and decreased the expression of Nanog, although transfection at mRNA level was more efficient. In addition to these findings, the results of knockdown experiments further confirmed the inevitable role of Dazl in GC differentiation. Therefore, this study confirmed the capacity of goat BM-MSCs for differentiation into germ-like cells, but the researchers did not confirm the functionality of the produced germ-like cells and their ability to enter meiosis as well as participation in the spermatogenesis process [53].

In 2015, Tamadon *et al.* reported that transplantation of hamster BM-MSCs into the testes of busulfan-treated infertile hamsters restored sepermatogenesis. However, their evaluation lacked molecular analyses and immunostaining of the tissues to detect GC markers. Their main analytical techniques were H-E staining and histomorphometric, which do not seem to provide enough support for their conclusions. Moreover, they did not show any data on participation of donor cells in the regeneration of germinal epithelium and gamete production [54].

In one of our studies published in 2016, we investigated and compared the efficiency of three different inductive methods for derivation of mGCs from BM-MSCs in the laboratory. Ram BM-MScs were divided into three distinct

study groups. The cells of the first group were treated with 10 μM RA for 14 days, the second group was treated with 10 μM RA for 21 days and the cells of the third group were treated with 10 ng/ml TGFb1 for 21 days. Following the treatments, the expression of GC markers was evaluated using real time RT-PCR, immunocytochemistry and ELISA. Our results revealed that a number of cells in all three groups showed GC characteristics, but TGFb1 had the highest effect in induction of GC features in ram BM-MSCs. Moreover, after confirmation of generation of GCs in the cultures, the cells of each treatment group were labeled with PKH26 and transplanted into testes of ram lambs. The transplanted testes were evaluated two month later. Our results showed that donor cells of each group survived in the testes and a number of them migrated to the germinal epithelium, settled on the basement membrane of the seminiferous tubules and expressed GC-specific marker, Pgp9.5. Moreover, some donor-derived colonies, which expressed Pgp9.5, were also observed. Overall, based on the *in vitro* and *in vivo* results, TGFb1 treatment was the most efficient method for induction of BM-MSCs to differentiate into mGC. Unfortunately, no further differentiation of donor cells toward the spermatocyte or spermatid stages was observed in the recipient testes. Although these findings were important and interesting, we could not verify having functional GCs with the potency for participation in spermatogenesis process [17].

In a recent study by Li *et al.*, they evaluated the role of overexpression of different genes for induction of BM-MSC for differentiation into GCs. In this study, goat BM-MSCs were isolated and characterized, and then transfected with plasmids carrying Stra8, Boule and Dazl transgenes in separated groups. In addition, one group of cells was transfected with all these three transgenes together. Two days after transfection, the cells were evaluated with real time RT-PCR, immunocytochemistry and Western blot analysis to check for the expression of a number of specific markers related to different stages of GC developmental process. The results showed that the overexpression of each of the three-abovementioned genes increased the expression of GC-specific markers from different stages of development, like PGC markers (Oct4, Nanog, Stella and c-Kit), pre-meiotic genes (Mvh, Dazl, Piwil2, Stra8, Boule, Rnf17) and also meiotic marker (Scp3) in goat BM-MSCs. Moreover, co-overexpression of the three genes had the highest inductive effect for differentiation into GCs. The authors indicated that all stages of GC development process from PGCs to post meiotic stage had occurred in the culture, though they did not further check the cells for DNA content and fertilizing capacity [55].

Recently, we have evaluated the fate of untreated autologous BM-MSCs after transplantation into the testes of infertile male rats. Rat BM samples were collected from the tibia bones under anesthesia. MSCs were isolated from whole BM cultures. Infertility was induced in each donor rat with a single injection of busulfan (40 mg/kg BW) and after 28 days, characterized BM-MSCs were labeled with PKH-26 and transplanted into the testes seminiferous tubules via the rete testes by thin pipettes. Transplanted testes were evaluated 4, 6 and 8 weeks after transplantation. Our results showed that donor cells survived in the testes of the three treatment groups, though their numbers decreased dramatically over time. We observed that a number of BM-MSCs migrated to the germinal epithelium and localized at the basement membrane and expressed spermatogonia-specific markers, Dazl and Stella. Therefore, we concluded that a number of transplanted BM-MSCs differentiated into mGCs in the testes successfully, although their numbers had severely decreased from the time of transplantation after week 8 and no further differentiation into the stages beyond spermatogonia stage (spermatocytes and spermatids) was observed in any of the treatment groups. Moreover, the evaluation of epididymal contents revealed no PKH26 positive sperms in recipient rats. Therefore, BM-MSCs could differentiate into mGCs in the seminiferous tubules, but they couldn't participate in spermatogenesis to restore the host fertility [56].

4.2. Lung-derived MSCs

In another 2009 study, Hua and his colleagues derived some male germ-like cells from MSCs of human fetal lung with the same treatment as their previous work (10 μM RA and adult goat testis extracts for 10-15 days). Their analyses showed that the treatment of the cells caused induction of the expression of some GC-specific genes like Vasa, Scp3 and Acr. Moreover, they observed that a number of sperm-like cells, which were positive for a post-meiotic marker, Ema1, existed in the culture. Overall, they concluded that human fetal lung MSCs differentiated into mGCs in the laboratory. Although they claimed that they observed a number of spermatid-like cells in the culture and also a number of treated cells expressed SCP3 (meiotic marker) and Ema1, they didn't perform further investigation in order to confirm the occurrence of meiosis and formation of haploid mGCs in the culture [21].

4.3. UC-MSCs

A group of researchers in 2010 indicated that human UC Wharton's jelly-derived MSCs can differentiate into mGCs *in vitro*. They treated male Wharton's jelly-derived MSCs with inductive medium consisted of high glucose Dulbecco's modified essential media (H-DMEM) supplemented with 5% fetal bovine serum (FBS), 50% filtered testicular-cell conditioned (TCC) medium, 2 μM RA and 1 μM testosterone. They found that a number of GC markers like Oct4, CD49f, Stella and c-kit started to be expressed on day 3 of treatment, while the expression of Vasa was first observed on day 14 of the treatment. Although they stated that MSCs of umbilical Wharton's jelly can differentiate into mGCs, they did not evaluate their functionality and ability to enter meiosis, and to form haploid gametes [22].

In a study in 2014, Li and colleagues isolated and purified SSEA-1^+ MSCs from cultures of human UC-MSCs and then treated the cells with 12.5 ng/ml BMP4 for 7-14 days. At the end of the treatment, immunocytochemistry and real time RT-PCR were performed for detecting the expression of GC-specific markers. Their results showed that the treatment of SSEA-1^+ MSCs with BMP4, not only upregulated the expression of PGC-specific genes like SSEA-1, PRDM1, Stella, Sox2 and c-Kit, but also caused increase in the expression of mGC markers like Stra8, Plzf and Vasa and also meiotic markers like Scp3 and Acrosin. Moreover, it was showed that some of the treated cells showed sperm-like

morphology, which was strongly positive for Acrosin. Further evaluation revealed that SSEA-1$^-$ MSCs did not show GC differentiation capacity. The researchers concluded that SSEA-1$^+$ MSCs successfully differentiated into both PGCs and mGCs, and they claimed that they observed a number of sperm-like cells in the culture, but they did not examine their chromosomal contents nor fertility capacity [57].

In 2015, a research team preformed a study on human Wharton's jelly-derived MSCs. After isolation and characterization of MSCs of Wharton's jelly, passage 3 to 4 cells were divided into three treatment groups. Group 1 cells were treated with 10 ng/ml BMP4 (for 4 days), followed by the treatment with 1 μM RA for 17 days (total of 21 days); group 2 cells were treated with 1 μM RA together with 50% TCC for 21 days, and the third group was treated with a mixture comprised of 1 μM RA and 50% placental cells conditioned medium (PCC) for 21 days. At the end of the treatment period, the treated cells were evaluated for characteristics of mGCs. Real time RT-PCR revealed that c-Kit was upregulated significantly in both BMP4+RA and RA+TCC groups, while it was downregulated in RA+PCC group compared to untreated control cells. Moreover, the expression levels of Vasa, piwil2 and Dazl increased in all three treatment groups, though the elevation was significantly higher in RA+TCC than the other groups. Therefore, the researchers concluded that TCC had some hormones and growth factors caused differentiation of Wharton's jelly-derived MSCs to mGCs. Nonetheless, the researchers did not check the expression of the other GC-specific markers, nor functionality of the generated mGCs [58].

In an *in vivo* study in 2015, Chen *et al*. evaluated the capacity of human UC-MSCs for reconstruction of testis germinal epithelium. For this purpose, after isolation and characterization of UC-MSCs, the cells were transplanted into the testes of busulfan-induced male mice and the transplanted testes were evaluated after 30, 60 and 120 days. The evaluation showed that donor cells survived at least for 120 days in the testes. From day 30 to 60, the transplanted UC-MSCs gradually migrated to the basement membrane of the seminiferous tubules and by day 120, a number of donor cells returned to luminal space again. Tubular diameter in the transplanted testes in any evaluated time was significantly greater than that in non-transplanted testes. Moreover, the transplanted cells expressed Oct4, Itgα6, Mvh and c-Kit, which confirmed the differentiation of human UC-MSCs into mGCs in the testes. However, they did not test the expression of meiotic markers nor confirmed the generation of haploid cells [59].

Another group of researchers investigated the capacity of human Wharton's jelly-derived MSCs for differentiation into mGCs with two different induction methods. In one of the treatment groups, MSCs were treated with 10 ng/ml BMP4 for 4 days and then with 1 μM RA for 17 days. The cells of the second group were first treated with 1 μM RA for 7 days and then co-cultured with placental cells (Human amniotic epithelial cells and human chorionic plate cells) for 14 days. At the end of these treatments, real time RT-PCR and immunocytochemistry were performed for detecting possible changes in the expression of GC-specific markers in the treated cells. Their results indicated that although both methods induced upregulation in almost all of the tested genes [POU5F1 (Oct4), Fragilis, Ddx4 (Vasa), Plzf and Piwil2 (Mili), Stra8, Dazl, Itgb1, Itga6] and also expression of some markers (c-Kit, Ddx4 and SSEA4) at protein level, treating the cells with BMP4+RA was more effective than the co-culture method for stimulating Wharton's jelly MSCs to differentiate into mGCs [60]. Similar to some other groups, the authors did not check the functionality of their produced GCs.

4.4. Adipose Tissue MSCs

In an *in vivo* study, Cakici *et al*. tested the potency of rat adipose tissue-derived MSCs for restoring spermatogenesis in experimentally infertile male rats with busulfan injection. In this study, they isolated MSCs from peritoneal adipose tissue and after characterization, they made transgenic GFP$^+$ cells using a standard transfection kit. Recipient male rats were made infertile using two injections of busulfan (15 mg/kg with 14 day intervals), and 1×10^6 transgenic MSCs were injected into the left testis of each recipient rat via the rete testis. After 12 weeks, the researchers evaluated the testes by morphological analyses and immunohistichemistry for the GC marker, Vasa. Their results showed that adipose tissue-derived MSCs not only supported endogenous SSCs for the recovery of spermatogenesis, but also transdifferentiated into GFP$^+$ SSCs, which further differentiated into GFP$^+$ sperm cells. Moreover, they claimed that the transplanted rats obtained their fertility after 12 weeks and could produce a number of viable offspring after mating with female rats. In addition, they confirmed that these offspring carried GFP transgene in some cells of their bodies. Although the study was well designed with outstanding findings, the researchers did not evaluate the expression of so many other GC-specific markers. Moreover, they did not further investigate the factors involved in inducing MSCs into fully differentiated sperm [61].

4.5. Amniotic Membrane-Derived MSCs

Recently, we isolated and characterized MSCs from amniotic membrane of pregnant mice and then treated them with a two-step approach. At first, the cells were treated with 25 ng/ml BMP4 for 5 days and then treated with 1 μM RA for the following 12 days. At the end of the treatment, the expression of GC-specific markers was evaluated using real time RT-PCR analysis, immunocytochemistry and folw cytometry. Real time RT-PCR showed that except for the Oct4, all other tested genes (Itgb1, Dazl, Stra8, Piwil2, Mvh and c-Kit) were upregulated post-treatment, and immunostaining confirmed the expression of Mvh and Dazl proteins in a number of treated cells. Flow cytometry revealed that a considerable number of treated MSCs expressed these markers and that there was a significant difference between the percentage of Mvh and Dazl expressing cells between treated and untreated groups. Although our results confirmed the presence of germ-like cells in the culture derived from mouse amniotic membrane MSCs, we did not check the expression of meiotic markers and/or the functionality of the produced germ-like cells [62].

Analyzing the results of conducted studies reported above shows that the findings are controversial to some ex-

tent despite being promising. Since most of the conducted studies show an absence of conformational and functional techniques in the evaluation of the produced germ-like cells, their results do not render a completely established procedure for generation of GCs from ASCs. Moreover, it seems that spermatogenesis is very complex and many aspects of this process are still unknown. Although mGCs were generated from ASCs in some studies (*in vitro* and/or *in vivo*), their functionality and potency for producing fertile gametes with normal karyotypes are debatable. In the end, despite all the promising results, it must be admitted that much more detailed investigations should be performed in order to generate functional GCs with the ability to produce normal gametes from ASCs. The summary of the studies on the derivation of male GCs from different types of ASCs are shown in Table **1**.

5. FEMALE PGCs FROM ASCs

5.1. BM-Derived Cells

In 2007, Bukovsky *et al*. reported that transplantation of BM-derived immune cells (via the tail vein), however did not differentiate directly to germ-like cells, but helped with the emergence of new PGCs from ovarian surface epithelium SCs in normal adult female rats. The researchers also indicated the generation of PGCs from medullary precursors in neonatally estrogenized adult female rats as well [63].

5.2. Amniotic Fluid Stem Cells (AFSCs)

Antonucci *et al*. in 2014 reported that they had successfully isolated and cultured a group of cells from human AF, which expressed characteristics of pluripotent cells and formed embryoid bodies (EBs) in suspension cultures. These EBs were positive for ALP and vastly expressed pluripotency markers Oct4 and Sox2. Moreover, EB cells expressed specific markers related to three germ layers and also markers of ESCs, FGF4 and DAPPA4. Additionally, EBs expressed PGC-specific markers, Fragilis, Stella, Vasa, c-Kit, and Rnf17. The authors concluded that human AFSCs expressed many shared markers with PGCs and ESCs, which may be important for their usage in cell therapy. However, they did not check their differentiation capacity into GCs [64].

5.3. UC-MSCs

Asgari and colleagues co-cultured human UC-MSCs and placental cells for 14 days and then evaluated the expression of four PGC markers, Oct4, c-Kit, SSEA4 and Vasa, as well as two oocyte markers, GDF9 and ZP3, by immunostaining and real time RT-PCR. The findings of these experiments were also compared with the effects of RA treatment on the expression of the aforesaid markers. The results showed that co-culture of UC-MSCs with placental cells increased the expression of Oct4, SSEA4, c-Kit and Vasa significantly compared to their expression in RA-treated cells. Moreover, GDF9 and ZP3 were also upregulated, though it was not statistically significant. The researchers concluded that co-cultured human UC-MSCs showed higher tendency to differentiate into female PGCs than RA-treated cells. They concluded that this observation was probably a result of the activity of some factors such as TGFα and β and also bFGF, secreted by placental (including both amniotic and chorionic) cells [65].

6. FEMALE ADULT GERM CELLs (fGCs) FROM ASCs

The old belief of a finite number of female gametes was challenged after a landmark paper published by Johnson *et al*. in 2004. This research confirmed that ovaries of postnatal mammals contained some germline stem cells, which renewed the follicular pool of ovaries [66]. Therefore, scientists hypothesized that maybe the generation of female gametes could be possible in the laboratory and several research groups focused on isolation of ovary GCs and induction of differentiation into gametes in those cells. Nonetheless, a number of researchers began to study the generation of fGCs or gametes from other sources of SCs. There are some valuable research papers on derivation of fGCs from ASCs isolated from various tissues.

6.1. BM and Peripheral Blood (PB) Stem Cells

In a very interesting study by Johnson and his team, they showed that BM and PB consisted of a group of cells, which were potentially GCs and could establish new folliculogenesis and oocyte production in the ovaries of sterile female mice. Researchers firstly found a group of adhesive cells in the BM, which expressed GC markers, and then transplanted them to mutant sterile and chemotherapy-sterilized female mice via the tail vein. Evaluating the transplanted ovaries showed that several hundreds of donor-derived oocyte-containing follicles were generated in the recipient ovaries. Further, they isolated PB mononuclear cells and transplanted them to other sterile mice. Their assessments revealed that PB transplantation led to the generation of a considerable number of donor-derived oocytes in the ovaries of recipient female animals.

Overall, from the results of their experiments, they concluded that both BM and PB possessed putative GCs, which could regenerate the oocyte pool of the ovaries in infertile females. Although they found the same pool of fGCs in the BM and PB of human females, they did not conduct any investigations on human cases using these cell types. Even though these results were very important, especially because they introduced BM and PB as potential sources of fGCs, as stated by authors of the paper, they did not check the ability of donor-derived oocytes for normal fertilization and development into viable offspring [67].

Lee *et al.* in 2007 conducted a study on mouse folliculogenesis. They performed BM transplantation in experimentally infertile female mice after high-dose chemotherapy with single injections of busulfan and cyclophosfamide. They assessed the effects of BM transplantation at 1 week and 2 months after chemotherapy on restoration of ovarian function. They used transgene (GFP+) donor cells and transplanted the cells via the tail vein. Their evaluations revealed that BM transplantation had considerable effects on restoration of ovarian function, mating and having live-birth pregnancies. In addition, they showed that BM transplantation 1 week following chemotherapy was more effective than in 2 months after chemotherapy. The authors also evaluated the

Table 1. Reports on derivation of male germline cells from adult stem cells (ASCs).

Research Team	Year	Utilized Stem Cell Type	Treatment	Produced Germ-Like Cells
Nayernia *et al*.	2006	Mouse BM-MSCs*	1 μM RA* for 1, 2 and 3 weeks	Male adult GCs*
Drusenheimer *et al*.	2007	Human BM-MSCs	1 or 10 μM RA, for 15 days	Male adult GCs
Lue et. al	2007	Whole BM cells	Transplantation into the testis and evaluation 10-12 weeks later	Male adult GCs, primary spermatocytes
Horn *et al*.	2008	BM mononuclear cells	Transplantation into the testis and evaluation 15 days later	None of the transplanted cells were alive and no differentiation was observed
Lassalle *et al*.	2008	Whole BM cells	Transplantation into the testis and evaluation 3, 6 and 8-9 months later	No differentiation into GCs
Hua *et al*.	2009	Human fetal BM-MSCs	10 μM RA and adult goat testis extract for 10-15 days	Male adult GCs
Hua *et al*.	2009	Human fetal lung MSCs	10 μM RA and adult goat testis extracts for 10-15 days	Male adult GCs Spermatid-like cells
Huang *et al*.	2010	Wharton's jelly-derived MSCs	Testicular-cell conditioned medium, 2 μM RA and 1 μM testosterone	Male adult GCs
Mazaheri *et al*.	2011	Mouse BM-MSCs	0.01, 0.1, 1, 5, 25, 50, and 100 ng/ml BMP4 for 4 days	PGC*-like cells
Ghasemzadeh-Hasankolai *et al*.	2012	Ram BM-MSCs	0.14 μg/ml Zinc sulfate for 21 days	No differentiation
Shirazi *et al*.	2012	Mouse BM pluripotent (SSEA-1+) cells	20 ng/ml BMP4 for 4 days	PGCs
Monsefi *et al*.	2013	Rat BM-MSCs	Transplantation into the testis of busulfan-treated male rats and evaluation 8 weeks later	Male adult GCs (SSCs*), spermatocytes, spematids and spermatozoids
Cacki et l.	2013	Rat adipose tissue- MSCs	Transplantation into the testis of busulfan-treated male rats and evaluation 12 weeks later	SSCs, spermatocytes, spematids and fertile sperm cells
Hosseinzadeh Shirzeily *et al*.	2013	Mouse BM-MSCs and adipose-derived MSCs	10 μM RA for 7 days	Male adult GCs
Latifpour *et al*.	2014	Human UC-MSCs*	20 ng/ml BMP4 for 4 days + 14 days with 10 μM RA	PGCs
Ghasemzadeh-Hasankolai *et al*.	2014	Ram BM-MSCs	1, 5 and 10 μM RA for 21 days	Male adult GCs
Ghasemzadeh-Hasankolai *et al*.	2014	Ram BM-MSCs	100 ng/ml BMP4, 100 ng/ml BMP8b and 10 ng/ml TGFb1 for 21 days in separate groups	Male adult GCs PGCs
Zhang *et al*.	2014	Rat BM-MSCs	20 μM RA for 3 days and then transplantation them into the testes of busulfan-treated rats	Male adult GCs
Li *et al*.	2014	Human SSEA-1+ UC-MSCs	12.5 ng/ml BMP4 for 7-14 days	PGCs male adult GCs Sperm-like cells

Table (1) contd….

Research Team	Year	Utilized Stem Cell Type	Treatment	Produced Germ-Like Cells
Yan *et al.*	2015	Goat BM-MSCs	G1*: 1 μM RA, 25 ng/ml BMP4 and RA+BMP4 in separate groups for 14 days. G2: Overexpression of Dazl by transfection	Male adult GCs Male adult GCs
Amidi *et al.*	2015	human Wharton's jelly MSCs	G1: 10 ng/ml BMP4 for 4 days + 1 μM RA for 17 days G2: 1 μM RA + 50% testicular cells conditioned medium for 21 days G3: 1 μM RA + 50% placental cells conditioned medium for 21 days	Male adult GCs
Chen *et al.*	2015	Human UC-MSCs	Transplantation into the testis of busulfan-treated male mice and evaluation 30, 60 and 120 days later	Male adult GCs
Tamadon *et al.*	2015	Hamster BM-MSCs	Transplantation into the testis of busulfan-treated male hamsters and evaluation 35 days later	The authors did not show formation of donor-derived germ-like cells but reported restoration of spermatogenesis
Nejad *et al.*	2015	Human Wharton's jelly-derived MSCs	G1: 10 ng/ml BMP4 for 4 days and then for 17 days with 1 μM RA for 17 days G2: 1 μM RA for 7 days and then co-culturing with placental cells	Male adult GCs
Ghasemzadeh-Hasankolai *et al.*	2016	Ram BM-MSCs	G1: 10 μM RA for 14 days G2: 10 μM RA for 21 days G3: 10 ng/ml TGFb1 for 21 days	Male adult GCs (The cells homed at the basement membrane of testis after transplantation)
Li *et al.*	2016	goat BM-MSCs	G1: Stra8 overexpression G2: Boule overexpression G3: Dazl overexpression G4: overexpression of Stra8, Boule and Dazl together	Treated cells expressed GC-specific markers from PGCs to post meiotic GCs
Afsartala *et al.*	2016	Mouse amniotic membrane MSCs	25 ng/ml BMP4 for 5 days and then 1 μM RA for the following 12 days	Male adult GCs
Ghasemzadeh-Hasankolai *et al.*	2016	autologous rat BM-MSCs	Transplantation into the testis of busulfan-treated male rats and evaluation 4, 6 and 8 weeks later	Male adult GCs
Li *et al.*	`2016	Human UC-MSCs	overexpression of CD61 (Itgb3) and treatment with 12.5 ng/ml BMP4 for 7 days	PGCs
Wei *et al.*	2016	canine adipose MSCs	12.5 ng/ml BMP4 for 7 days	PGCs

BM-MSCs: bone marrow mesenchymal stem cells; GCs: germ cells; RA: retinoic acid; PGCs: primordial germ cells; SSCs: spermatogonial stem cells; UC-MSCs: umbilical cord mesenchymal stem cells; G: study group

effects of BM transplantation after chemotherapy on the number of follicles in recipients' ovaries. Thus, they transplanted GFP^+-BM cells after chemotherapy and assessed recipient ovaries after 2 months. They found that although the total number of follicles in the ovaries of BM-transplanted mice was lower than non-treated control animals, it was considerably higher than that in mice received chemotherapy drugs without BM transplantation. Furthermore, they indicated that there were a number of donor-derived oocytes in the immature follicle pool in the recipient

ovaries (≈ 1.4%), but they were never seen in antral or Graafian stage and never ovulated. This was also confirmed by their findings that the mice had donor BM-derived oocytes in their ovaries, but the origins of all offspring from chemotherapy-induced females, whose fertility was rescued by BM transplantation, were recipient GCs. All these results were very interesting because they showed that BM cells differentiated into fGCs (although they were not functional), and more importantly played a supportive role for endogenous GCs and restoration of fertility. Despite the valuable achievements, it would be helpful if the researchers checked the donor-derived oocyte fertilization capacity *in vitro,* and figured out the gene expression profile and chromosomal contents of the donor-derived GCs and gametes [68].

In a study in 2012, Ghadami *et al.* transplanted whole BM cells to FSHR (-/-) female mice. FSHR (-/-) or FORKO (follitropin receptor knockout) female mice have small non-functional ovaries, thin uterus wall, high follicle stimulating hormone (FSH) and low estrogen levels and are sterile due to the absence of folliculogenesis. The results showed that BM transplantation caused significant changes in total body and reproductive organs weights, as well as in hormonal levels of the treated animals. Both total body and reproductive organs alone showed significant weight gains in the treated group compared to the control group. Hormonal assay revealed that serum FSH level, which is high in FORKO female mice, decreases significantly after BM transplantation. Moreover, BM transplantation caused considerable elevation in serum estrogen level, which is lower than normal in FORKO females. RT-PCR showed that follicle stimulating hormone receptor (FSHR) expression was induced in ovaries of the recipient females while it was absent in untreated females. Researchers suggested that FSHR expression was a consequence of a decrease in serum FSH. Evaluations showed that BM transplantation stimulated folliculogenesis in FORKO mice so that the total number of follicles and the number of antral follicles would be both higher in the transplanted group compared to the control group. Moreover, ovarian cycles were induced in the transplanted animals following BM transplantation. Despite all these interesting data, which confirm the restoration of follicolugenesis and stroidogenesis in sterile female mice, transplanted FORKO females never ovulated oocytes nor got pregnant [69].

6.2. Skin-Derived Stem Cells

In an interesting study in 2006, Dyce *et al.* isolated porcine skin SCs and then treated them with differentiation medium comprised of DMEM supplemented with 5% FBS, 5% filtered porcine follicular fluid, 0.23 mM sodium pyruvate, 0.1 mM β-mercaptoethanol, 0.1 mM non-essential amino acids and 2 mM l-glutamine. After 30-40 days, a number of suspended aggregates were formed from the attached cells to the poly-D-lysine surface of cell culture dish. After another 10-15 days in the culture, a number of aggregates showed large cells in their centers, resembling comolus-oocyte complexes (COCs). These cell aggregates clearly expressed Oct4, GDF9, Dazl and Vasa transcripts. In the next step, cell aggregates were collected and cultured in oocyte growth medium, which consisted of TCM 199 supplemented with 3 mg/ml BSA, 1 ng/ml EGF, 5 µl/ml insulin transferring selenium, 0.005 international units (IU) FSH, 0.23 mM pyruvic acid, 0.003 IU LH and 1 mg/ml fetuin, for 5-10 days. In this step, the researchers observed that some cell aggregates extruded some large cells, which resembled oocytes and seemed surrounded by a structure similar to zona pellucida. Molecular analyses revealed that these large cells obviously expressed a number of fGC-specific markers, Vasa, Oct4, Scp3, c-Mos, ZPA and ZPC at mRNA level, and ZPC and Scp3 at protein level as well. In addition, they showed that some of the cells around the large cells were positive for P450-arom, which is a marker of granulosa cells. Furthermore, it was shown that the treatment of the cells caused them to secrete steroid hormones, namely estradiol and progesterone in the culture. Interestingly, it seemed that some of the oocyte-like cells parthenogenically changed to blastocyte-like compartments, whose cells expressed Oct4. The researchers concluded that since a few subpopulations of skin SCs showed the ability of differentiation into fGCs, maybe a number of PGCs were lost during their developmental process, located in the skin, and therefore could now produce female gamete-like cells in the culture. However, the authors did not check for the expression of other fGC markers either at mRNA or protein levels, and did not test the fertilization capacity of the cells. Besides, they did not evaluate the produced oocyte-like cells epigenetically [14].

In 2009, Linher and colleagues tested the capability of porcine skin SCs for differentiation into fGCs. They isolated SCs from the skin of porcine fetuses. Skin SCs were non-adherent cells. After characterization, the cells were cultured in poly-D-lysine treated dishes and treated with differentiation medium that consisted of DMEM, penicillin/streptomycin, 5% heat inactivated fetal bovine serum (FBS), 5% porcine follicular fluid, 0.23 mM sodium pyruvate, 2 mM L-glutamine, 0.1 mM b-mercaptoethanol, and 0.1 mM non-essential amino acids for 50 days. Evaluations showed that some of the attached fibroblast-like skin SCs acquired a new morphology and became round shaped and blebbed from days 15 to 30 of this treatment, while their sizes were greater than untreated cells. These cells were still proliferative and some of them gradually detached from the culture dish and became floating as single cells or clusters of cells. The researchers reported that these cells resembled natural PGCs. Moreover, they showed that morphologically distinct cells were positive for ALP. Furthermore, RT-PCR revealed that undifferentiated skin SCs expressed GC markers (Oct4, Fragilis, Stella, Dazl, Vasa and c-kit) innately, which is expected for pluripotent SCs. Therefore, semi-quantitative real time PCR was performed in days 0, 20, 25 and 30 after treatment, and indicated that except for Vasa and c-kit, which were downregulated moderately, all the other markers were upregulated significantly after the treatment with differentiation medium. Immunostaining on day 30 of treatment revealed that the produced PGC-like cells expressed Oct4, Vasa, Stella, c-kit and Dazl proteins. Control cells were negative for all of the tested markers. Epigenetic evaluation showed that *in vitro*-derived PGC-like cells underwent imprint erasure and had the same methylation pattern in H19 DMRI. To investigate the capacity of produced PGC-like cells, the cells on day 25 of treatment were collected, transduced lentivirally with Dazl-GFP transgene and cultured for a longer period. Evaluations showed that a number of either cell aggregates or large oocyte-like cells

(OLCs) were formed in the cell cultures after 5 to 15 days. These Dazl-GFP positive OLCs were surrounded by negative cells, which were similar to granulosa cells. RT-PCR revealed that OLCs expressed fGC-specific markers- Oct4, Vasa and GDF9, while there was no expression in the control group. Although, all these findings were important and helpful, the authors did not test the expression of many other GC markers at mRNA and protein levels. Moreover, the developmental competence and fertilization capacity of the produced cells were not evaluated [24].

Dyce and co-workers in another study examined the ability of skin SCs for differentiation into fGCs, alone and in co-culture with ovarian cells (OV). They isolated SCs from the skin of newborn female Oct4-GFP transgenic mice. The isolated SCs were then divided into two treatment groups, SC only and SCs+OV' however, an OV only group was considered as a positive control. The treatment groups were cultured in differentiation medium that consisted of TCM199 (no antibiotics) supplemented with 0.05 IU FSH, 0.03 IU LH, 5 ml/ml ITS, 1 ng/ml EGF, 0.23 mM sodium pyruvate, 3 mg/ml BSA, and 1 mg/ml Fetuin, for 12 days. After the treatment of non-adherent skin SCs, and also SCs+OV cells, a number of cell aggregates were formed, and contained some large GFP^+ OLCs. Flow cytometric analysis showed that the percentage of GFP+ cells increased in SC group after treatment, which confirms the generation of positive cells *in vitro*. After dissociation of these OLCs, the authors observed that they were very similar to oocytes of the positive control group and both appeared to be surrounded by a structure similar to zona pellucida. The size of OLCs produced in treatment groups was almost similar to the oocytes of adult female mice. Molecular analysis indicated that despite large variations, the expression levels of fGC-specific markers, Oct4, GDF9, Vasa, Dazl and Figα, in the treatment group were comparable to the positive control group. Interestingly, OLCs derived from SCs showed higher levels of Sox2 and Fragilis than the oocytes of adult mice, while the expression levels of Nanog and Stella were higher in oocytes compared to the OLCs. It was also shown that among Zp1, Zp2 and Zp3, only Zp3 was expressed in SC-derived OLCs. Furthermore, immunostaining confirmed the expression of Dazl, Vasa and Stella proteins. Investigation for the occurrence of meiosis revealed that SC-derived OLCs did not express Rec8, while they expressed mRNA of other meiotic markers, such as Scp3 and DMC1. Some of the OLCs seemed to have a germinal vesicle. The researchers concluded that OLCs entered into the meiosis process but did not further differentiate beyond the M1 phase. In the other part of the study, researchers mixed skin SCs with OV and after a 72-hour culture in the laboratory, transplanted them under the kidney capsules of immunedeficient recipient mice. They found that after 5-16 weeks, a number of SCs differentiated into follicle-like structures with Oct4-GFP^+ OLCs. Although all these findings are interesting and helpful, the authors did not evaluate the developmental competence and fertilization capacity of the produced OLCs [13].

Lai *et al*. in 2014 showed that skin-derived (S) MSCs helped to restore fertility in busulfan + cyclophosfamide-treated infertile female mice. In this study, one week after inducing infertility by chemotherapy, female mice received male and female SMSCs in two different groups from the tail vein. The results showed that transplantation of both male and female SMSCs led to weight gain in both total body and reproductive organs. Moreover, transplantation of SMSCs contributed to new folliculogenesis, and thus follicles at different stages of development were detected from 2 weeks to 2 months after transplantation in the ovaries of both male and female SMSCs-transplanted mice. Surprisingly, the transplanted mice showed higher pregnancy rates and fecundity in comparison to the untreated control mice. No donor-derived GCs were observed in the recipient ovaries and the authors concluded that MSCs altered the adverse effects of chemotherapy on the ovaries [23].

In an investigation by Ge *et al*. in 2015, it was indicated that female human fetus skin-derived stem cells (SDSCs) differentiated into GC-like cells. They found that fhSDSCs after 4 days of culture in EB culture medium, comprised of TCM 199, supplemented with 3 mg/ml BSA, 1 mg/ml fetuin, 5 μ l/ml insulin transferring selenium, 1 ng/ml EGF (Epidermal Growth Factor), 0.23 mM pyruvic acid, 30 ng/ml BMP4, and 20 ng/ml Activin A expressed GC-specific markers, Dazl and Vasa. Further treatment of the cells with OLCs differentiation medium, which contains DMEM/High glucose supplemented with 5% FBS, 5% filtered porcine follicular fluid, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 0.23 mM sodium pyruvate, and 0.1 mM β -mercaptoethanol, penicillin (100 U/ml), streptomycin (100 mg/ml), changed the morphology of the cells and formed some vesicular structures in the cells. Moreover, both Dazl and Vasa were overexpressed along with the emerging of AMH expression, which indicated the formation of some granulosa cells in the culture. Therefore, the authors concluded that some fGCs were produced in the culture. The authors also showed that the treatment of human fetal SDSCs with a second differentiation medium containing DMEM: F12 (1:1) basic, supplemented with 15% FBS, 1500 U/ml LIF, 5 μ l/ml ITS, 10 ng/ml EGF, 50 international units (mIU) FSH, 2% B27 supplement, penicillin (100 U/ml), streptomycin (100 mg/ml), induced Scp3 overexpression. They concluded that meiosis has taken place in their system. Ploidy analysis and fluorescent in situ hybridization (FISH) analysis revealed that indeed a number of 1N cells existed in the culture. Interestingly, the researchers claimed that these 1N cells were male GCs because they expressed SCP3 marker. Thus, they reported that they could produce both male and female GCs from female human fetus SDSCs. It should be noted that SCP3 not only is expressed in male GCs but also has been shown that oocytes also express this meiotic marker [70]. Although these findings were truly valuable, the researchers did not check the OLCs fertilizing capacity and also chromosomal content or the occurrence of meiosis or existence of any polar bodies. As they said in their paper, they did not examine the 1N cells fertilization capacity [15].

6.3. Pancreatic Stem Cells

In an investigation, Danner at al. showed that pancreatic (P)SCs can spontaneously form OLCs in cell culture. In this study, scientists established a clonal SC line from passage 75 (P75) PSCs, which could form cell aggregates after culture as hanging drops. They found that after culturing, the cells aggregated in the culture dish, and some floating single cells

were released into the culture medium. Morphological evaluations showed that these cells resembled oocytes. Molecular analyses showed that among four study groups, P8 and P75 PSCs, clonal cell line and OLCs, P8 cells expressed all of the examined SC and GC markers (Oct4, Nestin, CD9, SCp3, DMC1 and Vasa), except for SSEA1 and GDF9. P75 SCs did not express SCP3 and DMC1. Finally, clonal cells and OLCs expressed all of the tested markers. Furthermore, immunostaining showed that OLCs vigorously expressed Oct4, SCP3 and DMC1 proteins, which were very weak or negative in primary cell population. In their conclusion, researchers presented an interesting interpretation of the expression of GC-specific markers in PSCs and their ability for differentiation into fGCs. They claimed that it is possible that a number of PGCs get "lost" during their migration from the wall of the yolk sac to the primary gonads, and locate in other organs like pancreas. Probably these cells can maintain their GC capacity and could be active again any time in the right culture conditions. Although this was a really interesting description, the researchers did not check the expression of other fGC-specific markers at neither mRNA nor protein levels. In this study, OLC maturation, fertilization capacity and chromosomal content (to check for meiosis) were not examined [71].

6.4. Muscle MSCs

In an interesting study Lv *et al.* tested the ability of porcine muscle-derived (PM)-MSCs for differentiation into fGCs. In this study, researchers isolated MSCs from porcine muscle tissue and after characterization of the cells by differentiation capacity test and confirmation of their pluripotency, the cells were treated by 10% and 20% bovine follicular fluid (FF) for 5-18 days. Their data showed that a number of attached fibroblastic PM-MSCs changed into round and oval shaped cells and then some of these cells detached from the culture dish and grew larger as floating single cells or in clusters. These large cells resembled PGCs. Some of these large cells became larger (40-60 μm in diameter) and a few of them generated oocyte-like structures. Molecular analyses revealed that the expression levels of GC-specific markers Figla, Vasa, Gdf9, Scp3, ZP1 and ZP3 were increased in PM-MSCs treated with 20% FF in comparison with the cells treated with 10% FF and untreated PM-MSCs. In addition, the expression levels of mesoderm marker, Desmin, pluripotency marker, Oct4, and proliferation marker, PCNA, were lower in 20% FF-treated cells compared to the two other groups. Furthermore, the level of estradiol was higher in 20% FF cultures than in 10% FF and untreated control groups. In another part of the study, the authors evaluated the role of a small molecule, Reversine, in differentiation induction in PM-MSCs toward GCs. Therefore, PM-MSCs were treated with 1μM Reversine for 2 days and then for 10 days with 20% FF in the absence of Reversine. Evaluations revealed that Reversin-treated PM-MSCs acquired larger sizes than both untreated PM-MSCs and DMSO (solvent of Reversine)-treated cells. Three to ten days after treatment with FF, some large female germ-like cells appeared in the cultures. The number of fGCs was significantly more than the cells in the two other groups. The generated fGCs clearly expressed Stra8 and Zp3 proteins. Quantitative RT-PCR indicated that the expression of Gdf9, Dazl, Vasa, Scp3, Figla and Zp2 were upregulated in Reversine-treated PMMSCs in comparison with the other groups. Furthermore, immunocytochemistry showed that similar to porcine oocytes, *in vitro*-derived large round or oval GC's were positive for Vasa, Dazl, Stra8, Scp3, Zp2 and Zp3 and were negative for Oct4. On the other hand, Vasa, Dazl, Stra8 and Zp2 were not expressed in untreated PMMSCs. The authors then mixed pretreated PMMSCs with 20% FF for 10 days with the cell recovered from ovaries (by enzymatic digestion), and transplanted them under kidney capsule. Four weeks later they evaluated the grafts. They observed that a number of BrdU-labeled PMMSCs expressed Vasa, Dazl, Stra8 and Scp3, while some of them expressed Figla, Zp2 and Zp3. The authors concluded that PMMSCs were capable of differentiation into fGCs, and that treatment with FF and Reversine promoted the differentiation process. Overall, it seems that muscle MSCs would be a new source of SCs for use in cell therapy procedures, although the researchers did not evaluate the potency of *in vitro*-derived OLCs for fertilization and formation of viable embryos [72].

6.5. Amniotic Fluid Stem Cells (AFSCs)

Lai and colleagues published a research article in 2013, and they explain how they isolated human AFSCs in 18th week of pregnancy and let the cells form colonies. They harvested colonies and cultured them in EB media enriched with 5% human FF for 7-14 days to stimulate EB formation. The produced EBs were then cultured in differentiation medium, which consisted of EB medium supplemented with a germ cell factor cocktail containing: human SCF 100 ng/ml, bFGF 20 ng/ml, SDF1 20 ng/ml, CYP26 inhibitor R115866 1 mM, BMP4 50 ng/ml, and N-acetylcysteine 1 mg/ml, forskolin 5 mM, RA 1 mM for another 7-14 days. Molecular evaluation on P1 to P6 of untreated human AFSCs revealed high expression levels of Oct4, Stella, Scp1 and Scp3, and low expression levels of Stra8, c-Mos and ZPC. They also had high but variable expression levels of Dazl, Vasa and GDF9. At protein level, just Oct4 was expressed by untreated human AFSCs. Moreover, it was found that under standard differentiation situation, without GC factor cocktail or human FF, Oct4 and Nanog were dowregulated, while Blimp1, Stra8 and Stella were upregulated. Interestingly, the expression of Dazl was not detected in untreated cells at the end of the experiment. In contrast, after treatment with differentiation media with GC factor cocktail or human FF, the expression of Blimp1, Stella, Dazl, Vasa, Stra8, Scp3 and c-Mos clearly increased in EB cells, whereas the levels of Oct4 and Nanog expression decreased. In line with these data, immunocytochemistry confirmed the increase in the expression of Blimp1, Stella, Dazl, Scp3, and ZPC. The cells in the control group expressed only Blimp1, Stella and ZPC. Scp3 was specifically expressed in the treated cells. Next, they transfected human AFSCs with EGFP and transplanted them into the ovaries of chemotherapeutically sterilized female mice. Two months after transplantation, histological analysis revealed that similar to the ovaries of non-sterilized mice, the transplanted ovaries possessed a large number of oocytes in different developmental stages, and $EGFP^+$ cells surrounded some of them. Immunohistochemistry showed that these cells were positive for FSHR, a granulosa cell marker. Although transplantation of

AFSCs improved the histology of the sterilized ovaries, and many $EGFP^+$ somatic cells that were derived from transplanted cells were observed near the oocytes, no donor-derived fGC was observed in recipient ovaries in the end. Therefore, the authors concluded that human AFSCs restored ovarian function by differentiation into granulose cells and improvement of the ovarian niche. No additional experiment was performed in order to check for the functionality of the *in vitro* produced fGCs [73].

In another study again using human AFSCs, Yu and co-workers isolated SCs from AF of second-trimester pregnant women. AFSCs expressed MSCs characteristics and showed potency for multilineage differentiation. These cells were treated with GC-inductive medium, which consisted of alpha-MEM supplemented with 5% FBS, 5% porcine FF, 0.1 mM β-mercaptoethanol, and 2 mM L-glutamine, 1% NEAA, for 15 days. This treatment caused the formation of some cell aggregates, which were highly positive for ALP and showed elevated expression levels of GC-specific markers, Figla, Dazl, Ifitm3 and Gdf9. In addition, the expression of a number of GC-specific genes like Oct4 and Vasa were induced after treatment with GC medium. From these results, the researchers concluded that they had some primordial follicle-like structures in the culture. A number of cell aggregates, which were considered as COCs were excluded from these structures. In addition, the cell aggregates were treated with OLC maturation medium that consisted of TCM 199 supplemented with 3 mg/mL BSA (bovine serum albumin), 10 U/mL human chorionic gonadotropin (hCG), equine chorionic gonadotropin (eCG), 2.5 U/mL FSH, 0.23 mM pyruvic acid, 10 ng/mL epidermal growth factor (EGF), and 1% insulin-transferrin-selenium for 7 days. This treatment resulted in the formation of OLCs, which were surrounded by granulose-like cells. These structures were detected next to the treated cell aggregates. Floating OLCs were in different sizes ranging from 50 to 120 μm in diameter. Expression of Zp2 was detected in OLCs while untreated AFSCs did not express this marker. Moreover, the expression levels of Zp1 and Zp3 were upregulated in OLCs compared to the AFSCs. Production of estradiol was also evaluated during the treatment with OLC medium and was shown to be significantly elevated due to the treatment. Similarly, the expression levels of steroidogenic enzymes (P450, Cyp17 and Fshr) were significantly upregulated in follicle-like structures derived from human AFSCs after the treatment with GC medium. Molecular analysis revealed that some haploid cells were formed after the treatment, which expressed meiotic markers, Scp3, Dmc1 and Foxl2. Researchers in this study reported the formation of some embryo-like multicellular structures and suggested the parthenogenesis of OLCs for their production. Therefore, these findings were truly exciting, but the number of fGC-specific markers, which were evaluated in this study, were very few and also the researchers did not check the fertilization potency of the produced OLCs [74].

6.6. UC-MSCs

In an interesting research project, Qiu and colleagues tested the ability of male and female human UC-MSCs for differentiation into fGCs and OLCs under the treatment with cattle FF containing inductive medium. After isolation and characterization of UC-MSCs, the cells were treated for 7-14 days with 20% cattle FF containing differentiation medium. They observed that after 4 days, a number of round cells were formed in both male and female cell cultures, though the number of these round cells in the female culture was more than that in the male culture. At the end of the treatment period (day 14), a number of large round cells similar to oocytes were formed in both male and female cultures and interestingly the size of the female UC-MSC-derived OLCs (40-50 μM) was clearly greater than that of OLCs derived from male UC-MSC (25-35 μM). Both male and female cells expressed high levels of fGC and meiotic markers like Stella, Dazl, GDF9, ZP1, ZP3 and Stra8 after induction with differentiation medium. Real time RT-PCR showed that induced male cells had the peak of gene expression level (for all markers) on day 7 and then gene expression levels were decreased. In contrast, in female cells, all markers except for Dazl reached the peak on day 14. Furthermore, mRNA levels of GDF9 and ZP2 were higher in the female treatment group than the male group. Researchers showed that treatment with cattle FF containing medium caused an increase in the amount of estradiol in both treatment groups, while female group had a higher level of estradiol compared to the male group. Immunostaining of OLCs from both male and female treatment groups showed that they were positive for Oct4, Vasa, Dazl, ZP2, Zp3 and Stra8. Researchers also transplanted the cultured UC-MSCs into seminiferous tubules and kidney capsule of mouse to see if they had the capacity to differentiate into GCs *in vivo*. They observed that a mouth after transplantation, there were a number of male UC-MSCs in the seminiferous tubules, which expressed Vasa and Dazl. Furthermore, transplanted female HUMSCs in the kidneys were positive for Vasa, Dazl and ZP2 after 1-2 months. Overall, the researchers concluded that FF induced differentiation into fGCs in both male and female UC-MSCs, though female cells showed more potency for differentiation into OLCs. Moreover, they stated that *in vitro*-derived female gamete-like cells did not reach maturity in the culture [75].

Hu *et al*. (2015) used first trimester human UC-MSCs to see if they have the capacity to differentiate into fGCs. For this purpose, they treated the characterized cells for 24 days with a differentiation medium comprised of α-MEM supplemented with 10% FBS, 25% human FF, 150 mIU luteinizing hormone (LH), 150 mIU FSH, and 300 pg/ml of estradiol. The morphology of the cells together with the expression of PGC-specific genes e.g., Oct4, SSEA1, Blimp1, PRDM14, TFAP2C, Vasa, Stella, Dazl, Ifitm3, and oocyte markers Scp3, GDF9, ZP1, 2 and 3 were evaluated on days 0, 7 and 24 of the treatment period with real time RT-PCR, immunocytochemistry and western blotting. On day 7, the researchers found some round PGC-like cells in the culture, which expressed elevated levels of all tested PGC markers. Moreover, expression levels of most oocyte markers, except for ZP2 and 3, increased on day 7 compared to day 0. Upregulation of Scp3 and GDF9 was not significant. After day 7, PGC-like cells continued to produce some primordial follicle-like structures and later COCs and OLCs in the culture. Assessment on day 24 revealed that there were some OLCs in the culture ranging from 50-120 μM in diameter, and except for PRDM1, which was slightly upregulated, expression levels of all the other PGC markers went down severely in the produced OLCs. In contrast, oocyte markers

were all upregulated significantly in these cells. Additionally, the researchers reported that the amount of estradiol increased during the treatment process, which was a consequence of the human UC-MSC-derived somatic granulose-like cells activity in COCs. This was further supported by the elevated amounts of LIF and VEGF proteins in the cultures during differentiation process. Overall, this report demonstrated that first trimester human UC-MSCs successfully differentiated into PGCs, granulosa-like cells and also OLCs. Furthermore, the authors stated that they did not see any differences between male and female HUMSCs regarding the efficiency of differentiation into GCs. Despite these remarkable findings, the authors did not show chromosomal contents, developmental competence or fertilization capacity of the produced OLCs [76].

6.7. Endometrial (En) MSCs

A recent study by Lai *et al.* showed the ability of EnMSCs to differentiate into OLCs *in vitro*. In this study, researchers isolated MSCs from menstrual blood of women and after confirming their stemness through differentiation tests, they treated them with differentiation medium comprised of DMEM/F12 supplemented with 10% ES-FBS, 20% filtered human FF, 1% glutamine and 1% penicillin/ streptomycin, 4 ng/ml bFGF, 0.23mM sodium pyruvate, 0.1mM non-essential amino acids, 2mM L-glutamine, and 0.1mM β-mercaptoethanol, for 1 day, 1 week, 2 weeks and 4 weeks. At the end of treatment, the cells' morphology and expression of GC-specific genes were assessed. After 2 weeks of induction, the fibroblastic En-MSCs were changed into round shaped cells and some cell aggregates were also formed in the culture. Some of the round cells detached from the cell aggregates and formed floating spheroidal cells gradually. After 4 weeks of differentiation induction, some cell aggregates were similar to follicle-like structures. Even though RT-PCR showed that fGC-specific markers, Oct4, NANOG, Gdf9, Bimp1, Dazl, Stella, Stra8, Vasa, Scp1, Scp3, ZpA, and ZpC, were expressed in both treated and un-treated EnMSCs, real time RT-PCR revealed that, the expression levels of Scp3, Dazl, Stella and Gdf9 were significantly upregulated by the treatment until week 4. Immunocytochemistry revealed that large floating spheroidal OLCs were generated in the culture of EnMSCs after 4 weeks of treatment, expressed Bimp1, Dazl, Stella, Scp3, and ZpC, while no positive cells were observed in the untreated culture. Moreover, evaluations indicated that granulose cell-specific markers, FshR and LHR were expressed by a number of cells in the cells aggregates, which suggested differentiation of a number of treated EnMSCs into granulose-like cells in the culture. Stimulation of these cell aggregates with FSH and LH showed that they reacted to the stimulation by secretion of both estradiol and progesterone. Researchers then co-cultured EnMSCs with granulosa cells isolated from the FF in induction medium for 21-28 days. They observed that a number of OLCs were formed in the culture and a few of them changed to multicellular structures resembling parthenogenic embryos. At the end, researchers concluded that EnMSCs successfully differentiated into fGCs, gamete-like cells and also granulosa-like cells under stimulation of FF. Despite these interesting results, they did not evaluate the fertilization capacity of the produced OLCs, as well as generation of viable embryos to create a successful pregnancies [77].

Considering the variability of the cell types that have been used in various studies, different research teams have made a good progress and so many useful reports have been published. Although none of these valuable studies could generate functional fGCs or gametes from ACSs, the results are completely promising. It seems that just like the male cells, a lack of knowledge on the female GC biology and gametogenesis, is the main reason for the incomplete protocols available. These issues can be addressed by detailed and creative research studies. Table **2** summarizes the information reported on generation of female germ line cells from different types of ASCs.

CONCLUSION

Different types of ASCs from various sources have been utilized for generation of GCs in the laboratory setting. Nevertheless, the vast majority of studies have used MSCs, and regardless of the different sources from which these cells were isolated, almost all of them showed the capacity to differentiate into germ-like cells both *in vitro* and *in vivo*. Most studies used MSCs from the BM probably because of their ease of isolation and availability.

Moreover, the review of literature shows that, various ASCs can differentiate into germ-like cells under appropriate conditions, while they express GC-specific markers at mRNA or protein levels. However, as stated earlier, none of the research groups could produce functional GCs, which can be used for treatment of infertility. Although all the conducted studies were fabricated based on the events and mechanisms, which took place in the body and had interesting results, the final goal is yet to be achieved. Apparently, some important factors, which play crucial roles, are still unknown. Therefore, despite valuable and helpful research that has been done so far, there are still some dark zones in GC biology (like GC inductive factors, mechanism of entering into meiosis, etc.), which lead to low efficiency in mimicking the developmental process of GCs in the laboratory. Detailed investigation on GC developmental biology and determination and characterization of the factors and mechanisms involved in specification of GCs and gamete production are still necessary.

PERSPECTIVES AND FUTURE DIRECTIONS

To expand our knowledge in the field of GC development, some important research topics would be: identifying factors involved in specification of PGCs, entering into meiosis, maintaining stemness, renewal and differentiation of SSCs, different stages of gametogenesis, epigenetic changes of GCs in the culture, and GC fertilization capacity.

There are still uncovered data on emergence and specification of GCs. Despite some good available information, we have not exactly characterized the factors, which determine the fate of a group of embryonic cells as PGCs. Another important and unexplained event in the developmental process of GCs is meiosis. Up to now, one of the greatest barriers in

Table 2. Reports on derivation of female germline cells from adult stem cells (ASCs).

Research Team	Year	Utilized Stem Cell Type	Treatment	Produced Germ-Like Cells
Johnson *et al.*	2005	Mouse BM* and PB* mononuclear cells	Transplantation to sterile female mice via the tail vein	New donor-derived follicles and oocytes in recipient ovaries
Dyce *et al.*	2006	porcine skin SCs*	5% filtered porcine follicular fluid, 0.23 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM l-glutamine and 0.1 mM β-mercaptoethanol for 30-40 days	Oocyte-like cells
Lee *et al.*	2007	Mouse whole BM cells	Transplantation to chemotherapeutical infertile female mice via the tail vein	New donor-derived follicles and oocytes in recipient ovaries and restoration of infertility by reactivation of endogenous folliculogenesis
Danner *et al.*	2007	Pancreatic SCs	Spontaneously	Oocyte-like cells
Linher *et al.*	2009	porcine skin SCs	5% porcine follicular fluid, 0.23 mM sodium pyruvate, 0.1 mM non-essential amino acids, 2 mM L-glutamine, and 0.1 mM b-mercaptoethanol, for 50 days	PGCs and Oocyte-like cells
Dyce *et al.*	2011	Mice skin SCs	G1*: SCs G2: co-culture of SCs with ovarian cells Both treated with TCM199 (no antibiotics) supplemented with 0.05 IU FSH, 0.03 IU LH, 3 mg/ml BSA, 5 ml/ml ITS, 0.23 mM sodium pyruvate, 1 mg/ml Fetuin, and 1 ng/ml EGF, for 12 days G3: transplantation of SCs+ ovarian cells after 3 days co-culture under the kidney capsule and evaluation 5-16 weeks later	Oocyte-like cells (both *in vitro* and *in vivo*)
Ghadami *et al.*	2012	Mouse whole BM cells	Transplantation to FORKO (follitropin receptor knockout) female mice	Support and reactivation of endogenous folliculogenesis
Lv *et al.*	2012	Porcine muscle-derived (PM)-MSCs	G1: 10% bovine follicular fluid G2: 20% bovine follicular fluid both for 5-18 days G3: s days treatment with Reversine* and then for 10 days with 20% bovine follicular fluid	PGCs and oocyte-like cells
Lai *et al.*	2013	Human amniotic fluid stem cells (hAFSCs)	G1: EB* media supplemented with 5% human follicular fluid for 7-14 days to stimulate EB formation and then treatment of the produced EBs with human SCF 100 ng/ml, SDF1 20 ng/ml, bFGF 20 ng/ml, BMP4 50 ng/ml (all R&D Systems, Minneapolis, Minnesota, USA), N-acetylcysteine 1 mg/ml, forskolin 5 mM, retinoic acid 1 mM and CYP26 inhibitor R115866 1 mM for further 7-14 days G2: transplantation of hAFSCs into the ovaries of chemotherapeutically sterilized female mice	G1: generation of some germ-like cells G2: differentiation of the transplanted cells into granulosa cells
Qiu *et al.*	2013	Male and female human UC-MSCs*	20% bovine follicular fluid for 7-14 days	Oocyte-like cells

Table (2) contd….

Research Team	Year	Utilized Stem Cell Type	Treatment	Produced Germ-Like Cells
Antonucci *et al.*	2014	Human amniotic fluid stem cells (hAFSCs)	No treatment	EB-like structures a number of which expressed PGC markers
Yu *et al.*	2014	Human amniotic fluid stem cells (hAFSCs)	Medium supplemented with alpha-MEM supplemented with 5% FBS, 5% porcine follicular fluid, 2 mM L-glutamine, 1% NEAA, and 0.1 mM β-mercaptoethanol, for 15 days and then with a medium comprised of TCM 199 supplemented with 3 mg/mL BSA, 2.5 U/mL FSH, 10 U/mL hCG, eCG, 0.23 mM pyruvic acid, 10 ng/mL EGF and 1% insulin-transferrin-selenium for 7 days.	Follicular-like structures and oocyte-like cells
Lai *et al.*	2014	Male and female skin-derived (S)-MSCs	Transplantation into busulfan + cyclophosfamide treated infertile female mice via the tail vein	No donor-derived GCs just support of the endogenous folliculogenesis and ovarian function
Asgari *et al.*	2015	Human UC-MSCs	Co-culturing with placental cells for 14 days	PGCs
Ge *et al.*	2015	Human fetus skin SCs	G1: TCM 199, supplemented with 3 mg/ml BSA, 1 mg/ml fetuin, 5 µ l/ml insulin transferring selenium, 0.23 mM pyruvic acid, 1 ng/ml EGF, 20 ng/ml Activin A, and 30 ng/ml BMP4 for 4 days and further treatment with oocyte-like differentiation medium containing DMEM/High glucose supplemented with 5% FBS, 5% filtered porcine follicular fluid, 0.23 mM sodium pyruvate, 2 mM L-glutamine, 0.1 mM non-essential amino acids, and 0.1 mM β -mercaptoethanol, penicillin (100 U/ml), streptomycin (100 mg/ml) G2: 1500 U/ml LIF, 50 mIU FSH, 10 ng/ml EGF, 2% B27 supplement, 5 µ l/ml ITS, penicillin (100 U/ml), streptomycin (100 mg/ml)	PGCs fGCs Oocyte-like cells 1N GCs
Hu *et al.*	2015	Human UC-MSCs	25% human follicular fluid, 150 mIU FSH, 150 mIU LH and 300 pg/ml of estradiol for 24 days	PGCs Oocyte-like cells
Lai *et al.*	2016	Endometrial- MSCs	20% filtered human follicular fluid, 4 ng/ml bFGF, 0.23mM sodium pyruvate, 0.1mM non-essential amino acids, 2mM L-glutamine, and 0.1mM β-mercaptoethanol, for 1 day, 1 week, 2 weeks and 4 weeks	Oocyte-like cells

PB: peripheral blood; BM-MSCs: bone marrow mesenchymal stem cells; GCs: germ cells; SCs: stem cells; PGCs: primordial germ cells; SSCs: spermatogonial stem cells; UC-MSCs: umbilical cord mesenchymal stem cells; Reversine: is a small molecule, G: study group.

the way of achieving gametes form SCs has been meiosis. It is not exactly known what factors control meiotic division and how we can mimic and control this phenomenon in the laboratory conditions. However, a very useful research paper has described the gold standards for proof of *in vitro* meiosis [78].

Even though we have so much good information on mechanisms of stemness maintenance, renewal process and triggering of differentiation in various SCs, there are still some unknown aspects in these areas, especially for SSCs and female germline SCs. Since testis is a closed and highly specialized environment, which is strictly controlled, investigation and understanding of so many events, which take place in it are nearly impossible. Moreover, the biology and function of sertoli cells, the only cell types that are in direct contact with SSCs in the testis and control their behavior have not been completely understood yet. Furthermore, numbers and types of components secreted by sertoli cells, SSC-sertoli cell interactions, and also the events that take place and control each developmental stage during gametogenesis procedure have not been fully understood. On the other hand, a similar role is conceivable for somatic cells of

the ovary, granolusa cells, which should be fully investigated in detail. Recent studies on transcriptomic and proteomic analyses of gametogenesis are such valuable work and should be continued in order to have a better understanding of these processes [79-81].

One of the most important issues in the field of *in vitro* gametogenesis is epigenetic change in the cells and their imprinting states. Knowing the epigenetic modifications in an *in vitro*-derived gamete is really crucial because it can directly affect the cells' fate and fertilization capacity. As can be seen, so many un-resolved problems are still remaining in gametogenesis.

A number of research teams have recently proposed an idea focusing on the construction of an artificial gonad using tissue engineering techniques. Although this hypothesis is in its first steps and all studies have been conducted on animal models, their results were promising and interesting [82-86]. MSCs, are multi/pluripotent undifferentiated cells, which reside in almost all tissues, can be simply isolated from tissue biopsies and have the capacity to differentiate into mesodermal and non-mesodermal cell types as well as germ-like cells [25, 87, 88]. Because of these characteristics and also some other important features like immunosuppressive activity, MSCs are vastly under consideration for cell therapy and tissue-engineering purposes [89-92]. Regarding these data, testis and/or ovary tissue engineering using MSCs can be attractive fields of research to find new techniques for treatment of infertility in both males and females.

Accomplishments in each of these fields will certainly help to achieve a standard system of *in vitro* gametogenesis using ASCs. Undoubtedly, results of these research studies will lead to set up a method for treatment of infertility using SC therapy in near future.

LIST OF ABBREVIATIONS

SCs = Stem cells
ASCs = Adult stem cells
BM = Bone marrow
MSCs = Mesenchymal stem cells
GCs = Germ cells
ESCs = Embryonic stem cells
iPSC = Induced pluripotent stem cells
PGCs = Primordial germ cells
UC-MSCs = Umbilical cord mesenchymal stem cells
EBs = Embryoid bodies
ALP = Alkaline phosphatase
TGF = Transforming growth factor
SSCs = Spermatogonial stem cells
mGCs = Male adult germ cells
fGCs = Female adult germ cells
RT-PCR = Reverse transcription polymerase chain reaction
FBS = Fetal bovine serum
TCC = Testicular-cell conditioned medium
PCC = Placental cell conditioned medium
AFSCs = Amniotic fluid stem cells
FSHR = Follicle stimulating hormone receptor
COCs = Comolus-oocyte complexes
OLCs = Oocyte-like cells
OV = Ovarian cells
IU = International units
SDSCs = Skin-derived stem cells
PSCs = Pancreatic stem cells
PM-MSCs = Porcine muscle-derived mesenchymal stem cells
FF = Follicular fluid
hCG = Human chorionic gonadotropin
EGF = Epidermal growth factor
EnMSCs = Endometrial mesenchymal stem cells

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

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REFERENCES

[1] Zhou Q, Wang M, Yuan Y, *et al*. Complete Meiosis from Embryonic Stem Cell-Derived Germ Cells *In vitro*. Cell Stem Cell 2016; 18(3): 330-40.
[2] Geijsen N, Horoschak M, Kim K, *et al*. Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature 2004; 427(6970): 148-54.
[3] Hubner K, Fuhrmann G, Christenson LK, *et al*. Derivation of oocytes from mouse embryonic stem cells. Science 2003; 300(5623): 1251-6.
[4] Toyooka Y, Tsunekawa N, Akasu R, *et al*. Embryonic stem cells can form germ cells *in vitro*. Proc Natl Acad Sci USA 2003; 100(20): 11457-62.
[5] Aflatoonian B, Moore H. Germ cells from mouse and human embryonic stem cells. Reproduction 2006; 132(5): 699-707.
[6] West FD, Mumaw JL, Gallegos-Cardenas A, *et al*. Human haploid cells differentiated from meiotic competent clonal germ cell lines that originated from embryonic stem cells. Stem Cells Dev 2011; 20(6): 1079-88.
[7] Nayernia K, Nolte J, Michelmann HW, *et al*. *In vitro*-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev Cell 2006; 11(1): 125-32.
[8] Teramura T, Takehara T, Kawata N, *et al*. Primate embryonic stem cells proceed to early gametogenesis *in vitro*. Cloning Stem Cells 2007; 9(2): 144-56.
[9] Sasaki K, Yokobayashi S, Nakamura T, *et al*. Robust *in vitro* induction of human germ cell fate from pluripotent stem cells. Cell Stem Cell 2015; 17(2): 178-94.
[10] Cai H, Xia X, Wang L, *et al*. *In vitro* and *in vivo* differentiation of induced pluripotent stem cells into male germ cells. Biochem Biophys Res Commun 2013; 433(3): 286-91.
[11] Zhu Y, Hu HL, Li P, *et al*. Generation of male germ cells from induced pluripotent stem cells (iPS cells): An *in vitro* and *in vivo* study. Asian J Androl 2012; 14(4): 574-9.
[12] Yang S, Bo J, Hu H, *et al*. Derivation of male germ cells from induced pluripotent stem cells *in vitro* and in reconstituted seminiferous tubules. Cell Prolif 2012; 45(2): 91-100.
[13] Dyce PW, Liu J, Tayade C, *et al*. *In vitro* and *in vivo* germ line potential of stem cells derived from newborn mouse skin. PLoS One 2011; 6(5): e20339.
[14] Dyce PW, Wen L, Li J. *In vitro* germline potential of stem cells derived from fetal porcine skin. Nat Cell Biol 2006; 8(4): 384-90.
[15] Ge W, Ma HG, Cheng SF, *et al*. Differentiation of early germ cells from human skin-derived stem cells without exogenous gene integration. Sci Rep 2015; 5: 13822.

[16] Ghasemzadeh-Hasankolaei M, Eslaminejad MB, Batavani R, *et al.* Comparison of the efficacy of three concentrations of retinoic acid for transdifferentiation induction in sheep marrow-derived mesenchymal stem cells into male germ cells. Andrologia 2014; 46(1): 24-35.

[17] Ghasemzadeh-Hasankolaei M, Eslaminejad MB, Sedighi-Gilani M. Derivation of male germ cells from ram bone marrow mesenchymal stem cells by three different methods and evaluation of their fate after transplantation into the testis. *In vitro* Cell Dev Biol Anim 2016; 52(1): 49-61.

[18] Ghasemzadeh-Hasankolaei M, Sedighi-Gilani MA, Eslaminejad MB. Induction of ram bone marrow mesenchymal stem cells into germ cell lineage using transforming growth factor-beta superfamily growth factors. Reprod Domest Anim 2014; 49(4): 588-98.

[19] Ghasemzadeh-Hasankolai M, Batavani R, Eslaminejad MB, *et al.* Effect of zinc ions on differentiation of bone marrow-derived mesenchymal stem cells to male germ cells and some germ cell-specific gene expression in rams. Biol Trace Elem Res 2012; 150(1-3): 137-46.

[20] Hua J, Pan S, Yang C, *et al.* Derivation of male germ cell-like lineage from human fetal bone marrow stem cells. Reprod Biomed Online 2009; 19(1): 99-105.

[21] Hua J, Yu H, Dong W, *et al.* Characterization of mesenchymal stem cells (MSCs) from human fetal lung: potential differentiation of germ cells. Tissue Cell 2009; 41(6): 448-55.

[22] Huang P, Lin LM, Wu XY, *et al.* Differentiation of human umbilical cord Wharton's jelly-derived mesenchymal stem cells into germ-like cells *in vitro*. J Cell Biochem 2010; 109(4): 747-54.

[23] Lai D, Wang F, Dong Z, *et al*. Skin-derived mesenchymal stem cells help restore function to ovaries in a premature ovarian failure mouse model. PLoS One 2014; 9(5): e98749.

[24] Linher K, Dyce P, Li J. Primordial germ cell-like cells differentiated *in vitro* from skin-derived stem cells. PLoS One 2009; 4(12): e8263.

[25] Nayernia K, Lee JH, Drusenheimer N, *et al.* Derivation of male germ cells from bone marrow stem cells. Lab Invest 2006; 86(7): 654-63.

[26] Drusenheimer N, Wulf G, Nolte J, *et al.* Putative human male germ cells from bone marrow stem cells. Soc Reprod Fertil Suppl 2007; 63: 69-76.

[27] Hyun I. The bioethics of stem cell research and therapy. J Clin Invest 2010; 120(1): 71-5.

[28] Lo B, Parham L. Ethical issues in stem cell research. Endocr Rev 2009; 30(3): 204-13.

[29] Medvedev SP, Shevchenko AI, Zakian SM. Induced pluripotent stem cells: Problems and advantages when applying them in regenerative medicine. Acta Naturae 2010; 2(2): 18-28.

[30] Hayashi K, de Sousa Lopes SM, Surani MA. Germ cell specification in mice. Science 2007; 316(5823): 394-6.

[31] Hayashi K, Ohta H, Kurimoto K, *et al.* Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells. Cell 2011; 146(4): 519-32.

[32] Lesch BJ, Page DC. Genetics of germ cell development. Nat Rev Genet 2012; 13(11): 781-94.

[33] Jung D, Kee K. Insights into female germ cell biology: from *in vivo* development to *in vitro* derivations. Asian J Androl 2015; 17(3): 415-20.

[34] Stukenborg JB, Kjartansdottir KR, Reda A, *et al.* Male germ cell development in humans. Horm Res Paediatr 2014; 81(1): 2-12.

[35] Ewen-Campen B, Schwager EE, Extavour CG. The molecular machinery of germ line specification. Mol Reprod Dev 2010; 77(1): 3-18.

[36] Matsui Y. The molecular mechanisms regulating germ cell development and potential. J Androl 2010; 31(1): 61-5.

[37] Kocer A, Reichmann J, Best D, *et al*. Germ cell sex determination in mammals. Mol Hum Reprod 2009; 15(4): 205-13.

[38] Bowles J, Koopman P. Sex determination in mammalian germ cells: extrinsic versus intrinsic factors. Reproduction 2010; 139(6): 943-58.

[39] Spiller CM, Bowles J. Sex determination in mammalian germ cells. Asian J Androl 2015; 17(3): 427-32.

[40] Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. Development 2007; 134(19): 3401-11.

[41] Mazaheri Z, Movahedin M, Rahbarizadeh F, *et al.* Different doses of bone morphogenetic protein 4 promote the expression of early germ cell-specific gene in bone marrow mesenchymal stem cells. *In vitro* Cell Dev Biol Anim 2011; 47(8): 521-5.

[42] Shirazi R, Zarnani AH, Soleimani M, *et al.* BMP4 can generate primordial germ cells from bone-marrow-derived pluripotent stem cells. Cell Biol Int 2012; 36(12): 1185-93.

[43] Latifpour M, Shakiba Y, Amidi F, *et al.* Differentiation of human umbilical cord matrix-derived mesenchymal stem cells into germ-like cells. Avicenna J Med Biotechnol 2014; 6(4): 218-27.

[44] Li B, Liu W, Zhuang M, *et al.* Overexpression of CD61 promotes hUC-MSC differentiation into male germ-like cells. Cell Prolif 2016; 49(1): 36-47.

[45] Wei Y, Fang J, Cai S, *et al.* Primordial germ cell-like cells derived from canine adipose mesenchymal stem cells. Cell Prolif 2016; 49(4): 503-11.

[46] Lue Y, Erkkila K, Liu PY, *et al.* Fate of bone marrow stem cells transplanted into the testis: potential implication for men with testicular failure. Am J Pathol 2007; 170(3): 899-908.

[47] Horn MM, Paz AH, Duarte ME, *et al*. Germinative testicular cells and bone marrow mononuclear cells transplanted to a rat model of testicular degeneration. Cloning Stem Cells 2008; 10(4): 543-6.

[48] Lassalle B, Mouthon MA, Riou L, *et al*. Bone marrow-derived stem cells do not reconstitute spermatogenesis *in vivo*. Stem Cells 2008; 26(5): 1385-6.

[49] Yamaguchi S, Miura C, Kikuchi K, *et al*. Zinc is an essential trace element for spermatogenesis. Proc Natl Acad Sci USA 2009; 106(26): 10859-64.

[50] Monsefi M, Fereydouni B, Rohani L, *et al.* Mesenchymal stem cells repair germinal cells of seminiferous tubules of sterile rats. Iran J Reprod Med 2013; 11(7): 537-44.

[51] Hosseinzadeh Shirzeily M, Pasbakhsh P, Amidi F, *et al.* Comparison of differentiation potential of male mouse adipose tissue and bone marrow derived-mesenchymal stem cells into germ cells. Iran J Reprod Med 2013; 11(12): 965-76.

[52] Zhang D, Liu X, Peng J, *et al.* Potential spermatogenesis recovery with bone marrow mesenchymal stem cells in an azoospermic rat model. Int J Mol Sci 2014; 15(8): 13151-65.

[53] Yan G, Fan Y, Li P, *et al.* Ectopic expression of DAZL gene in goat bone marrow-derived mesenchymal stem cells enhances the trans-differentiation to putative germ cells compared to the exogenous treatment of retinoic acid or bone morphogenetic protein 4 signalling molecules. Cell Biol Int 2015; 39(1): 74-83.

[54] Tamadon A, Mehrabani D, Rahmanifar F, *et al*. Induction of spermatogenesis by bone marrow-derived mesenchymal stem cells in busulfan-induced azoospermia in hamster. Int J Stem Cells 2015; 8(2): 134-45.

[55] Li PZ, Yan GY, Han L, *et al.* Overexpression of STRA8, BOULE, and DAZL genes promotes goat bone marrow-derived mesenchymal stem cells *in vitro* transdifferentiation toward putative male germ cells. Reprod Sci 2017; 24(2): 300-12.

[56] Ghasemzadeh-Hasankolaei M, Batavani R, Eslaminejad MB, *et al.* Transplantation of autologous bone marrow mesenchymal stem cells into the testes of infertile male rats and new germ cell formation. Int J Stem Cells 2016; 9(2): 250-263.

[57] Li N, Pan S, Zhu H, *et al*. BMP4 promotes SSEA-1 hUC-MSC differentiation into male germ-like cells *in vitro*. Cell Prolif 2014; 47(4): 299-309.

[58] Amidi F, Ataie Nejad N, Agha Hoseini M, *et al. In vitro* differentiation process of human Wharton's jelly mesenchymal stem cells to male germ cells in the presence of gonadal and non-gonadal conditioned media with retinoic acid. *In vitro* Cell Dev Biol Anim 2015; 51(10): 1093-101.

[59] Chen H, Tang QL, Wu XY, *et al*. Differentiation of human umbilical cord mesenchymal stem cells into germ-like cells in mouse seminiferous tubules. Mol Med Rep 2015; 12(1): 819-28.

[60] Nejad NA, Amidi F, Hoseini MA, *et al.* Male germ-like cell differentiation potential of human umbilical cord Wharton's jelly-derived mesenchymal stem cells in co-culture with human placenta cells in presence of BMP4 and retinoic acid. Iran J Basic Med Sci 2015; 18(4): 325-33.

[61] Cakici C, Buyrukcu B, Duruksu G, *et al.* Recovery of fertility in azoospermia rats after injection of adipose-tissue-derived mesenchymal stem cells: the sperm generation. Biomed Res Int 2013; 2013: 529589.

[62] Afsartala Z, Rezvanfar MA, Hodjat M, *et al*. Amniotic membrane mesenchymal stem cells can differentiate into germ cells *in vitro*. *In vitro* Cell Dev Biol Anim 2016; 52(10): 1060-71.

[63] Bukovsky A, Ayala ME, Dominguez R, *et al.* Bone marrow derived cells and alternative pathways of oogenesis in adult rodents. Cell Cycle 2007; 6(18): 2306-9.

[64] Antonucci I, Di Pietro R, Alfonsi M, *et al.* Human second trimester amniotic fluid cells are able to create embryoid body-like structures *in vitro* and to show typical expression profiles of embryonic and primordial germ cells. Cell Transplant 2014; 23(12): 1501-15.

[65] Asgari HR, Akbari M, Abbasi M, *et al.* Human Wharton's jelly-derived mesenchymal stem cells express oocyte developmental genes during co-culture with placental cells. Iran J Basic Med Sci 2015; 18(1): 22-9.

[66] Johnson J, Canning J, Kaneko T, *et al.* Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428(6979): 145-50.

[67] Johnson J, Bagley J, Skaznik-Wikiel M, *et al*. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 2005; 122(2): 303-15.

[68] Lee HJ, Selesniemi K, Niikura Y, *et al*. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. J Clin Oncol 2007; 25(22): 3198-204.

[69] Ghadami M, El-Demerdash E, Zhang D, *et al.* Bone marrow transplantation restores follicular maturation and steroid hormones production in a mouse model for primary ovarian failure. PLoS One 2012; 7(3): e32462.

[70] Bukovsky A. Ovarian stem cell niche and follicular renewal in mammals. Anat Rec (Hoboken) 2011; 294(8): 1284-306.

[71] Danner S, Kajahn J, Geismann C, *et al.* Derivation of oocyte-like cells from a clonal pancreatic stem cell line. Mol Hum Reprod 2007; 13(1): 11-20.

[72] Lv X, Zhu H, Bai Y, *et al.* Reversine promotes porcine muscle derived stem cells (PMDSCs) differentiation into female germ-like cells. J Cell Biochem 2012; 113(12): 3629-42.

[73] Lai D, Wang F, Chen Y, *et al*. Human amniotic fluid stem cells have a potential to recover ovarian function in mice with chemotherapy-induced sterility. BMC Dev Biol 2013; 13: 34.

[74] Yu X, Wang N, Qiang R, *et al.* Human amniotic fluid stem cells possess the potential to differentiate into primordial follicle oocytes *in vitro*. Biol Reprod 2014; 90(4): 73.

[75] Qiu P, Bai Y, Pan S, *et al*. Gender depended potentiality of differentiation of human umbilical cord mesenchymal stem cells into oocyte-Like cells *in vitro*. Cell Biochem Funct 2013; 31(5): 365-73.

[76] Hu X, Lu H, Cao S, *et al*. Stem cells derived from human first-trimester umbilical cord have the potential to differentiate into oocyte-like cells *in vitro*. Int J Mol Med 2015; 35(5): 1219-29.

[77] Lai D, Guo Y, Zhang Q, *et al.* Differentiation of human menstrual blood-derived endometrial mesenchymal stem cells into oocyte-like cells. Acta Biochim Biophys Sin (Shanghai) 2016; 48(11): 998-1005.

[78] Handel MA, Eppig JJ, Schimenti JC. Applying "gold standards" to *in-vitro*-derived germ cells. Cell 2014; 157(6): 1257-61.

[79] da Cruz I, Rodriguez-Casuriaga R, Santinaque FF, *et al.* Transcriptome analysis of highly purified mouse spermatogenic cell populations: gene expression signatures switch from meiotic-to postmeiotic-related processes at pachytene stage. BMC Genomics 2016; 17: 294.

[80] Djureinovic D, Fagerberg L, Hallstrom B, *et al.* The human testis-specific proteome defined by transcriptomics and antibody-based profiling. Mol Hum Reprod 2014; 20(6): 476-88.

[81] Guyonnet B, Dacheux F, Dacheux JL, *et al.* The epididymal transcriptome and proteome provide some insights into new epididymal regulations. J Androl 2011; 32(6): 651-64.

[82] Bi H, Wang X, Xing X, *et al*. Proliferated Leydig cells for engineered testis-like tissue regeneration with testosterone-secreting ability. Tissue Eng Regen Med 2014; 11(5): 379-86.

[83] Raya-Rivera AM, Baez C, Atala A, *et al.* Tissue engineered testicular prostheses with prolonged testosterone release. World J Urol 2008; 26(4): 351-8.

[84] Baert Y, De Kock J, Alves-Lopes JP, *et al.* Primary human testicular cells self-organize into organoids with testicular properties. Stem Cell Reports 2017; 8(1): 30-38.

[85] Mahmoud H. Concise review: Spermatogenesis in an artificial three-dimensional system. Stem Cells 2012; 30(11): 2355-60.

[86] Kim J, Perez AS, Claflin J, *et al*. Synthetic hydrogel supports the function and regeneration of artificial ovarian tissue in mice. NPJ Regen Med 2016; 1: 16010.

[87] da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006; 119(Pt 11): 2204-13.

[88] Marques-Mari AI, Lacham-Kaplan O, Medrano JV, *et al.* Differentiation of germ cells and gametes from stem cells. Hum Reprod Update 2009; 15(3): 379-90.

[89] Pountos I, Corscadden D, Emery P, *et al*. Mesenchymal stem cell tissue engineering: techniques for isolation, expansion and application. Injury 2007; 38 Suppl 4: S23-33.

[90] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 2007; 213(2): 341-7.

[91] Rosenbaum AJ, Grande DA, Dines JS. The use of mesenchymal stem cells in tissue engineering: A global assessment. Organogenesis 2008; 4(1): 23-7.

[92] Sage EK, Thakrar RM, Janes SM. Genetically modified mesenchymal stromal cells in cancer therapy. Cytotherapy 2016; 18(11): 1435-45.